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The effect of eight weeks of swimming training, cell and laser therapy on the expression of IL-2, IL-10 and STAT-3 genes in testicular tissue in azoospermia model mice.

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Abstract

Background: Azoospermia means lack of sperm in semen. One percent of all men and ten percent of infertile men suffer from azoospermia, the purpose of this study is to investigate the effects of eight weeks of swimming training, cell and laser therapy on the expression of IL-2, IL-10 and STAT-3 genes in testicular tissue. It is azoospermia in rats.

Methods: Forty 6- to 8-week-old rats were randomly selected, and then the azoospermia model was induced with Busulfan at a dose of 40 mg in mice. One month after induction of the model, stem cells were transplanted once in the vas deferens at the rate of one million cells per mouse. Then, one week after cell transplantation, a laser with a wavelength of 632.8 nm and a power of 10 mW and energy 3 joules were applied in three repetitions throughout the study period with an interval of once a week, and after improving the effects of surgery, swimming training were performed for 30 minutes and 5 days a week for 8 weeks.

Results: The results showed that induction of azoospermia model caused a significant increase in IL-2, IL-10 and STAT-3 genes compared to healthy group rats ($P \leq 0.05$). Also, performing the interventional methods of laser therapy, cell therapy and exercise significantly reduced the expression of these genes in the testicular tissue of rats compared to the patient group ($P \leq 0.05$).

Conclusion: Swimming exercise in combination with cell and laser therapy may help the fertility of azoospermia model rats by reducing testicular tissue inflammation signaling.

Keywords: Azoospermia, swimming training, cell therapy, laser therapy and IL-10/STAT-3 signaling

Introduction

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Male infertility is a multifactorial disorder that encompasses a wide range of diseases and is a symptom of a wide range of pathological conditions that affect both the sexual and other physiological systems, including the endocrine, nervous, hematological, and immune systems (1).

Diseases such as genital cancers in young men, bacterial infections, some genetic syndromes such as praderovirus (PWS), and mutations in fertility genes can cause azoospermia in men. Azoospermia means a lack of sperm in the semen. One percent of all men and ten percent of infertile men have azoospermia (2). In 30 percent of men who suffer from infertility, no specific cause can be found (pathological male infertility). Although cytokines are a means of communication between cells, data on their function in male infertility in the human reproductive system are relatively scarce. Recently, Loveland et al. (2017) reviewed the function of cytokines in the regulation of spermatogenesis (3), and reported that they described the molecular mechanisms of cytokine action with a focus on the oncogenic role of cytokines and evaluated the potential of cytokines as apoptotic targets or therapeutic agents. Uncontrolled cytokine production leads to severe pathological conditions such as bacterial toxic shock and autoimmune diseases. Cytokines are not only produced by immune cells. Fibroblasts, endothelial cells, fat cells, and cells in other tissues such as the ovary produce cytokines. In addition to their role in the immune system, they interfere with almost every biological process such as embryonic development, cognitive function, and stem cell differentiation. Based on their mode of action, they can be classified as stimulants for immune cells, proinflammatory cytokines, inflammatory cytokines, anti-inflammatory cytokines, and cytokines that act as growth factors. Furthermore, different cytokines can have synergistic effects or may antagonize each other. Finally, cytokines are characterized by a cascade effect, as they can stimulate the production of other cytokines from the target cell (4).

IL-10 is an immunosuppressive cytokine produced by quiescent mesenchymal stem cells. IL-10 expression can be further increased by TLR ligands and PEG2. IL-10 can inhibit the maturation of antigen presenting cells (APC) and the expression of MHC and co-stimulatory factors. IL-10 inhibits proinflammatory cytokine production, T cell proliferation, and memory T cell formation. IL-10 suppresses Th17 production and promotes Treg formation. IL-10 exerts its anti-inflammatory effects through the JAK1-TYK2-STAT3-SOCS3 pathway (5).

Physical activity, which is considered a suitable practical solution for various diseases today, reduces cardiac risk factors, prevents myocardial damage, and increases cardiac function (6). Among aerobic exercises, low-intensity aerobic swimming is one of the exercises that is safe and usable in various physiological conditions. It is also used in most physiological, biochemical, and molecular response studies because of the lack of weight bearing in water compared to non-aquatic exercise (7). Slow to moderate exercise gradually improves metabolic activity due to increased blood flow, but vigorous activity decreases blood flow due to the diversion of blood flow to the active muscles (7). Reduced physical activity can reduce sex hormone levels, sperm production, and fertility, and can also cause a decrease in testicular sex hormone levels and sperm count (8).

