The effects of vitamin C and vitamin B12 on improving spermatogenesis in mice subjected to long-term scrotal heat stress

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Objective: Scrotal hyperthermia poses a significant threat to spermatogenesis and fertility in mammalian species. This study investigated the effects of vitamin B12 and vitamin C on spermatogenesis in adult male mice subjected to long-term scrotal hyperthermia. The rationale is based on the sensitivity of germ cells and epididymal sperm to increased scrotal temperatures. While various factors, both internal and external, can raise the testicular temperature, this study focused on the potential therapeutic roles of vitamins B12 and C.

Methods: After inducing scrotal hyperthermia in mice, vitamin B12 and vitamin C were administered for 35 days. We assessed sperm parameters, serum testosterone levels, stereological parameters, the percentage of apoptotic cells, reactive oxygen species (ROS) levels, and glutathione (GSH) levels. Additionally, real-time polymerase chain reaction was used to analyze the expression of the c-kit, stimulated by retinoic acid gene 8 (Stra8), and proliferating cell nuclear antigen (Pcna) genes.

Results: Vitamin C was more effective than vitamin B12 in improving sperm parameters and enhancing stereological parameters. The study showed a significant decrease in apoptotic cells and a beneficial modulation of ROS and GSH levels following vitamin administration. Moreover, both vitamins positively affected the expression levels of the c-kit, Stra8, and Pcna genes.

Conclusion: This research deepens our understanding of the combined impact of vitamins B12 and C in mitigating the effects of scrotal hyperthermia, providing insights into potential therapeutic strategies for heat stress-related infertility. The findings highlight the importance of considering vitamin supplementation as a practical approach to counter the detrimental effects of elevated scrotal temperatures on male reproductive health.

Keywords: Scrotal hyperthermia; Spermatogenesis; Vitamin B 12; Vitamin C

Introduction

In mammalian species, spermatogenesis occurs optimally at a temperature range that is approximately 2 to 4 °C lower than the core body temperature, which is essential for supporting both spermatogenesis and fertility [1]. To maintain this critical temperature difference, the testes are located outside the abdominal cavity, housed within the scrotum [2]. Studies have shown that germ cells in the testes and sperm in the epididymis are highly sensitive to increases in scrotal temperature [3].

Several factors can contribute to an increase in testicular tempera-
tture, falling into two primary categories: internal and external factors. Internal factors include cryptorchidism, varicocele, and prolonged fever exceeding 39 °C [4,5]. Meanwhile, external factors encompass lifestyle choices such as clothing, frequent hot baths and saunas, cycling, prolonged laptop use, and occupational hazards encountered by workers such as welders, kiln operators, and long-haul truck drivers [6].

Numerous studies have demonstrated that heat stress can lead to a range of detrimental effects. These include cell death in both germ and somatic cells, a reduction in testicular tissue volume, a decrease in the quality and quantity of sperm parameters, and ultimately, a disruption of spermatogenesis. This disruption occurs through the induction of apoptosis and oxidative stress signaling pathways [7].

Based on these findings, various approaches have been explored to counter infertility linked to heat stress. These include the use of specific drugs, flavonoid compounds, nanoparticles containing antioxidants, and androgens [8,9].

Vitamins are increasingly recognized as important treatments for a variety of health conditions. Vitamin B12, in particular, is essential for DNA synthesis and cell division [10], and it plays a vital role in the testicular environment, which is characterized by constant mitotic activity [11,12]. Studies on rodents with vitamin B12 deficiency have revealed significant testicular abnormalities, including decreased testicular weight, atrophy of the spermatogenic tubules, and a lack of spermatids and sperm [13]. In humans, studies have shown that high doses of vitamin B12 can improve spermatogenesis in individuals with oligospermia [13].

Vitamin C, a well-known natural antioxidant, plays a crucial role in numerous biological functions, notably its effect on semen quality and human fertility. Its capacity to neutralize free radicals helps shield spermatogenesis from the damaging influence of reactive oxygen species (ROS), thereby preserving sperm health. Previous studies have highlighted the beneficial effects of vitamin C on sperm count and quality, in addition to its function in boosting testosterone levels [14].

Despite this wealth of knowledge, the combined effect of these two vitamins in mitigating the effects of scrotal hyperthermia as a treatment strategy remains largely unexplored. Therefore, this study aimed to investigate and compare the impact of vitamins B12 and C on spermatogenesis in the testicular tissue of adult male mice after exposure to scrotal hyperthermia.

