



# Therapeutic Effects of Exosome Therapy and Photobiomodulation Therapy on the Spermatogenesis Arrest in Male Mice After Scrotum Hyperthermia

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## Abstract

**Introduction:** In men, several factors cause infertility, among which we can mention damage to sperm due to high temperature. So far, various treatments have been proposed for it, but they have not been highly effective. The current study aimed to evaluate the effect of exosome therapy (EXO) and photobiomodulation therapy (PBMT) on spermatogenesis arrest in male mice after scrotum hyperthermia.

**Methods:** In this experimental study, the animals were divided into four groups: control, scrotal hyperthermia, scrotal hyperthermia+EXO (100 µL/d) (mice were treated for 30 days), scrotal hyperthermia+PBMT (laser of 0.03 J/cm<sup>2</sup> for 30 seconds/for 30 days). Hyperthermia was induced by exposure to the temperature of 43 °C for 20 minute every day for 5 times. After 6 weeks, the animals were sacrificed.

**Results:** The treated groups showed a significant increase in sperm parameters, as compared to the hyperthermic groups. Moreover, these favorable effects were observed in relation to the volume of testicular tissue, the number of germ cells, Leydig cells and Sertoli cells, and the level of testosterone. Research on antioxidants showed a significant reduction in oxidized glutathione (GSSG) and reactive oxygen species (ROS) in the treatment groups in comparison to the hyperthermia group ( $P<0.001$ ). Also, there has been a significant increase in the amount of hydrogen peroxide enzyme observed in the hyperthermia group as opposed to the treatment group ( $P<0.001$ ).

**Conclusion:** These findings show that EXO and PBMT can improve spermatogenesis caused by hyperthermia, reduce ROS and GSSG, and increase glutathione (GSH) and sperm quality.

**Keywords:** Photobiomodulation therapy; Extracellular vesicles; Cord blood plasma; Spermatogenesis; Hyperthermia.



## Introduction

Infertility is defined as the incapability of conceiving a child through regular intercourse without the use of any form of contraception. It has been found that approximately 20%-30% of infertility cases are attributed to male factors, which are primarily linked to testicular and sclerotic disorders.<sup>1,2</sup> The maturation of sperm and the process of spermatogenesis are heavily reliant on the temperature of the testis; thus, thermal conditions possess the ability to act as a crucial factor in impeding the progression of spermatogenesis.<sup>3</sup> The effects of elevated temperature are comparable to the physiological changes that occur during cellular aging and the decline in peripheral gland function within the male reproductive system.<sup>4</sup> To prevent such occurrences, it is imperative to position

the testis outside the human body or within the scrotum where a temperature differential of approximately 2 to 5 °C lower than the central body temperature can be achieved.<sup>5</sup> An elevation in the temperature of the scrotum precipitates the advent of thermal stress, thus leading to the arrest of spermatogenesis, provocation of testicular atrophy, aberration of diverse sperm indices, and ultimately culminating in male infertility.<sup>6</sup> Several internal and external factors play a role in increasing the temperature of the scrotum. Extrinsic factors include personal habits such as taking hot baths, tight underwear, and occupational factors such as driving and working in furnaces. Varicocele, cryptorchidism, and high fever are also internal factors.<sup>7,8</sup> Until the present day, a plethora of approaches have been employed to address hyperthermia

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scrotomy-induced ailments. These include methods such as the transplantation of pluripotent stem cells,<sup>9</sup> the utilization of vitamin B,<sup>10</sup> fisetin administration,<sup>11</sup> iron particle-based curcumin delivery,<sup>7</sup> and the use of androgens, drugs, and anti-inflammatory compounds. Exosome (EXO), which belong to a specific category of extracellular vesicles (EVs), are characterized by their spherical morphology and two lipid bilayer membranes. Their size typically ranges from 30 to 150 nm in diameter.<sup>12</sup> EXO are comprised of various proteins, membrane transporters, and tetraspanins, specifically CD9, CD63, and CD81. These components do not incite an immune response, as their structural resemblance to the plasma membrane prevents such a reaction. Additionally, due to their diminutive size, EXO can easily traverse biological obstacles such as the blood-brain barrier.<sup>13,14</sup> EXO are extracellular vesicles that are released from a diverse array of both healthy and pathological cell types, thus rendering their ubiquitous presence throughout a vast assortment of bodily fluids, such as blood, plasma, synovial fluid, and amniotic fluid.<sup>15</sup> Mesenchymal stem cells procured from umbilical cord blood are recognized as the most rudimentary and nascent cells amongst diverse assemblages of mesenchymal stem cells gathered from disparate tissues. The EXO obtained from these cells constitute the most prevalent manifestation of secreted membrane vesicles that convey a plethora of proteins and RNAs for intercellular interchanges.<sup>16</sup> There exist photoreceptors that are sensitive to a low-intensity laser in the membranes of the mitochondria. In cells experiencing oxidative stress, the generation of nitric acid induces perturbation in the mitochondrial respiratory chain. The photobiomodulation can counteract the consequences of nitric oxide and restore the functionality of the electron transport chain.<sup>17</sup> Furthermore, photobiomodulation therapy (PBMT) technology has been identified as one of the efficacious measures in the management of male infertility. PBMT has been observed to substantially enhance spermatozoa's viability, mobility, and motility by maintaining DNA and acrosome integrity.<sup>18,19</sup> Such research may prove useful in introducing novel treatment modalities for males with infertility disorders due to hyperthermic scrotomy, as well as the development of targeted pharmaceuticals. Thus, the objective of this inquiry is to examine the effects of EXO derived from umbilical cord blood and PBMT on spermatogenesis in mice subjected to hyperthermic scrotomy.

