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Preclinical and Clinical Applications of Photobiomodulation Therapy in Sperm Motility: A Narrative Review

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Abstract

About 50% of infertility problems are related to male factors and reduced sperm motility. The important factor that affects the structure and function of sperm is reactive oxygen species (ROS), and over-concentration of ROS reduces the quality and motility of sperm. Photobiomodulation therapy (PBMT) using red to near-infrared (NIR) light is useful in oxidative stress restoration. It plays a therapeutic role in disorders such as asthenospermia, oligospermia cases, and cryopreserved sperm. It also enhances the metabolic capacity of sperm and increases the low-level and non-harmful intracellular content of Ca^{2+}, nitric oxide (NO), and ROS in the stressed cells. Likewise, it modulates survival intracellular pathways and maintains the motility, viability, DNA, and acrosome integrity of sperm. This article reviews the state-of-the-art preclinical and clinical evidence regarding the efficacy of semen PBMT.

Keywords: Sperm motility; Reactive oxygen species; Photobiomodulation therapy; Cryopreservation; Asthenozoospermia.

Introduction

Today infertility is the most important issue in many countries having population health problems. The rate of infertility in couples is different in various countries throughout the world.

Infertility influences about 20.2% of couples in Iran,[1] 10% in Europe, 15% in the USA, and 17% in Canada. The infertility rate in the world is 12%-15% while this rate is much higher in Iran.[2] Studies have shown that about 30% to 80% of male infertility cases are related to the adverse effects of oxidative stress. Controlled reactive oxygen species (ROS) concentrations can promote spermatozoal activities under normal situations (e.g. hyperactivation, sperm maturation, acrosome reaction, capacitation, and fertilization). However, increased physiological ROS concentrations lead to oxidative stress that causes molecular damage, sperm motility reduction, and lipid peroxidation, and finally, it may provide a basis for male infertility. Several factors such as genetic abnormalities,[3] lifestyle,[4] varicocele,[5] drugs [6], prolonged exposure of the testis to heat, hypoxia,[7] and dysfunction in energy metabolism may contribute to oxidative stress in testicular tissue of spermatozoa.[8] Sperm motility, capacitation, and acrosome response are tightly regulated by adenosine triphosphate (ATP), intracellular Ca^{2+}, and nitric oxide (NO) production mitochondria.[9] Normal physiological levels of ROS regulate the intracellular cascades, and hence, it mediates necessary physiological mechanisms (e.g. hyperactivation, sperm maturation, acrosome reaction, capacitation, and fertilization), while increased physiological ROS concentrations lead to oxidative stress.[8,9] In addition, an increase in the ROS level has also been shown in 40% of infertile men's semen samples.[10] Photobiomodulation (PBM) has been widely used in clinical methods as fertilizing potential, and its safety and positive effects have been proven in many studies.[11] Generally, this light-based method engages the exposure of a low-level laser which exploits the various visible to near-infrared (NIR) (600–1100 nm) wavelength ranges.[12] In this spectrum range, light easily

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penetrates the tissues, Many structures and molecules are affected, mainly those involved in oxygen delivery, energy production, and light absorption. The mechanical basis of the use of photobiomodulation therapy (PBMT) is related to the intracellular metabolism upregulation by higher ATP production, augmentation of some metabolic pathways, and reduction or induction of ROS.[13-15] PBM beneficial effects depend on time, irradiated area, and other treatment parameters (e.g. dose). The present review aims to provide studies regarding the efficacy of PBMT in the ROS level and sperm motility.

ATP and Sperm Motility

Motility is one of the most features of sperm cells, and it is also very essential for men's fertility.[1]

A lot of energy is needed by the highly specialized cells called spermatozoa. A direct correlation exists between the rate at which ATP is converted to energy and the beat frequency of the flagellum.[16] It is widely acknowledged that there are two primary metabolic routes through which spermatozoa generate ATP: oxidative phosphorylation in the mitochondria and glycolysis in the head and principal piece.[17] Multiple ATP-producing biological pathways are required for the flagellar mobility of the sperm cell, according to the proteome analysis of asthenozoospermic men. Men with asthenozoospermia were shown to have down-regulated levels of the enzymes involved in glycolysis, the tricarboxylic acid cycle, pyruvate metabolism, ketone metabolism, oxidative phosphorylation, and the beta-oxidation of fatty acids. These results suggest that several metabolic pathways may help regulate sperm motility.[18]

Two motility types, including activated motility and hyperactivated motility, are seen in spermatozoa at the fertilization site. Both of these motility types need a sufficient energy supply of ATP type which is used by the flagellar dynein-ATPase.