Methods

1. Animals

In this study, we obtained a total of 50 adult male mice from the Naval Medical Research Institute (NMRI). Each mouse had a body weight ranging from 25 to 30 g and was maintained under standard laboratory conditions. The Ethics Committee at Shahid Beheshti University of Medical Sciences (IR.SBMU.AEC.1402.12) conducted a thorough review and granted approval for the research protocol.

2. Scrotal hyperthermia model

To induce hyperthermia in mice, we exposed them to a temperature of 43 °C for 20 minutes on alternating days over a period of 5 weeks. The mice were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg). We immersed their lower body, including the scrotum and hind legs, in a water bath. Subsequently, the mice were dried and returned to their cages. In the control group, the animals were anesthetized but maintained at room temperature for the duration of the experiment.

3. Drug loading and release

In the group subjected to hyperthermia, animals received 3 µg of vitamin B12 and 10 mL/kg of vitamin C based on their body weight. As both vitamins are water-soluble, they did not require a solvent for administration. The injections were administered intraperitoneally over a period of 35 days.

4. Study design

The animals were divided into four groups: (sham) control, hyperthermia, and two treatment groups. In the sham group (control), the animals were placed in water at a temperature of 35 °C five times and were injected intraperitoneally with saline for 35 days, with a 48-hour interval between each session, for a duration of 20 minutes each time. The same procedure was performed in the hyperthermia and treatment groups, but at a temperature of 43 °C. No additional interventions were performed in the hyperthermia group. In the group treated with vitamin B12, 3 µg of vitamin B12 was injected intraperitoneally after inducing hyperthermia. In the group treated with vitamin C, 10 mL/kg of vitamin C was injected intraperitoneally for 35 days following hyperthermia induction.

5. Semen analysis

To collect sperm samples post-treatment, the tails of the epididymides were scraped and immersed in 1 mL of Ham’s F-10 medium (Sigma-Aldrich Product No. N6635). This was followed by a 20-minute incubation at 37 °C. Subsequently, a 10 µL aliquot of the sample was placed on a microscope slide and sperm motility was assessed using an inverted microscope. In this study, approximately 100 sperm were counted in each chamber. The evaluation of sperm motility was based on the criteria set forth by the World Health Organization (1999 to 2010), which include (1) progressive motility, (2) non-progressive motility, and (3) immotility. A counting chamber was used to measure the sperm count [15,16].
6. Measurement of blood testosterone levels

After the conclusion of the experiment, blood samples were collected from the animals’ hearts while they were deeply anesthetized to assess hormone levels. These samples were subsequently centrifuged at 6,000 ×g for 5 minutes at 4 °C and preserved at −80 °C for future analysis. To determine the serum testosterone levels in the blood, a mouse-specific enzyme-linked immunosorbent assay kit (catalog no. CSB-E11162r) was utilized.

7. Stereological studies

After the experimental period ended and the animals were euthanized, their testicles were extracted and preserved in Bouin’s fixative solution. Once the samples were adequately fixed, they underwent dehydration and embedding in a tissue processor. Following embedding in paraffin, sections of 5 and 10 microns were prepared from the samples and readied for hematoxylin and eosin staining.

The Cavalieri method was employed in the stereological analysis to determine the volume of testicular tissue. The calculation was performed using the formula [17,18]:

\[ V = \Sigma P \times t \times \frac{a}{p} \]

\( \Sigma P \) is the total number of points hitting the testis sections, \( a/p \) indicates the area associated with each sample point, and \( t \) represents the distance between tissue sections.

To quantify the total number of testicular cells, encompassing spermatogonia, primary spermatocytes, spermatids, as well as Sertoli and Leydig cells, researchers employ the optical dissector method. The cell count is determined using the formula provided below [17,18].

\[ N_v = \left[ \frac{\Sigma Q}{\Sigma P} \times \frac{a}{f} \times \frac{t}{BA} \right] \]

In the formula, \( \Sigma Q \) represents the overall count of cells, \( h \) denotes the thickness of the tissue under consideration, \( a/f \) represents the area per counting frame, and \( \Sigma P \) signifies the cumulative count of all the frames used for counting in all fields. Additionally, \( h \) corresponds to the height of the dissector, \( t \) represents the actual section thickness measured using a microcator, and \( BA \) is the thickness of the tissue section.

\[ N_{(total)} = N_v \times V_{(total)} \]

8. TUNEL staining

This technique is capable of detecting apoptotic cells by analyzing DNA fragmentation in cell and tissue suspensions. A terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) kit was used to assess the level of apoptosis according to the provided instructions. After slide preparation, the samples were examined under a fluorescence microscope for counting. The number of TUNEL-positive cells across different groups was then compared.