## Materials and Methods

### Animals

For the current investigation, a group of 24 male adult mice (NMRI) with a weight range of 25-30 g were chosen. The subjects were situated within customary laboratory settings that incorporated a regulated 12-hour light and dark cycle, with unrestricted availability of food and

water. The present examination has received the official sanction of the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.LASER.REC.1402.003).

### Testicular Hyperthermia Induction

The mice were subjected to anesthesia via an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/kg).<sup>7,20,21</sup> The subsequent step involved submerging the lower extremities, comprising the scrotum and hind legs, into a bath of elevated temperature measuring 43 °C. The induction of hyperthermia was carried out every other day over the course of five days, with each session lasting 20 minutes.<sup>22</sup>

### Photobiomodulation Therapy

The parameters of photobiomodulation are presented in this section. The wavelength utilized was 890 nm, with a pulse frequency of 80 Hz. The spot size was 1 cm<sup>2</sup>. In the experimental group, both testes were subjected to infrared laser irradiation for a duration of 30 seconds, and the energy density was 0.03 J/cm<sup>2</sup>. This protocol was repeated every other day over a period of 30 days.<sup>23</sup>

### EXO Extraction

The samples were centrifuged at 1500 rpm for 15 minutes at 4 °C. After that, plasma supernatant was collected, and successive centrifugations were performed to isolate EXO. Finally, centrifugation was performed for 90 minutes, at 60 000 g at 4 °C, after which the supernatant was carefully decanted and the pellet was resuspended with 1 µL of sterile PBS. The desired suspension was subsequently centrifuged at 1000 g for 30 minutes, and then the PBS was gently removed. The EXO were then stored at -80 °C to maintain their integrity.

### Dynamic Light Scattering

The present technique involves the collection of scattered light emitted by suspended particles, which subsequently creates a fluctuation in light intensity. In order to determine the particle size, the dynamic light scattering (DLS) method was employed in this study. The calculation for the particle size was performed by utilizing the formula below:

$$d_h = \frac{KT}{3\pi\mu D}$$

Where  $d_h$ : hydrodynamic diameter, K: Boltzmann constant, T: absolute temperature,  $\mu$ : viscosity, D: diffusion coefficient.

### Identification of the Molecular Markers of EXO by Flowcytometry

In this study, the flowcytometry method was used to track and examine three markers CD9, CD63, and CD81. To

prepare the sample, the EXO were suitably diluted to a volume of 1 cc. Following this, the sample was subjected to fluorochrome preparation and the subsequent reading was conducted via flow cytometry.

### Scanning Electron Microscopy

The utilization of an electron microscope to produce images of the surface sample of EXO offers a comprehensive analysis of the particles' morphology and size. To facilitate the observation process via scanning electron microscopy (SEM), the exosome suspension was initially fixed by using a 1% glutaraldehyde solution before being strategically placed on a carbon-coated grid at room temperature for the purpose of drying. Subsequently, the grids underwent a double wash process with sterile PBS for 5 minutes each before being stained with a 2% uranyl acetate dye for 20 minutes. Ultimately, the morphology of EXO was meticulously scrutinized by using an electron microscope.<sup>24</sup>

### Study Design

Healthy Adult mice were divided into four groups: Controls: The animals belonging to this particular assemblage remained undamaged; scrotal hyperthermia (Hyp): The members of this particular group were subjected to a temperature of 43°C for a duration of 20 minutes every other day over a span of 5 weeks; hyperthermia + EXO (Hyp + EXO): Mice were treated for 30 days with exosome (100 µL/d); hyperthermia + PBMT (Hyp + PBMT): Scrotal hyperthermia receiving infrared laser of 0.03 J/cm<sup>2</sup> for 30 seconds for each testis, and this procedure was performed every other day for a total of 30 days.

### Semen Analysis

After a duration of six weeks, the animals were sacrificed and the samples of sperm were procured from the caudal region of the epididymis. The samples were subsequently transferred to a single milliliter of Ham's F-10 medium. After a period of incubation lasting 20 minutes at a temperature of 37 °C, a sample measuring 10 µL was deposited onto a slide. Following this, the motility of sperm was examined through the use of a microscope. To assess the potential for survival, the sperm cells underwent examination utilizing the eosin-nigrosine staining method.

### Serum Testosterone

Blood specimens were collected from the cardiac region subsequent to the administration of anesthesia. The specimens underwent centrifugation at 6000 g for a duration of 5 minutes at a temperature of 4 °C. The specimens were maintained at -80 °C until their use. The blood testosterone serum level was measured by means of employing an ELISA kit.