In a review study by Wamond et al. (2017), it was reported that after a period of low- to moderate-intensity aerobic exercise, the level of azoospermia improved by modulating genes involved in the disease and reducing the level of oxidative stress and inflammation in these patients, and increasing sperm quality and fertility (9). Although possible mechanisms have been proposed, the results of

studies on the relationship between physical activity and the level of azoospermia are inconclusive. On the other hand, in the current decade, the emerging field of stem cell therapy has rapidly become a new era of regenerative medicine. The diverse potential of stem cells is the focus of research by many scientists in molecular biology, genetic engineering, and even general medicine to develop new approaches to the treatment of a number of diseases that have always been a challenge for clinics (10). Stem cells have been introduced as promising new therapeutic agents in the treatment of degenerative diseases due to their high differentiation potential while maintaining the ability to self-proliferate and maintain the characteristics of their cells of origin (11). Among the various types of cell therapy, stromal/mesenchymal stem cell (MSC) therapy is increasingly being developed as a new method for treating structural defects that require repair and regeneration (12). Based on in vitro studies, mesenchymal stem cells from various tissue sources have been differentiated into germ cells or gamete precursor cells in both males and females by simple methods. On the other hand, the therapeutic effects of mesenchymal stem cells have been evaluated for the treatment of animal models of azoospermia induced by chemical compounds or surgery (13). The results of these studies confirmed the successful transplantation or exon transplantation of MSCs into the seminiferous tubules. It has also been reported that exosomes secreted by MSCs are able to induce spermatogenesis in the testes of infertile animal models (14). Despite numerous advances in the treatment of reproductive diseases in men and women with the help of mesenchymal stem cells or their exosomes, no clinical trials have been completed on the treatment of azoospermia.

Also, laser therapy, which is a new method of physiotherapy, is an example of interdisciplinary medicine based on research in the fields of physiology, biophysics, and biochemistry (15). Laser therapy is widely used in modern medicine due to its high efficiency, ease of use, and lack of side effects. The natural and biological effect of this method on tissues and the attempt to promote tissue repair or reduce inflammation depends on the energy dose (15, 16), which involves the application of light in continuous or pulsed wave modes in the near-infrared range (600–1100 nm). It is a non-invasive treatment that uses energy density and wavelength to penetrate different tissue layers, thereby activating different cellular mechanisms and generating new systems and responses (16). The specific effect of laser radiation on sperm has been studied in animal models. In these studies, which used lasers with different powers, different radiation energies, and different radiation durations, the results showed that the use of low-power, low-energy-density lasers has the most favorable effect on sperm quantitative and qualitative parameters. For example, Tajali et al. (2022) showed in a study that using a laser with an energy density of 8 J/cm2 is beneficial in strengthening germ cells and producing sperm (17). Considering the above cases and the positive effects of physical activity, cell therapy, and laser therapy in the treatment of infertility and determining the combined effects of these interventions, this study investigated the effects of eight weeks of swimming training, cell therapy, and laser therapy on the expression of IL-2, IL-10, and STAT-3 genes in testicular tissue in azoospermia model mice.

Materials and methods

The samples of the present study consisted of male Wistar rats that were randomly selected. Given that the subjects were under control in the laboratory in terms of many variables, the present study was of an experimental type.