9. Measurement of ROS levels

The testicular cells were first separated using ethylene-diamine-tetraacetic acid (EDTA)/trypsin. Following this, the samples were centrifuged at 1,200 rpm and 4 °C for 5 minutes using phosphate-buffered saline. Afterward, 100 μL of dichlorodihydroflouresein diacetate with a concentration of 20 μM was added to the sample and incubated in the dark at 37 °C for 45 minutes. Finally, the samples were analyzed using a spectrofluorometer with a wavelength of 488 nm [19].

10. Measurement of glutathione levels

To determine glutathione (GSH) levels, the spectrofluorometric method is employed. A suspension of testicular cells (0.5 mL) was stained with O-phthalaldehyde (OPA) and N-ethyl-maleimide (NEM). The cells were then centrifuged at 1,000 rpm for 1 minute, and the resulting cell sediment was dissolved in 2 mL of fresh culture medium. Subsequently, the cells were washed twice to eliminate the fluorescent dye from the medium. To measure each sample, a Shimadzu RF 5,000 U fluorescence spectrophotometer was utilized, with an excitation wavelength of 495 nm and an emission wavelength of 530 nm [19].

11. Real-time polymerase chain reaction

This method involved observing polymerase chain reaction (PCR) in real-time by measuring the amount of fluorescence emitted and recording it in a detector. We used this method to semi-quantitatively study the expression of c-kit, proliferating cell nuclear antigen (Pcna), and stimulated by retinoic acid gene 8 (Stra8) genes.

First, we extracted whole RNA samples and added DNase to eliminate any remaining genomic DNA. Then, following the instructions provided with the kit, we synthesized cDNA in a 20-μL volume at a temperature of 42 °C for 60 minutes. We used SYBR Green I dye for labeling. This dye binds to and replaces the DNA minor groove during the annealing and DNA replication stage. As the amount of double-stranded DNA increases, more fluorescent light is emitted and measured by the device.

To investigate the genes, we used a pair of forward and reverse primers designed with Primer 3 Plus software for the exon-exon junction. These primers allowed us to distinguish cDNA from genomic DNA. Before conducting the tests, we confirmed the PCR primers using the Primer-Blast method available at www.ncbi.nlm.nih.gov/tools/primer-blast (Table 1).

12. Statistical analysis

The mean±standard deviation of the quantitative data was ob-
of the hyperthermia group, along with a statistically significant difference from the control group. Additionally, sperm motility was significantly reduced in the hyperthermia group in comparison to the hyperthermia +vitamins and control groups (p<0.0001). Furthermore, the groups receiving treatment showed a greater improvement in sperm motility than the hyperthermia group (p<0.0001). The research also indicated that vitamin C was more effective in improving these parameters than vitamin B12 (p<0.001). Despite the lack of a significant difference between the two vitamins in these parameters, vitamin C showed superior restorative effects (Figure 1).

2. Vitamin C outperformed vitamin B12 in improving stereological parameters in mice subjected to long-term scrotal hyperthermia

Based on a stereological assessment of the histological studies, the hyperthermia group exhibited a significant decrease in testis volume compared to both the hyperthermia+vitamins and control groups (p<0.0001). There was no significant difference between vitamin C and vitamin B12 (p<0.0001). When comparing the hyperthermia group to the hyperthermia+vitamins and control groups, the hyperthermia group demonstrated a significant reduction in the number of spermatogonia, primary spermatocytes, and round spermatids. However, vitamin C treatment significantly increased the counts of spermatogonia, primary spermatocytes, and round spermatids following sustained scrotal hyperthermia (p<0.0001). In contrast, the number of Sertoli cells did not differ significantly between the vitamin-treated groups. The number of Leydig cells decreased significantly in the hyperthermia group compared to the hyperthermia+vitamins and control groups. Notably, there was a significant increase in