### Tissue Preparation

The testis samples were excised after the ritualistic sacrifice of the animals, then diligently preserved in Bouin's solution for 48 hours, followed by a transfer into formalin. After the fixation, the tissue samples were transferred to a tissue processor, where they underwent paraffin embedding. We proceeded to create consecutive sections using a microtome. The thickness of 5 µm was utilized to make an estimation of the volume, while the thickness of 25 µm was utilized to estimate the number. The specimens were subsequently subjected to H&E staining for visualization and analysis.<sup>25</sup>

### Volume of Testicle

The assessment of the overall volume of the testis was conducted by employing the Cavalieri method. The estimation of the testis volume was achieved through the utilization of the following formula:

$$V = \Sigma P \times a/p \times t$$

Where the  $\Sigma P$  is the number of points counted and the  $a/p$  is an area of probe points divided by magnification.<sup>20</sup>

### The Length of the Seminiferous

The subsequent equation was employed to evaluate the measurement of the seminiferous tubules and their length density.<sup>7</sup>

$$Lv = \frac{2\Sigma Q}{\Sigma P \times \frac{a}{f}}$$

The aggregate quantity of the seminiferous tubules, represented by  $\Sigma Q$ , is determined by the computation of the area per frame, denoted by  $a/f$ , and the total points superimposed on the testis tissue, signified by  $\Sigma P$ . To estimate the comprehensive length of the seminiferous tubules, it was necessary to multiply the length density ( $Lv$ ) by the total volume of the testis.

### Testis Cells Number

To quantify the cellular population, the optical dissector method equation was employed as follows:

$$Nv = \frac{2\Sigma Q}{\Sigma P \times h \times \frac{a}{f}} \times \frac{t}{BA}$$

( $\Sigma Q$ ) is the number of cells, ( $\Sigma P$ ) is the number of counting frame grids in all fields, ( $a/f$ ) is the area of the frame, ( $h$ ) is the dissector height, ( $BA$ ) is the thickness of the microtome section, and ( $t$ ) is the real section thickness.

### GSH and GSSG Content Assessments

The maintenance of proper thiol metabolism is crucial for ensuring cellular safety. The spectrofluorometry method was employed in order to ascertain the levels of intracellular GSH and oxidized glutathione (GSSG), respectively. The 0.5 mL aliquots of the suspension containing testicular cells were subjected to staining with the utilization of o-phthalaldehyde and N-ethylmaleimide probe (5  $\mu$ M). Then the cells were centrifuged at 1000 rpm for one minute. The cellular sediment was subsequently reconstituted in 2 mL of the newly prepared incubation medium. The cells were subjected to a washing process to remove the fluorescent dye. To quantify the magnitude of each specimen, a Shimadzu RF5000U fluorescence spectrophotometer, calibrated for 495 nm excitation and 530 nm emission wavelengths, was employed in utilizing quartz cuvettes.<sup>26</sup>

### ROS Assay

After the separation of testicular cells using EDTA/trypsin, the resultant samples were subjected to centrifugation with PBS at 1200 revolutions per minute at 4 °C for a duration of 5 minutes. Subsequently, DCFDA was introduced to the samples at a concentration of 20  $\mu$ M/100  $\mu$ L and incubated in the dark at 37 °C for a

period of 45 minutes. Finally, the samples were subjected to analysis using a flow cytometer with a wavelength of 495 nm.<sup>27</sup>