Calcium and Sperm Motility

The main functions of ion channels and transporters located in the sperm tail membrane consist of regulation of the calcium concentration, membrane voltage, and intracellular pH of spermatozoa ($[Ca^{2+}]$), and they are necessary for sperm maintenance and fertility.[19,20]

Calcium ion is another significant element that acts as an intracellular second messenger. It is necessary for different functions of sperm, such as spermatogenesis, capacitation, acrosome reaction, fertilization, and sperm activity and hyperactivity. The homeostasis of calcium ions in sperm is strictly controlled by the calcium pump because of plasma membrane Ca^{2+}-ATPase activation.[21] The activity of mitochondria adjusts the Ca^{2+} signals. For instance, the control of the Ca^{2+} signal of mitochondria is essential to regulate both the cellular membrane voltage and particularly pH gradients which drive the generation of ATP.[22] Eventually, dysfunction in mitochondria is related to infertility and asthenospermia.[23,24] Mitochondrial dysfunction is the subject of many types of disorders in organs that need a lot of respiratory energy.[25]

In eukaryotic organisms, cilia and flagella consist of a scaffold of a pair of similar microtubules whose extension of the cellular membrane covers them, and they are powered by dynein ATPase motors. It is well known that augmented ciliary beating and accelerated forward swimming are related to membrane hyperpolarization, whereas ciliary reversal and backward swimming result from depolarization[26]. Intracellular calcium concentration ($[Ca^{2+}]$) causes depolarization or hyperpolarization by the activation of specific channels in different cell regions (e.g. voltage-dependent Ca^{2+} channels, Ca^{2+} stores). Also, calcium regulates ciliary movement through the NO pathway and the phosphorylation and dephosphorylation of ciliary proteins.[27]

Reactive Oxygen Species and Sperm Motility

Although ROS can exert useful effects via the regulation of signaling cascades of vital cells, they are considered toxic metabolites. High ROS concentration induces oxidative stress and has harmful effects on the quality of semen, and it has been related to many types of men fertility complications such as cryptorchidism, varicocele, infection of the urogenital tract, presence of bacteria, and idiopathic infertility.[28] In seminal plasma, ROS has two sources, Endogenous (varicocele, leucocytes, and immature spermatozoa) and exogenous (alcohol, poisons, radiation, long heat exposure, and smoking), which induce DNA damage in sperm,[29,30] peroxidation of lipid,[31] and disorder in the structure and function of sperm.[30]

The mechanism of ROS production and the effects of oxidative stress on sperm are summarized in Figure 1.

Spermatozoa can mainly produce ROS in two ways: (1) the oxidase system of nicotinamide adenine dinucleotide phosphate that produces ROS at the plasma membrane of sperm, and (2) the production of ROS at the mitochondrial level.