The statistical sample of this study included male Wistar rats, aged about 6 to 8 weeks, of which 40 were purchased from the Tehran Laboratory Animal Research and Reproduction Center. The sample size in our study was determined based on previous experimental studies using rodent models of azoospermia, where a similar number (5 Rats per group) was sufficient to detect statistically significant differences in gene expression (23). After the subjects were transferred to the laboratory environment and after a week of adaptation to the new environment, they were kept in groups of 5 in transparent polycarbonate cages in an environment with an average temperature of 22 ± 1.4 °C, humidity of 55%, and a 12:12 hour light-dark cycle. Animal care was carried out in accordance with the guidelines of the International Institute of Health and the protocols of this study, in compliance with the principles of the Declaration of Helsinki and the rules of medical ethics. All animals were fed a standard packaged chow manufactured by Behparvar Company (Karaj, Iran), containing approximately 20% protein, 4% fat, 55% carbohydrate, 4% fiber, and supplemented with vitamins and minerals, at a rate of 10 g per 100 g of body weight daily, based on weekly weighing. Animals had free access to drinking water. No high-fat diet was used in this study. In order to create the azoospermia model, first, 6- to 8-week-old adult rats with an average weight of 220 to 250 g were used. Then, busulfan was injected intraperitoneally at a dose of 40 mg/kg of body weight of the rats (23). After confirmation of azoospermia, rats were randomly assigned to seven groups (n=6 per group) using a computer-generated random number table. Group allocation was performed by a laboratory assistant blinded to the interventions to minimize selection bias. After one month of model induction, the rats in each group were grouped as follows:

1- Healthy control group (kept for 8 weeks).

2- Patient control group, which remained in the cage for 8 weeks without any intervention one month after the creation of the model until the end of the study.

3- Patient group + low-power laser (one month after the creation of the azoospermia model, low-power laser with a wavelength of 632.8 nm and a power of 10 mW and an energy of 3 joules was applied three times during the entire study period, once a week, to the testicle area of the rats. And the rats were kept for 8 weeks until the end of the study)

4- Patient group + swimming exercise (one month after the creation of the azoospermia model, the rats swam for 30 minutes a day, 5 days a week, for 8 weeks).

5- Patient group + cells (one month after the creation of the azoospermia model, stem cells were transplanted once in the vas deferens area at a rate of one million cells per rat in the right testicle, and the rats were maintained for 8 weeks until the end of the study).

6- Patient group + cells + swimming exercise (One month after creating the azoospermia model, stem cells were transplanted once into the vas deferens area at a rate of one million cells per mouse. One week after cell transplantation, the mice swam for 30 minutes a day, 5 days a week, for 8 weeks).

7- Patient group + laser + swimming exercises (One month after creating the azoospermia model, low-power laser with a wavelength of 632.8 nm, a power of 10 milliwatts, and an energy of 3 joules was applied three times throughout the study period, once a week. After the wound in the cell transplant area on the abdomen healed, swimming exercises were performed for 5 days a week, each session lasting 30 minutes, for 8 weeks).

8- Sham group.

Tissue sampling from the testicular tissue of mice was performed under completely similar conditions and at baseline (two days after the end of the training period). To eliminate the acute effect of training, sampling was performed from the animals 48 hours after the last swimming training program. For this purpose, the animals were first anesthetized using intraperitoneal injection of ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg) and then killed. After killing, the transplanted tissues were evaluated for genetic studies.

Method of studying the genes studied in the study

To study the expression of the genes studied in the study in each group, the tissues were examined using the Real Time PCR technique. First, primer design was done, and then total RNA was extracted from the tissues and converted to cDNA. Then, the cDNA was amplified by PCR and examined for the expression of the mentioned genes.

Total RNA extraction

For molecular studies at the gene expression level, total RNA was first extracted from testicular tissues of all studied groups using Qiagen kits (Germany) according to the manufacturer's protocol. To eliminate potential genomic DNA contamination, all RNA samples were treated with DNase I (Fermentas, USA). RNA purity and concentration were assessed using a spectrophotometer (DPI-1, Qiagen), and only samples with A260/A280 ratios between 1.8–2.0 were used for downstream analysis.

Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using oligo-dT primers (MWG-Biotech, Germany) and reverse transcriptase enzyme (Fermentas, USA) following the manufacturer's instructions. For RT-qPCR, each reaction was performed using SYBR Green Master Mix (Applied Biosystems) on an ABI StepOne system (Applied Biosystems, Foster City, CA). The amplification protocol consisted of an initial denaturation at 94°C for 5 minutes, followed by 40 cycles of 94°C for 20 seconds, 58–60°C for 30 seconds, and 72°C for 30 seconds.