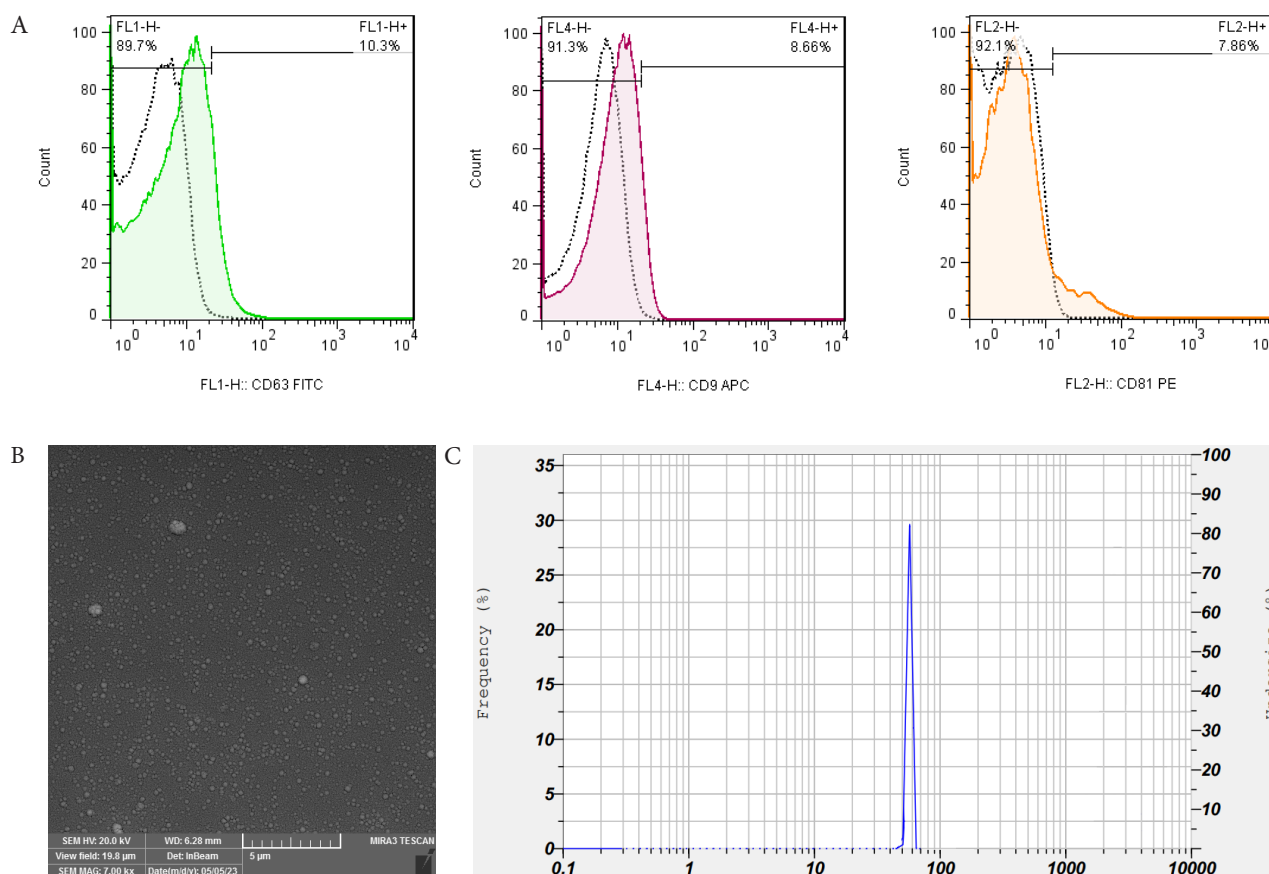
### Glutathione Peroxidase Assay

The activity of glutathione peroxidase (GPX) was conducted by the methodology outlined by Paglia and Valentine. The method involves the utilization of GPX enzyme to induce coloration in glutathione (GSH), followed by the reduction of GSH through the use of GSH reductase enzyme and measurement of light absorption at a wavelength of 340 nm.

### Results

#### Confirmation of EXO Using an Electron Microscope, DLS and Flowcytometry

The flowcytometry results indicate that CD9, CD63, and CD81 are recognized as markers for the identification of EXO. These markers have significant expression of EXO derived from cord blood plasma (Figure 1A). Using the SEM, an investigation was conducted on the configuration of EXO through microscopic examination. The resulting images showed a membrane of EXO characterized by spherical morphology (Figure 1B). The dimensions of EXO fall within the range of 40 to 100 nm, as evidenced



**Figure 1.** The results of flowcytometry show the expression of 89.7% CD63, 91.3% CD9 and 92.1% CD81 (A). SEM analysis showed that the spherical structure of EXO can be seen (B). The results of DLS show that the size range of the extracted particles is between 60 and 90 nm (C)

in the accompanying visual representations. This finding has been duly verified. The determination of the particle diameter present in the EXO suspension isolated from the cells was conducted through the utilization of the DLS technique. The particle dimensions, which measure 53.5 nm, are represented in Figure 1C.

### Semen Analysis

As illustrated in Figure 2, the Hyp group exhibited a notable reduction in sperm count, motility, and viability when contrasted with the control group. Furthermore, it was observed that the Hyp group exhibited a decreased normal morphology of sperm in comparison to the control group (Figure 2). Meanwhile, it was observed that the Hyp+EXO group and Hyp+PBMT group showed a significant improvement in the quality of sperm parameters compared to the Hyp group.