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### Table 1. Primer design

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>Product size (bp)</th>
<th>TM (°C)</th>
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<tbody>
<tr>
<td>c-kit</td>
<td>F: TGTAGAGAGAGGGAGGA</td>
<td>332</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>R: ATGTTGGAGATGGCTGTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stra8</td>
<td>F: GAACCTCACCACACGCTCAA</td>
<td>151</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>R: TCACCCGAGGGCATCTTTATT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pcna</td>
<td>F: CAGAACAATTCACCCAGCCTCC</td>
<td>160</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>R: TGGCAGGTTTCTCAAGACGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: CAGAACATCATCCCAGCCTCC</td>
<td>293</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>R: TTGGCAGGTTTCTCAAGACGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TM, temperature; Stra8, stimulated by retinoic acid gene 8; Pcna, proliferating cell nuclear antigen; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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**Figure 1.** (A, B) Mean±standard deviation of the total sperm count and sperm motility in the study groups (control, hyperthermia, vitamin B12, and vitamin C). a) p<0.001; b) p<0.0001.

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Leydig cells in the hyperthermia+vitamins group, with vitamin C showing the most pronounced effect \(p<0.0001\) (Figure 2).

3. Vitamin C improved serum testosterone levels to a greater extent than vitamin B12 in mice subjected to long-term scrotal hyperthermia.

The serum testosterone level was significantly lower in the hyper-

![Figure 2](image)

**Figure 2.** (A) Photomicrograph of the testis stained with hematoxylin and eosin (H&E), \(\times10\) (a: control group; b: hyperthermia group; c: vitamin B12 group; d: vitamin C group). (B) Mean±standard deviation of (a) testis volume and the total number of (b) spermatogonia (SG), (c) primary spermatocytes (PS), (d) spermatids, and (e) Sertoli cell (SC) and (f) Leyding cell (LC) in the study groups (control, hyperthermia, vitamin B12, and vitamin C). (g) Photomicrograph of the testis stained with H&E, \(\times40\) (scale bar=10 μm). ST, seminiferous tubule; IT, interstitial tissue; SPT, round spermatid. \(^a\) \(p<0.0001\).
thermia group than in both the hyperthermia+vitamins group and the control group. Our findings indicate that vitamin C treatment effectively elevated serum testosterone levels. No significant difference was observed in serum testosterone levels between the hyperthermia group and the group treated with vitamin B12. However, vitamin C supplementation resulted in serum testosterone levels that more closely approximated those of the control group ($p < 0.0001$) (Figure 3).

4. Administration of vitamins decreased the level of ROS and increased the level of GSH

ROS are small, unstable, and highly reactive molecules that have the potential to oxidize proteins, lipids, and DNA. ROS are generated through the incomplete reduction of oxygen involving an electron. A marked increase was observed in ROS levels in the hyperthermia group compared to the control group. Conversely, ROS levels were significantly reduced in the hyperthermia + vitamins group. There was no significant difference between the two hyperthermia+vitamin groups ($p < 0.0001$) (Figure 3). In contrast the level of GSH in the hyperthermia group decreased compared to the control group. The GSH level increased in the hyperthermia+vitamins group, and the increase in GSH levels was higher in the hyperthermia +vitamin C group ($p < 0.001, p < 0.0001$) (Figure 3).

5. Vitamin B12 and vitamin C reduced the percentage of apoptotic cells in mouse testes subjected to long-term scrotal hyperthermia

The results of the TUNEL assay revealed a significant increase in apoptotic cells in the hyperthermia group compared to both the hyperthermia+vitamins and control groups. Moreover, vitamin C was found to be more effective in reducing apoptotic cells than vitamin B12. In other words, vitamin C led to a greater decrease in apoptotic cells, approaching the levels observed in the control group ($p < 0.0001$) (Figure 4).

6. Vitamin administration increased the expression of c-kit, Stra8, and Pcna in mice subjected to long-term scrotal hyperthermia

The expression of all three genes (c-kit, Stra8, and Pcna) was decreased in the hyperthermia group compared to the control group. However, the expression of all three genes increased in the hyperthermia+vitamin C and hyperthermia+vitamin B12 groups and was close to the control group. Nonetheless, the increase in the expression of genes in the hyperthermia +vitamin C group was slightly greater than that in the hyperthermia+vitamin B12 group, although the difference was not significant ($p < 0.001, p < 0.01$) (Figure 5).