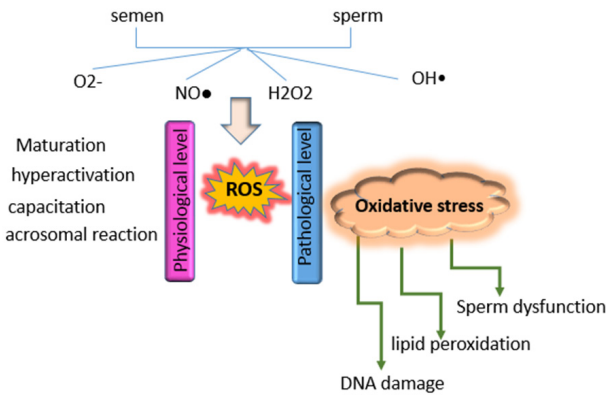

Figure 1. ROS Production and its Effects of Oxidative Stress on Sperm

Spermatozoa have many mitochondria because of their permanent need for energy for motility.[32] Elevating the level of dysfunctional spermatozoa in semen results in the production of ROS and affects sperm motility.[33] On the other side, the higher rate of unsaturated fatty acids in sperm and insufficient cells for repairing systems weaken it for lipid peroxidation at high ROS concentration, causing an efflux of ATP and flagellar impairment.[34] The main ROS in spermatozoa of humans is superoxide (O_2^-), and it reacts on its own via dismutation reactions to produce hydrogen peroxide (H_2O_2). If transitional metals (e.g. copper and iron) exist, hydroxyl radical (OH^-) production can be induced by H_2O_2 and O_2^- via the Haber–Weiss reaction, which can begin lipid peroxidation cascade, disrupt membrane fluidity, and impair sperm function.

Mechanism of Photobiomodulation on ATP, Ca and ROS

There are low-intensity laser-sensitive photoreceptors in the mitochondria membranes.[35,36] Photon absorption by the photoreceptors activates molecular signals in these receptors and alters the molecular configuration of the cells.[12,37] The signal between the nucleus and mitochondria affects many cellular activities in both pathological and normal conditions,[35,38] although the optimal result of PBM is seen in stressed cells more than it is seen in healthy cells.[39,40] It has already been proven that ROS overproduction occurs through the dysfunction of mitochondria in pathologic conditions.[41]

The PBM appears to be the initial stage of the recuperation of oxidative stress since the mitochondria are the primary site for interactions between red/NIR light cells. Additionally, Hamblin observed that the level of ROS decreased under cellular oxidative stress situations in the animal disease models after PBM.[13] The hypothetical mechanism supposed that in stressed cells, the NO produced in the mitochondria is connected to cytochrome c oxidase; therefore, it competitively moves oxygen, inhibits electron transport, and disrupts the respiratory chain.[42]

The PBM may cause the separation of NO from binding sites; hence, it leads to oxygen influx, resumes respiration, and generates ROS.[43,44] Besides, the concentrations of NO are raised because of photo-relaxation from other intracellular stores like nitrosylated myoglobin and hemoglobin.[6,45] Additionally, alteration in the respiratory chain changes Ca^{2+}ion flow between cytoplasm and mitochondria.[46,47] The signaling cascade which raises cytoprotection and proliferation of the cells begins with the increased low-level and non-harmful intracellular content of Ca^{2+}, NO as well as ROS.[48] The main underlying mechanism of PBMT in mitochondria is summarized in Figure 2.

Previous studies have stated that a laser increases the rate

Figure 2. Mechanism of Photobiomodulation Therapy in Mitochondria

of cyclooxygenase, Vmax, and ATP values in irradiated sperm, and also a direct relationship exists between the rate of cyclooxygenase and progressive sperm motility (PSM), and ATP level.[49,50] In vitro increase of human sperm motility following PBMT can be potentially useful for the technologies of assisted reproduction. Before the insemination of intrauterine periovulatory, the low-quality semen can be treated to raise the possibility of conception. Before the insemination of oocytes in vitro, the motility of poorer semen samples can be increased for maximizing fertilization of the oocyte. This probably avoids using an injection of intracytoplasmic sperm to attain fertilization.[51]

Dose-Dependent Effects of Photobiomodulation

Today assisted reproduction methods in both animals and humans depend upon direct sperm injection into the oocyte and using drugs to improve fertility. These two methods may not be successful in all cases, and they can be more effective by using additional devices for increasing fertility. Also, the ongoing methods might be used in association with accepted IVF techniques for the improvement of treatment outcomes.[52] Initially, Sato et al showed that the stimulation of laser light affects human spermatozoa with low energy density.[53]

Some studies have reported the probability of increasing sperm motility following laser irradiation in the sperm of bovines,[4,54] canines,[5] and humans[32,53,55,56] (Table 1).