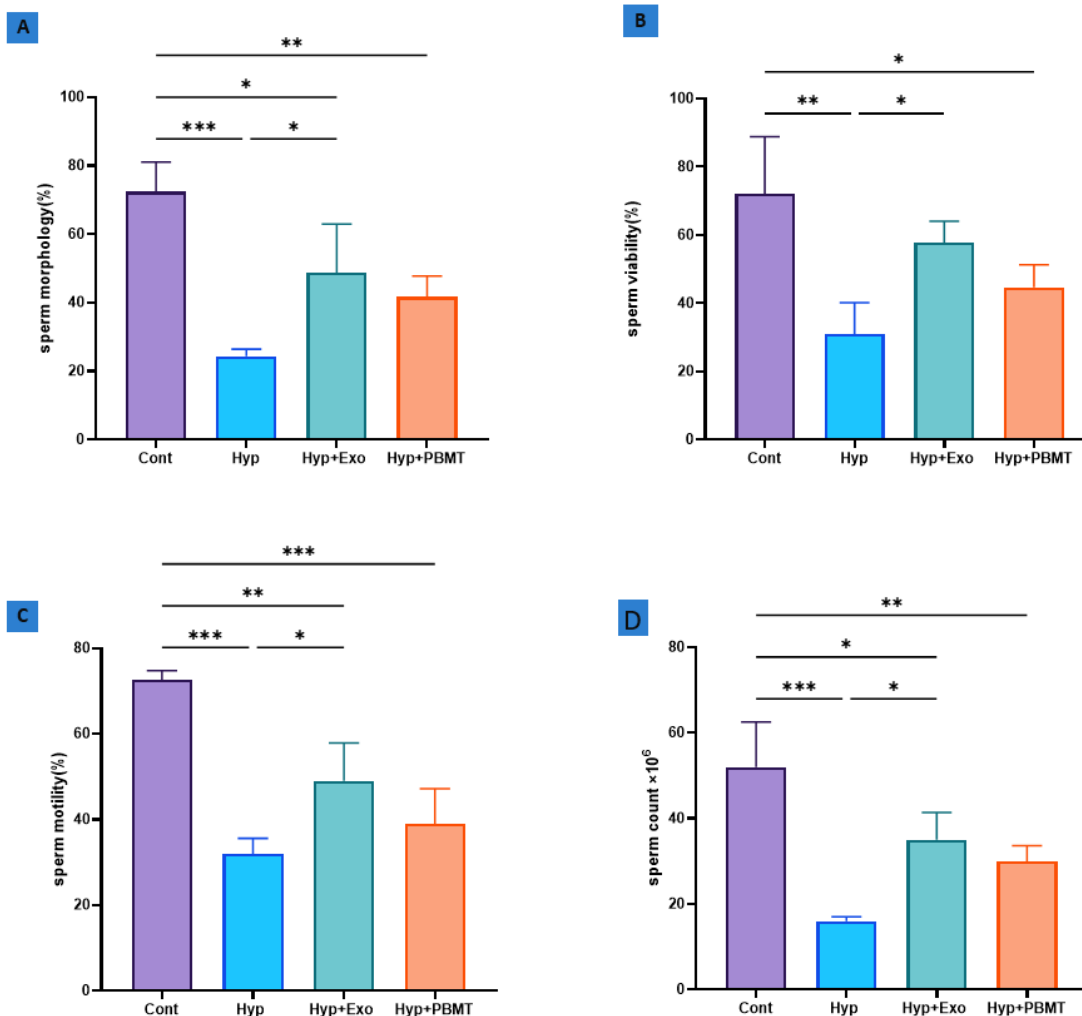
### Serum Testosterone Level

The utilization of the ELISA method for the quantification of testosterone serum level revealed a remarkable reduction in the hormone quantity within the Hyp

group as compared to the control group ( $****P < 0.0001$ ). Conversely, the amount of this hormone observed within the Hyp+EXO and Hyp+PBMT groups exhibited a marked similarity to the control group (Figure 3).

### The Stereological Analysis

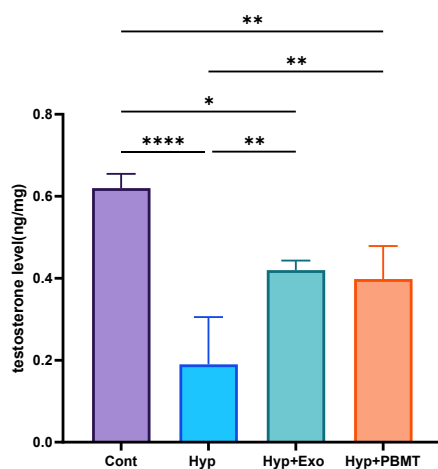
The results of stereological studies show that the density of cells in the seminiferous tubules decreases in the Hyp group compared to the control group. A notable number of vacant gaps can be discerned within the spermatogenic conduits in the Hyp group, and the intervals between said conduits are greater in the Hyp group compared to the control group, thereby suggesting a reduction in the dimensions of these conduits. These instances exhibited substantial amelioration in the treatment groups as opposed to the Hyp group (Figure 4). Based on the findings from the stereological analyses, it was observed that the comparison between the quantity of Sertoli cells and Leydig cells revealed a notable decrease in the hyperthermia group compared to the control group ( $****P < 0.0001$ ; Figure 5A). However, EXO and PBMT



**Figure 2.** The Effect of EXO Extracted From Umbilical Cord Blood and PBMT on Sperm Parameters. Parameters of morphology (A), survival (B), motility (C), and number (D) in different groups. (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ )



remarkably alleviated the corresponding and there was no significant difference between the two groups of Hyp+Exo and Hyp+PBMT. The number of cells involved in spermatogenesis, including spermatogonia, primary spermatocytes, and spermatids, exhibited a significant decline in the Hyp group when compared to the control group ( $****P < 0.0001$ ; Figure 5A), while there was a notable increase in the number of the specified cells in the Hyp + EXO and Hyp + PBMT groups in comparison to the Hyp group ( $***P < 0.001$ ; Figure 5B). Based on the results of the stereological analyses, it was observed that the volume exhibited a noteworthy decrease in the Hyp group in comparison to the control group, while the testis volume in the Hyp + EXO and Hyp + PBMT groups increased significantly compared to Hyp group



**Figure 3.** Mean  $\pm$  SD of the Serum Testosterone Level of Testis in Different Groups. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$