**Discussion**

Numerous studies have shown that hyperthermia in mammals can lead to toxicity and reproductive issues, which may result in fertility disorders [20,21]. These studies have found that hyperthermia worsens the impact on testicular tissue, causing abnormalities in sperm parameters such as motility, morphology, count, and viability, as well as damage to sperm DNA. This damage stems from disruptions in spermatogenesis that disturb the balance between oxidation and reduction, leading to oxidative stress. As a result, heat stress im-

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**Figure 3.** (A) Mean±standard deviation (SD) of the testosterone level in the study groups (control, hyperthermia, vitamin B12, and vitamin C). (B) Mean±SD of reactive oxygen species (ROS) generation in testicular tissue in the study groups (control, hyperthermia, vitamin B12, and vitamin C). (C) Mean±SD of glutathione (GSH) generation in testicular tissue in the study groups (control, hyperthermia, vitamin B12, and vitamin C). $^{a} p<0.001; ^{b} p<0.0001.$

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pairs spermatogenic cells, lowers testosterone levels, and ultimately reduces fertility and fertilization rates. Additionally, heat stress triggers oxidative stress, increases the expression of p53 and caspase-3 mRNA, and decreases Bcl-2 expression, thereby activating the apoptotic pathway [22]. Given the absence of definitive treatments for infertile patients with non-obstructive azoospermia, there is a pressing need to discover effective treatments or preventive agents to improve fertility.

To date, a range of methods including the administration of androgens, pharmaceuticals, and antioxidant compounds have been employed to address infertility. One treatment approach that has garnered interest involves the use of vitamins. This study specifically focused on the effects of vitamins B12 and C. A thorough understanding of the beneficial impacts of vitamins B12 and C could represent a significant advancement in the treatment of male infertility.

The results of this study offer valuable insights into the relative effectiveness of vitamin C and vitamin B12 in reducing the negative impact of chronic scrotal hyperthermia on spermatogenesis and testicular health in mice. These findings are significant for the development of potential treatments for heat-induced damage to the testes. The study observed marked differences in sperm parameters across the experimental groups. In the hyperthermia group, there was a

![Figure 4. (A) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) detection of apoptosis in the testis of the study groups. Photomicrograph showing the immunofluorescence of apoptotic cells expressed in the testis tissue (green) and counterstained with 4′,6-diamidino-2-phenylindole (DAPI; blue) in the study groups (control, hyperthermia, vitamin B12, and vitamin C). (B) Mean±standard deviation of apoptotic cells in the study groups (control, hyperthermia, vitamin B12, and vitamin C). a) p<0.01; b) p<0.001; c) p<0.0001.](image-url)
pronounced decrease in the total sperm count. Treatment with either vitamin C or vitamin B12 (hyperthermia+vitamins) led to a notable improvement in sperm counts when compared to the hyperthermia group, though the counts did not reach the levels seen in the control group. Vitamin C, in particular, showed a marginally better effect in improving spermatogenesis.

Sperm motility and viability were adversely affected by scrotal hyperthermia, with significant reductions observed in the hyperthermia group. Both vitamins facilitated the recovery of sperm motility, with vitamin C having a more pronounced effect, exceeding the restorative capabilities of vitamin B12. These findings align with previous research that underscores the importance of vitamin C in maintaining sperm function [23].

The study also evaluated serum testosterone levels, which are essential for male reproductive function. In the hyperthermia group, scrotal hyperthermia led to a marked decrease in serum testosterone compared to the hyperthermia+vitamins and control groups. Treatment with vitamin C was notably effective in enhancing testosterone levels, bringing them nearer to those observed in the control group. Conversely, treatment with vitamin B12 did not result in a significant elevation of serum testosterone levels when compared to the hyperthermia group. These findings are consistent with the recognized antioxidative and hormonal regulatory functions of vitamin C [23,24].

These findings indicate that vitamin C is more effective than vitamin B12 at reducing the negative impact of scrotal hyperthermia on testosterone production. The ability of vitamin C to normalize testosterone levels highlights its potential role in supporting reproductive health [24]. Stereological analysis of testicular tissue revealed several significant alterations caused by scrotal hyperthermia. Testicular volume markedly decreased in the hyperthermia group, but both vitamin C and vitamin B12 treatments provided some improvement. Notably, vitamin C had a marginally better effect, though the difference between the effects of the two vitamins was not statistically significant. Similar results have been observed in other studies investigating the influence of antioxidants on testicular volume [25].