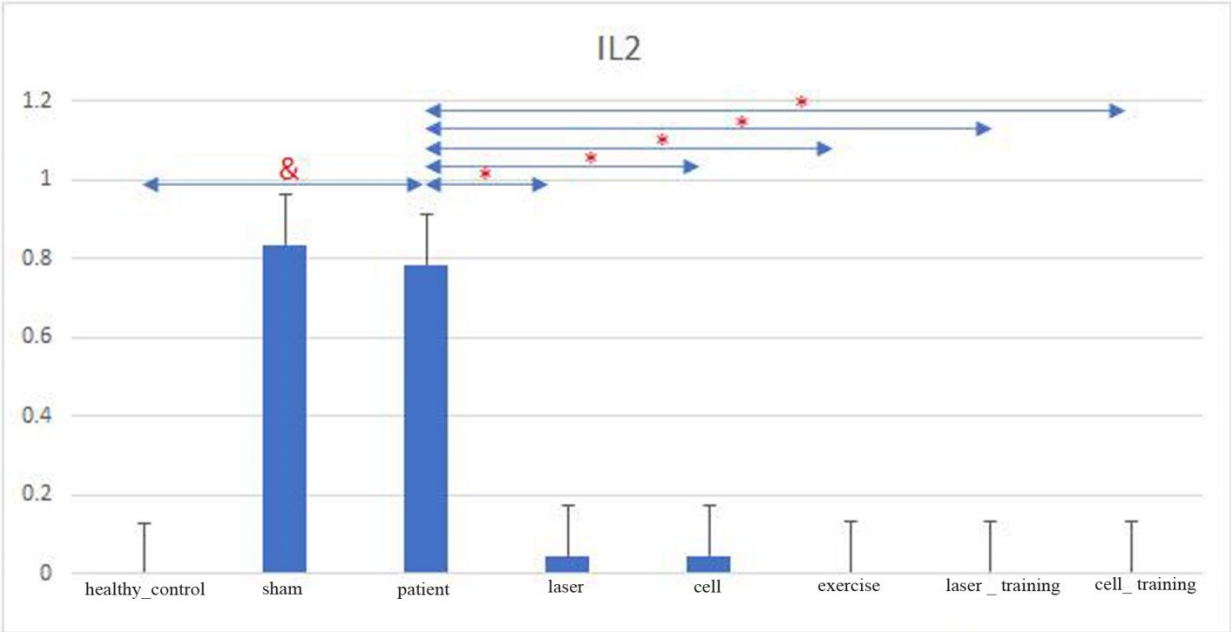
To ensure specificity and reliability of the results, melting curve analysis was performed for each gene to detect non-specific amplification or primer-dimer formation. Additionally, each qPCR run included negative controls without cDNA template to monitor for contamination. All samples were run in triplicate, and the mean Ct values were used for relative quantification using the 2^-ΔΔCt method. These steps ensured high accuracy, reproducibility, and validity of the gene expression data.

Statistical Analysis

To analyze the findings of this study, the Shapiro-Wilk test was used to examine the normal distribution of data, Levine's test for homogeneity of variances, one-way analysis of variance test, and Tukey's post hoc test were used to compare between different research groups. All calculations were performed using SPSS 22 statistical software at a significant level of $P \leq 0.05$.

Results

The average and standard deviation of the expression levels of the IL-2 gene in different research groups show that the highest levels of the IL-2 index are related to the sham group and the lowest levels belong to the healthy control group. Also, the results of one-way analysis of variance on the levels of IL-2 in different research groups indicate that; the calculated F value (146.870) and its significance at the p = 0.000 level indicate a significant difference between the levels of IL-2 in different research groups. At the 0.05 confidence level, the patient and sham groups had a significant increase compared to the healthy group, and the laser, cell, exercise, laser-exercise, and cell-exercise groups had a significant decrease compared to the patient and sham groups (Figure 1).