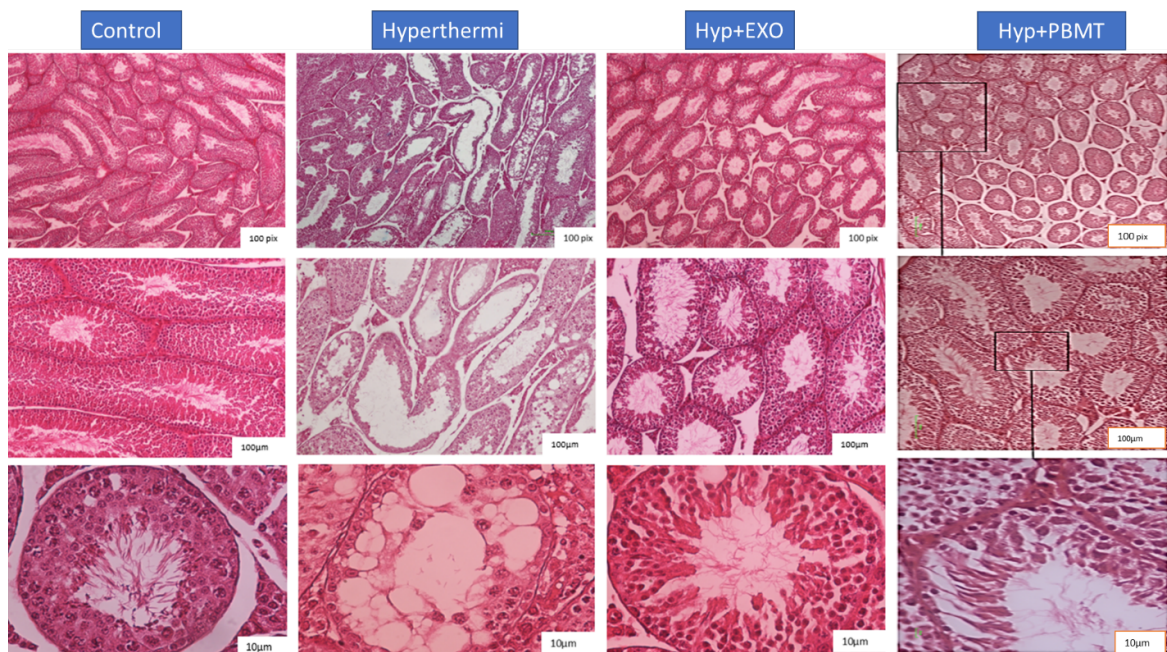
(Figure 5C;  $****P < 0.0001$ ).

### Measuring ROS, GSSG, GPX

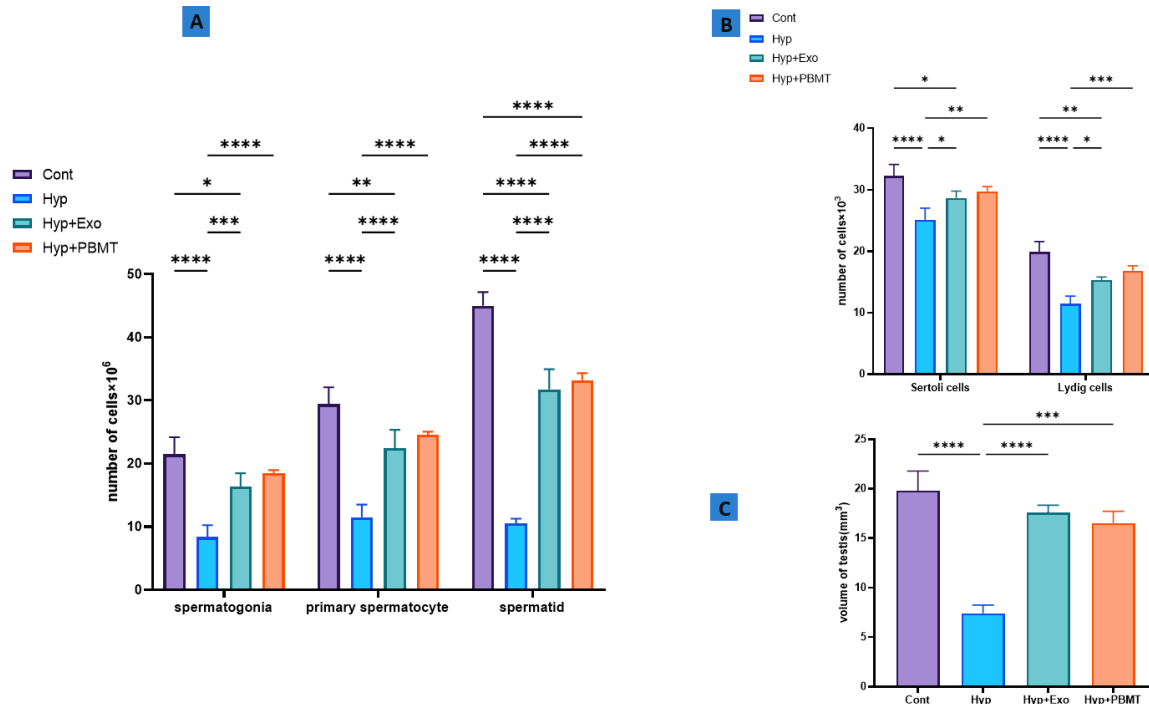
We measured the levels of ROS, GSSG, and GPX in the control and experimental groups as shown in Figure 6. The data illustrated in Figure 6A clearly demonstrate an elevation in the quantity of DCF in the Hyp group relative to the control group. Furthermore, the amount of DCF in the treatment groups exhibited a significant improvement when juxtaposed with that of the Hyp group. In general, these results indicate an increase in oxidative stress in the Hyp group compared to the control group and a relative improvement in oxidative stress in the Hyp + EXO and Hyp + PBMT groups. The findings illustrated in Figure 6B were attained through the examination of antioxidative properties, revealing a decrease in the quantity of GPX enzyme in the Hyp group compared to the control group. Figure 6C exhibits a notable escalation in the quantity of GSSG in the Hyp group in comparison to the control group. However, this quantity underwent a significant decrease in the treatment groups, resulting in an absence of any significant variation as compared to the control.