Furthermore, the number of testicular cells was significantly reduced in the hyperthermia group. Treatment with vitamin C resulted in a marked increase in the quantity of these cells, an effect that was significantly more pronounced than that seen with vitamin B12. This finding is consistent with prior studies that have highlighted the protective role of vitamin C in spermatogenesis [26]. The numbers of Sertoli and Leydig cells, which are crucial for spermatogenesis and testosterone synthesis respectively, also improved after vitamin therapy. Although vitamin C had a more notable impact on the proliferation of Leydig cells, the difference in efficacy between the two vitamins did not reach statistical significance. These findings underscore the potential of vitamin C to protect and restore the structure and function of testicular tissue after damage induced by hyperthermia [26].

Apoptosis, a form of programmed cell death, is essential for regulating cell populations in the testis. When excessive, apoptosis can interfere with spermatogenesis and compromise testicular function. Our study examined the effects of scrotal hyperthermia on the levels of apoptotic cells in the testis and assessed the efficacy of vitamin C and vitamin B12 in mitigating apoptosis.

Figure 5. Mean±standard deviation of the relative gene expression of (A) c-kit, (B) stimulated by retinoic acid gene 8 (Stra8), and (C) proliferating cell nuclear antigen (Pcna) in testis tissue in the study groups (control, hyperthermia, vitamin B12, and vitamin C). a) \( p<0.01; \) b) \( p<0.001; \) c) \( p<0.0001. \)
The TUNEL assay results revealed a significant increase in apoptotic cells in the hyperthermia group compared to both the hyperthermia+vitamins and control groups. Notably, treatment with vitamin C led to a more pronounced decrease in apoptotic cells than vitamin B12. This suggests that vitamin C is more effective at safeguarding testicular cells against apoptosis induced by hyperthermia. These findings are consistent with prior studies that have highlighted the anti-apoptotic properties of vitamin C in relation to testicular health [26].

The findings of this study are consistent with prior research, suggesting that scrotal hyperthermia results in elevated levels of ROS and reduced levels of GSH in testicular tissue. Furthermore, the results indicate that the consumption of vitamins C and B12 can counteract the increase in ROS levels, boost GSH levels, and help normalize these two parameters.

Additionally, we utilized RNA extraction to analyze the expression levels of the c-kit, Stra8, and Pcna genes. These genes play roles in the initiation of meiosis and the differentiation, migration, survival, proliferation, and maturation of spermatogonial cells and round spermatids [9]. Our study’s results suggest that scrotal hyperthermia, along with vitamin C and vitamin B12 treatment, leads to a significant reduction in transcriptional levels in mice. However, this reduction is offset by an upregulation of c-kit, Stra8, and Pcna gene expression, implying that the protective effects of vitamins on spermatogonia cells may be responsible for this outcome.

In summary, our study highlights the potential advantages of vitamin C and vitamin B12 in counteracting the detrimental effects of chronic scrotal hyperthermia on spermatogenesis, testosterone synthesis, testicular architecture, and apoptotic cell counts. Vitamin C demonstrated greater effectiveness in most of these aspects. These results could have implications for creating therapeutic approaches to mitigate heat-induced testicular damage and enhance male reproductive well-being. Additional research is required to clarify the mechanisms involved and to apply these findings to clinical practices for human fertility and reproductive health.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

This study was prepared from the thesis by Nafiseh Moeinian, an MSc student at the Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Registration No. 43005493).

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Funding acquisition: RMF. Project administration: RMF. Visualization: NM, RMF, MAA. Software: NM, HAA, HN, AA (Azar Afshar), RMF, MAA.
Validation: RMF, MAA. Investigation: NM, RMF, MAA. Writing-original draft: NM, FFF, MN, HAA, HN, AA (Azar Afshar), RS, FA, AA (Abbas Aliaghaei), RMF, MAA. Writing-review & editing: NM, RMF, MAA. Approval of final manuscript: NM, FFF, MN, HAA, HN, AA (Azar Afshar), RS, FA, AA (Abbas Aliaghaei), RMF, MAA.

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