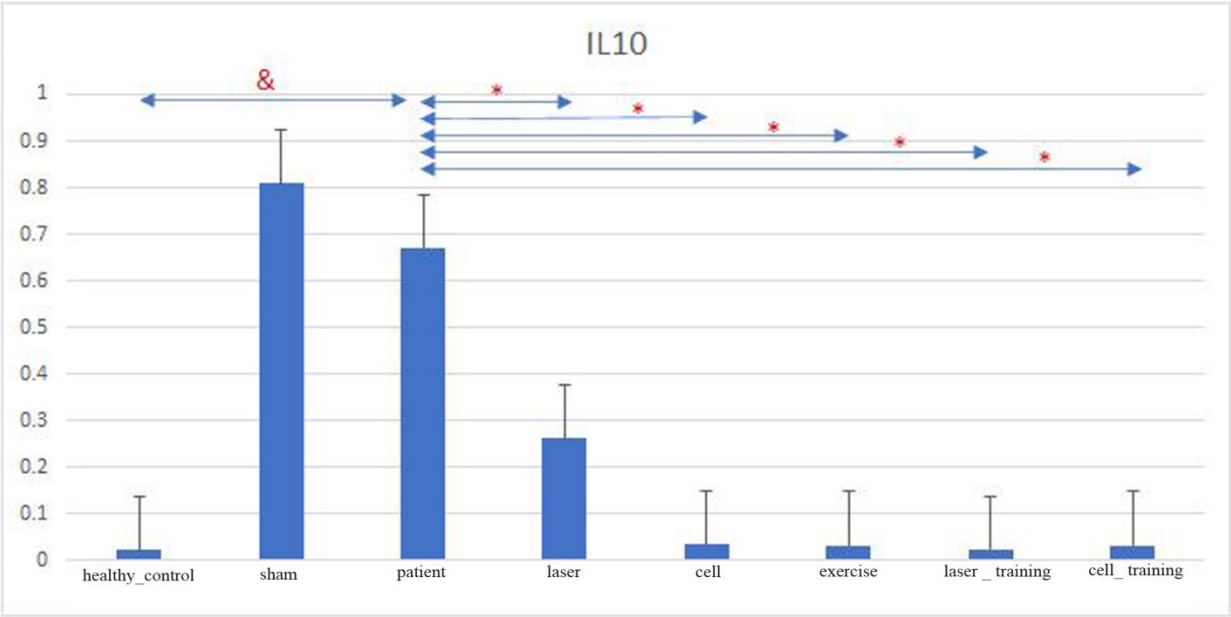


*Significant sign compared to the patient group & Significant sign compared to the healthy group

Figure 1. Comparison of mean IL2 levels in different study groups

The mean and standard deviation of IL-10 gene expression levels of different research groups show that the highest levels of IL-10 index are related to the sham group and the lowest levels belong to the healthy control group. The results of one-way analysis of variance on IL-10 levels of different research groups indicate that; the calculated F value (617.59) and its significance at the p = 0.001 level indicate a significant difference between IL-10 levels in different research groups. At the 0.05 confidence level, the patient and sham groups had a significant increase compared to the

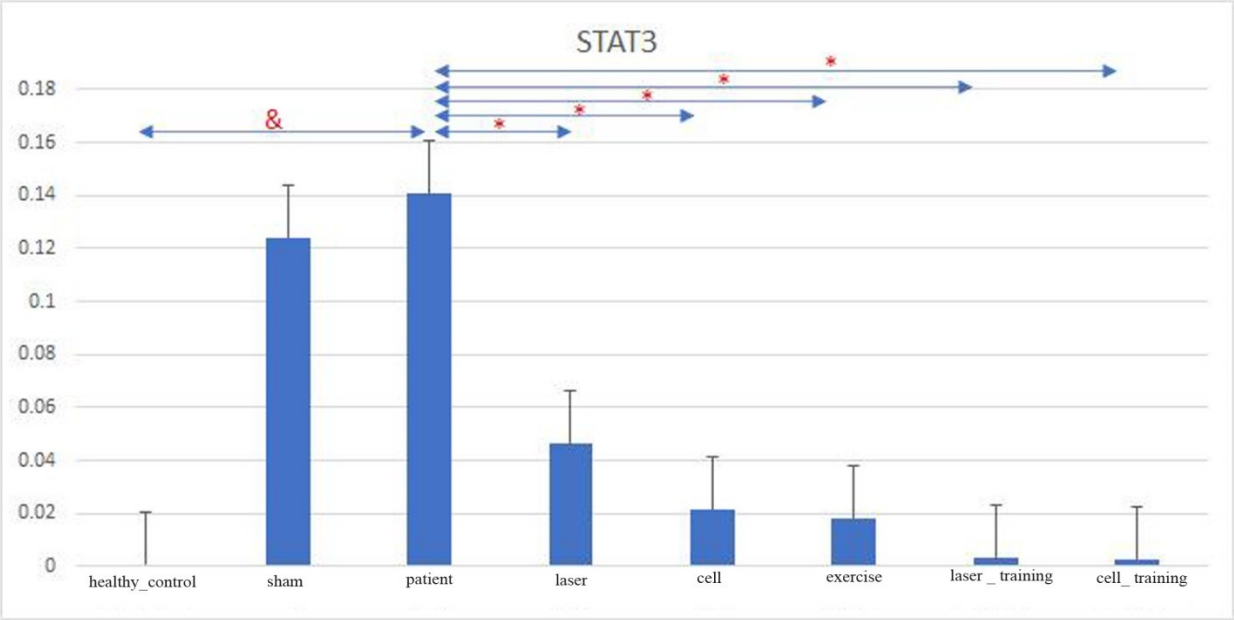
healthy group, and the laser, cell, exercise, laser-exercise, and cell-exercise groups had a significant decrease compared to the patient and sham groups (Figure 2).