### Discussion

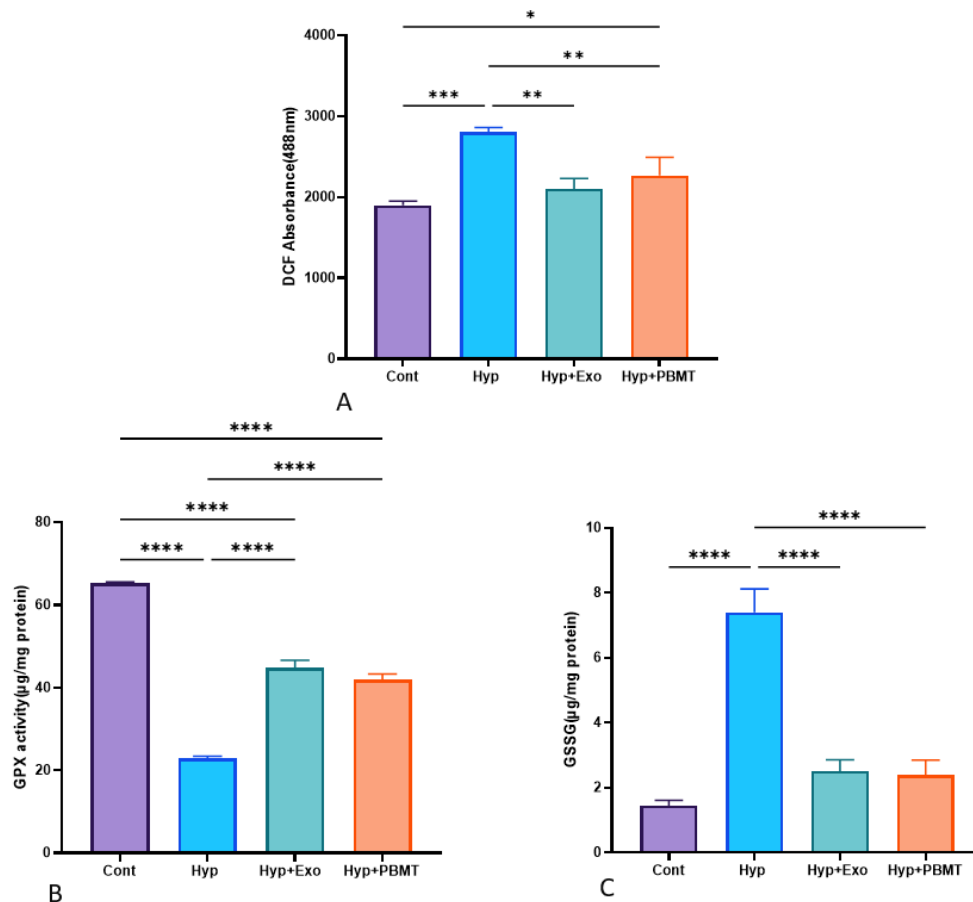
The increase in temperature in the testes can have detrimental effects on numerous factors that are crucial for the process of sperm production. In this study, we examined the effect of EXO derived from umbilical cord blood and PBMT on the complications arising from scrotum hyperthermia. The effects of scrotum hyperthermia on azoospermia development and testicular tissue damage revealed that there was a complete depletion of spermatogenic cells, cessation of spermatogenesis, and



**Figure 4.** Photomicrograph of the Seminephros Tubule in Testis Tissue Stained With H&E in Control and Experimental Groups (magnification: 4x, 10x, 40x)



**Figure 5.** The Effect of EXO and PBMT on the Total Number of Spermatogenic Cells in Scrotal Hyperthermia-Induced Mice. (A) Mean  $\pm$  SD of the total number of spermatogonia, the total number of primary spermatocytes, and the total number of round spermatids of testis in different groups. The effect of EXO and PBMT on the total number of Sertoli cells and Leydig cells in scrotal hyperthermia-induced mice. (B) Mean  $\pm$  SD of the total number of Sertoli cells and the total number of Leydig cells of testis in different groups. The effect of EXO and PBMT on testis volume. (C) Mean  $\pm$  SD of the testis volume. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$