*Significant sign compared to the patient group & Significant sign compared to the healthy group

Figure 2. Comparison of mean IL-10 levels in different study groups

The mean and standard deviation of the expression levels of the STAT3 gene in different research groups showed that the highest levels of STAT3 were in the patient group and the lowest levels were in the healthy control group. The results of one-way analysis of variance on the levels of STAT3 in different research groups indicated that; the calculated F value (72.031) and its significance at the p = 0.001 level indicate a significant difference between the levels of STAT3 in different research groups. At the 0.05 confidence level, the patient and sham groups had a significant increase compared to the healthy group, and the laser, cell, exercise, laser-exercise, and cell-exercise groups had a significant decrease compared to the patient and sham groups (Figure 3).



*Significant sign compared to the patient group & Significant sign compared to the healthy group

Figure 3. Comparison of average STAT3 levels in different study groups

Discussion

The results showed that the expression of IL-10, IL-2 and STAT3 genes in the testicular tissue of the patient and sham groups significantly increased compared to the healthy group, and the laser, cell, exercise, laser-exercise and cell-exercise groups significantly decreased compared to the patient and sham groups. No study was found that examined the expression of these genes in azoospermic samples. However, Karami et al. (2022) showed that IL-10 gene expression in the intestinal tissue of mice with fatty liver is significantly increased compared to other groups, so fatty liver causes inflammation in the intestinal tissue, which can then be balanced by increasing anti-inflammatory factors such as IL-10 (18). IL-10 has been reported to be released in response to inflammation. An excess increase in the pro-inflammatory cytokine IL-10 challenges the immune system to mount an inflammatory response, leading to inflammation. Physical activity follows a similar mechanism, which is not consistent with the current study (19). Studies have shown that the anti-inflammatory effects of physical activity are primarily mediated by IL-10 (20). Similarly, some studies consistent with the present study have shown the role of physical activity on the regulation of IL-10 gene expression. Several studies have shown the effects of high-intensity exercise on reducing fat in human and animal livers, which could help treat fatty liver patients (21), as the effect of high-intensity exercise on reducing hepatic steatosis and fibrosis was greater than that of moderate-intensity exercise. Overall, the molecular mechanism suggests that physical exercise downregulates IL-10 in the T cell pathway via Th2 by negatively regulating NF-kB activities (22). On the other hand, it has been proven that cytokine balance discrete from Th1 and Th2 through regular exercise. Contrary to the results of the present study, some studies have shown that very high-intensity interval training significantly increased IL-10 (23). Furthermore, a study