**Figure 6.** Comparison of DFC, GSSG, and GPX levels in different groups. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$

an increase in ROS production. Previously, many factors were employed to ameliorate the deleterious effects of heightened temperatures in the testicular region. These factors encompassed the utilization of iron oxide nanoparticles infused with curcumin<sup>7</sup> and laser therapy.<sup>23</sup> Furthermore, the use of PBMT on the azoospermia model stands as a novel treatment approach.<sup>28</sup> This study represents a novel investigation into the potential therapeutic effects of EXO isolated from umbilical cord blood in the management of hyperthermia scrotomy-induced disorders. The non-immunogenic nature of these particles presents a highly viable option for therapeutic use and the transfer of requisite cellular factors.<sup>29</sup> EXO derived from umbilical cord blood plasma exhibit a mitigating effect on apoptosis, consequently inhibiting the decline in the number of germ cells, Leydig cells, and Sertoli cells.<sup>30</sup> An extensive inquiry was conducted into EXO. Hasani et al demonstrated that the application of photobiomodulation, specifically at a wavelength of 890 nm and a dosage of 0.03 J/cm<sup>2</sup> for a duration of 30 seconds per testis, leads to enhanced sperm motility while mitigating the adverse effects of scrotal hyperthermia.<sup>23</sup> In the current investigation, we have also observed that PBMT can exert an influence on enhancing all elements of sperm quality. Our investigations demonstrate that EXO and PBMT exert a notable impact on the enhancement of both the qualitative and quantitative traits of sperm. Additionally, the stereological analyses conducted in this context reveal the amelioration of testicular tissues and a concurrent augmentation in the number of germ cells within this tissue. In PBMT, light is assimilated by diverse structures and molecules, with a primary focus on molecules that play a crucial role in energy generation and oxygen transportation. Therefore, it can increase ATP and reduce oxidative stress in cells.<sup>31</sup> Antioxidants have a broad impact in the field of andrology, as a consequence of the absence of cytoplasmic enzymes in sperms, which hinders their ability to rectify the harm incurred by oxidative stress. These substances safeguard sperms from anomalies triggered by ROS. Measuring the quantity of ROS, GSSG, and GPX may serve as a reliable indicator for the assessment of cytotoxicity. Ziaepour et al demonstrated that the use of PBMT with a lower intensity (0.03 J/cm<sup>2</sup>) led to an increase in the ratio of GSH to GSSG in mice with hyperthermia-induced azoospermia. Simultaneously, PBMT treatment reduced ROS levels, mitochondrial membrane potential, and lipid peroxidation in the azoospermia model.<sup>32</sup> The findings demonstrated a significant increase in the levels of GSSG and ROS in the hyperthermic group as compared to the control group. However, no significant difference was observed between the control and treatment groups. Additionally, the amount of hydrogen peroxide enzyme in the Hyp group decreased when compared to both control and treatment groups.

These observations mean the beneficial impact of EXO and PBMT in mitigating the oxidative stress induced by elevated temperature. Also, studies show that the level of ROS decreases during cases of cellular oxidative stress in animal disease models after using PBMT.<sup>33</sup> In a comprehensive investigation that assessed the impact of EXO with polycystic ovary syndrome, the findings demonstrated the advantageous effects of these particles in alleviating oxidative stress within the ovarian tissue.<sup>3</sup> The analysis of the data acquired from the measurement of serum testosterone levels in this study demonstrated that inducing hyperthermia in scrotomy could elicit deleterious effects on testosterone levels, leading to a reduction in its concentration. In fact, the testosterone levels in the Hyp groups were significantly lower than those in the control group. Testosterone reduction can be caused by the damage of Leydig cells due to hyperthermia. However, the administration of EXO caused a significant increase in the testosterone levels in the treated group as compared to the Hyp group. Previous research has also shown decreased testosterone levels as a result of hyperthermic scrotomy, thus confirming the findings of the present study.<sup>11,21,35</sup> The examination of hormones revealed that the application of PBMT at a dosage of 0.03 J/cm<sup>2</sup> significantly influenced the concentration of testosterone in the bloodstream.<sup>28</sup> The current study has yielded results that demonstrate the beneficial effects of EXO derived from umbilical cord blood and PBMT on the mechanisms of spermatogenesis and tissue regeneration in the testicles.

## Conclusion

In this study, novel therapeutic potential was demonstrated for PBMT and EXO derived from human umbilical cord blood in ameliorating hyperthermia scrotomy-induced damage. The utilization of these small extracellular vesicles, which contain various types of RNAs, proteins, and lipids, offers a non-cellular treatment approach devoid of the adverse effects associated with cell-based therapies. In addition, PBMT as a useful method for improving the function of the mitochondrial respiratory cycle can reduce the complications caused by scrotal hyperthermia. Based on the comprehensive analysis and comparison of the results obtained among the study groups, it can be inferred that PBMT and EXO enhance multiple sperm parameters, mitigate testicular tissue damage, elevate serum testosterone levels, and alleviate oxidative stress in the hyperthermia scrotomy cohort.

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## Authors' Contribution

**Conceptualization:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh.



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**Formal analysis:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh.

**Funding acquisition:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh,

**Investigation:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh, Shahram Darabi, Reza Soltani, Fakhroddin Aghajanzpour.

**Methodology:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh, Shahram Darabi.

**Project administration:** Hojjat Allah Abbaszadeh.

**Resources:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh, Hassan Rajabi-Maham.

**Software:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh, Hassan Rajabi-Maham.

**Supervision:** Hassan Rajabi-Maham, Hojjat Allah Abbaszadeh.

**Validation:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh.

**Visualization:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh.

**Writing—original draft:** Fakhrosadat Tabatabaee.

**Writing—review & editing:** Fakhrosadat Tabatabaee, Hojjat Allah Abbaszadeh.

### Competing Interests

The authors declare that they have no conflicts of interest related to this study.

### Ethical Approval

The research protocols employed in this study were reviewed and approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.LASER.REC.1402.003).

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