by Ranjbar et al. showed that IL-10 levels did not change significantly after 8 weeks of HIIT training in men with type 2 diabetes. Therefore, the anti-inflammatory effects of exercise appear to depend on its intensity and duration. Dornels et al. reported that the inflammatory response depends on the intensity of exercise, as they showed an increase in IL-10 in obese subjects after high-intensity exercise (24). In addition, the results of a study showed that the expression level of STAT3 gene in liver tissue in the HIIIT group was significantly increased compared to other groups. IL-10 enables efficient activation of the IL-10/JAK1/STAT3 signaling pathway, and STAT3 phosphorylation is essential for this. In addition, mutations in the IL-10 gene or its receptor (IL-10RA) impair STAT3 activation. Dysregulation and overexpression of anti-inflammatory factors increase the risk of infection, which is consistent with the results of the present study. It is possible that overexpression of IL-10 reduced STAT3 activity in the azoospermic group (25). As previously mentioned, exercise increases STAT3 activation through its phosphorylation. This is also consistent with the results of the present study. Studies have shown the effects of exercise to be correlated with increased STAT3 activity (26). Jia et al. have shown that interval training increased STAT3 activity in male rats, which is consistent with the results of the present study (27). The effects of the anti-inflammatory cytokine IL-10 are regulatory, and its overproduction and underproduction can impair immune function. Based on the results of the studies, exercise training balanced the expression of anti-inflammatory cytokine genes through a cellular mechanism. Therefore, exercise training may be helpful in the treatment of azoospermia (18). No study was found that examined the effect of stem cells on IL-2, IL-10, and STAT3 genes in the testicular tissue of azoospermia model. In 2023, Hu et al. evaluated that optimizing and manipulating immunomodulatory cells (such as mesenchymal stem cells) through effective biological factors (including IL-10) could be the main method for treating asthma (28). The IL-10/IL-10 receptor signaling pathway is central to the inflammation that leads to asthma, and the trans-signaling and transcriptional activator STAT3 is a key player in this pathway. STAT3 has been implicated in the pathogenesis of asthma, and IL-13-induced STAT3-dependent pathways in the lung were inhibited by inhaled corticosteroids. STAT3 is involved in the regulation of lung surfactant and the inflammatory response in lung fibrosis (29). IL-10 is a pleiotropic cytokine produced by various cell types, including lymphoid cells and myeloid cells. The broad anti-inflammatory activity of myeloid dendritic cells and macrophages expresses IL-10 by activating the early myeloid differentiation response and TRIF-dependent toll-like receptor (TLR) pathways (such as TLR3 and TLR4). In addition, tolerogenic dendritic cells produce large amounts of IL-10, which induces the development of T-reg. On the other hand, natural regulatory cells (nT-reg) produce IL-10 in response to IL-2, which is critical for immune homeostasis (30). IL-10 plays an important role in the suppression and management of allergic diseases, especially in asthma. IL-10, in combination with immune regulatory cells, plays a crucial role in successful allergen immunotherapy. The pathogenesis of asthma is associated with reduced IL-10 production. It has been shown that the number of IL-10-releasing and IL-10-producing monocytes is reduced in patients with severe asthma. Furthermore, the absence of IL-10 leads to increased eosinophilic airway inflammation and increased IL-5 synthesis. Mesenchymal stem cells can control inflammation and the pathophysiology of allergic asthma through their immunomodulatory effect, and this effect was enhanced by increasing IL-10 gene expression (31). Allergic asthma symptoms were controlled by the immunomodulatory capacity of MSCs, and the suppressive effect of IL-10

by the expressed gene could enhance the controlling output of asthma pathophysiology. Therefore, manipulation of MSCs as immunomodulatory cells through immunosuppressive biofactors (IL-10) could be a major method for controlling and treating asthma, as observed in the study by Hu et al. (28). MSCs transplanted into the testes of animal models of chemical or surgical azoospermia have been shown to induce spermatogenesis and/or differentiate MSCs into germ cells (11). MSC transplantation has been shown to improve the expression of testicular germ cell markers and could be proposed as a suitable method for the treatment of infertility. Several possible mechanisms for the restoration of testicular function during MSC-induced tissue regeneration have been demonstrated: 1) MSCs may be involved in the suppression of antisperm antibodies (ASA) (32). 2) MSCs can reduce factors that lead to infertility by reducing apoptosis (13). 3) MSCs can reduce oxidative stress (33).۴) MSCs can stimulate testosterone production (34) by differentiating into Leydig cells (35). 5) MSCs can differentiate into target cells (36). 6) Transplant cells secrete growth factors such as bone morphogenetic proteins (BMPs) and transforming growth factor-beta (TGF-β), which are male germ cell-inducing factors with the ability to stimulate the restoration of recipient cell function (11).۷)) MSCs connect with endogenous cells and restore the function of damaged senescent cells, and can alter the expression of some spermatogenesis-related miRNAs and their target genes (37). One study showed that the use of an appropriate laser dose could be an effective treatment for busulfan-induced azoospermia (17). Based on other reports (38, 39), these findings confirm that busulfan at an effective dose of 30 mg/kg reduces sperm count to create a model of azoospermia. Busulfan is a chemotherapy drug used to treat certain diseases such as leukemia, although it can cause male infertility. It is a potent chemical agent that preferentially destroys spermatogonial stem cells and disrupts spermatogenesis by affecting germ cells and Sertoli cells, which is accompanied by a severe reduction in reproductive epithelial cells and the presence of very few spermatogonial cells. So far, much research has been conducted to correct and eliminate the infertility effect of busulfan. In one study, the therapeutic effect of bone marrow-derived mesenchymal stem cells on busulfan-induced azoospermia showed that the epithelial tissue of the seminiferous tubules was normal and spermatogenesis was detectable in most of these tubules. These stem cells, which have differentiation potential, are a good candidate for therapeutic applications due to their secretion of anti-inflammatory cytokines and growth factors (40). In another study, a peripheral blood smear of these stem cells was used to treat busulfan-induced azoospermia. Histomorphological findings showed increased thickness of the seminiferous tubules of azoospermic men compared with controls (41). Overall, these studies demonstrate that the anti-fertility effect of busulfan is reversible and provide a pathway for the use of simpler and less risky treatments such as photobiomodulation. Light modulation can increase cellular metabolism after receiving energy and modulate cellular processes such as proliferation, differentiation, and tissue repair (17). Therefore, if given in the appropriate dose, light energy and power will have a natural and biological effect on the tissue and lead to the initiation of some processes in the living cell (16). Comparing the effectiveness of the three energy doses, the energy dose of 8 J/cm2 was more efficient compared to the doses of 2 and 4 J/cm2. This means that the greatest increase in testicular volume was observed with 8 J/cm2 laser irradiation, and the presence of germ cells in the seminiferous tubules was observed after extensive destruction due to the effect of busulfan, indicating stimulation of spermatogenesis and a balance between cell proliferation and differentiation. In general, studies have shown that light modulation using laser can increase sperm

motility (42, 43). These studies showed that laser light stimulates ATP production and reduces oxidative stress, which leads to increased sperm motility. Laser light affects the bipolar lipid bilayers of the cell membrane and the membranes of intracellular organelles, thus activating the electron transport chain and increasing ATP and generating the energy required for sperm motility by transporting calcium and other ions through ion channels on the mitochondrial membrane (44). In this study, laser therapy alone and in combination with swimming training resulted in a significant decrease in IL-2, IL-10, and STAT3 genes in the testicular tissue of azoospermia model rats, which seems to be a factor in increasing fertility in azoospermia model rats. The observed synergistic effects of aerobic exercise combined with laser therapy on mitophagic gene expression may be explained through several interconnected mechanisms. Aerobic exercise enhances mitochondrial biogenesis, improves mitochondrial membrane potential, and upregulates key mitophagy-related signaling pathways, including PINK1/Parkin and AMPK. Simultaneously, photobiomodulation (laser therapy) increases cellular ATP production, modulates reactive oxygen species (ROS) levels, and activates mitochondrial stress-response pathways, thereby promoting mitochondrial quality control. When applied together, exercise-induced signaling and laser-induced cellular energy enhancement likely converge to amplify mitophagic gene expression more effectively than either intervention alone. This synergistic effect is consistent with the reductions observed in IL-2, IL-10, and STAT3 expression in the combined exercise-laser groups, suggesting that the combination of interventions not only modulates inflammation but also promotes mitochondrial turnover and cellular homeostasis in the testicular tissue of azoospermic rats (23, 25, 44).

Limitations

One of the limitations of the present study is the relatively small sample size, which may reduce the statistical power and generalizability of the results. Future studies with larger sample sizes are recommended to validate and extend these findings. Another limitation of the study is the single-time-point tissue sampling, which may not fully reflect temporal dynamics in gene expression. Future studies with multiple sampling intervals are recommended to elucidate the kinetics of intervention effects on anti-inflammatory and mitophagic pathways.

Conclusion:

The results indicate an increase in testicular tissue inflammation in azoospermia model rats through an increase in IL-2, IL-10 and STAT3 genes, and each of the intervention methods was effective in reducing these genes, with the best results obtained in the combined exercise-cell and exercise-laser groups. Therefore, it can be said that swimming exercise in combination with cell therapy and laser therapy helps fertility in azoospermia model rats by reducing testicular tissue inflammation signaling.

Conflict of Interest

There are no conflicts of interest.

Acknowledgment

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