Normally, visible light irradiation (600 nm, 40 m W/cm^2 in 3 min.) created higher hyperactivated motility and produced ROS.[65] Nevertheless, previous studies assessed the PBM effect on normal and fresh sperm of humans only in the range of red to NIR light. They showed that PSM increases in the exposure of all experimental wavelengths. The shortest time of radiation (23 seconds) in the NIR laser at the energy density sample

Table 1. The Effects of Laser Irradiation on Sperm Motility of Bovines, Canines, and Humans

Study/Year	Specimen	Light Source	Wavelength	Irradiation parameter	Finding
Salman Yazdi et al, 2013[57]	Human	GaAlAs laser	830 nm	200 mW 4, 6, 10 J/cm² for 30, 45, 60 min.	The progressive motility significantly increased in all three doses. The highly irradiated group indicated better motility than the other groups. Not statistically significant increase in the level of DNA fragmentations in sperm cells. Reduce swelling cell percentage in the 10 J/cm² irradiation group
Ban Frangz et al, 2015[58]	Human	LED	850, 625, 660, 470 nm	2.16, 3.92, 5.06, 8.23 mW/cm²	An LED improved sperm motility in asthenozoospermia regardless of the wavelength. The improvement of sperm motility was the largest in 5.06 mW/cm² 470 nm semen samples.
Hasani et al, 2020[59]	Mice	Infrared laser	890 nm	0.03 J/cm², 30 s, 80 Hz	- The increasing level of serum testosterone. Decrease ROS production, as well as the expression of IL1-α, IL6, and TNF-α genes - Higher improvement in the number of spermatozoa
Espey et al, 2020[32]	Human	Pulsed laser probe	655 nm	25 mW/cm² 4, 6, and 10 J/cm²	- No significant effect on DNA fragmentation - Improved sperm motility and velocity in asthenozoospermic patients - Increase of progressive sperm motility at 6 J/cm² for asthenozoospermic patients - No change in expression of CD46 protein
Salama et al, 2015[60]	Human	LED	636.6-nm	496 mg/cm2, 1.241 J/cm² & 2.482 J/cm² for 2, 5 and 10 minutes	- Increase in sperm motility at the different doses, particularly at 5 min - The progressive motility declined with time(10 min) - Progressive motility higher in the normal semen samples than that in the asthenospermic samples post-irradiation
Iaffaldano et al, 2016 [49]	Ram	He-Ne laser	632.8 nm	6 m W, 3.96, 6.12 and 9 J/cm²	- Increases in sperm mass motility, progressive motility, ATP contents, and viability in a dose of 6.12 J/cm² - Lower DNA integrity in the semen samples irradiated at 9 J/cm²
Safian et al, 2021[61]	Human	Diode laser	810 nm	0.6 J/cm², 23 s, 0.0261 W/cm²	- Decrease ROS and lipid peroxidation - Increase sperm motility and modulate mitochondrial membrane potential
Firestone et al, 2013[62]	Human	Infrared	905 nm	50 m W/cm² 1.5 J/cm2 for 30 seconds	- Increase motility in oligospermia and asthenospermic samples - No increase in DNA damage - Significant changes in sperm motion kinetics
Safian et al, 2020[63]	Human	Diode laser	Red 630 nm	0.05 (W); 23, 46, 92 (s); 0.6, 1.2, 2.4 J/cm²	At 0.6 J/cm² had significantly decreased viability
			NIR 810 nm	0.05 (W); 23, 46, 92 (s); 0.6, 1.2, 2.4 J/cm²	- Increase PSM in all dose after 15 min - No increased DNA fragmentation index
			Red + NIR 630 + 810 nm	0.6, 1.2, 2.4 J/cm²	- Increased progressive sperm motility in all dose after 60 min - Increased DNA fragmentation index with 2.4 J/cm²
Preece et al, 2017[52]	Human	Red laser light	633 nm	5.66 mW/cm²	- Increases sperm swimming speed - ROS production level that does not cause significant DNA damage - No DNA damage to sperm cells
Fernandes et al, 2015[64]	Bull	AlGaInP	660 nm	30 mW, 4 and 6 J	- Increase in the percentage of live sperm cells in 4 and 6 - Maintained the integrity of the acrosome membrane of living cells in 4 and 6 J - Head side movement and mobile progress were higher in the 4j group compared with 6 J
Shahar et al, 2011[65]	Human	Visible light	600 nm	40 m W/cm2 for 3 min	- Hyper-activated motility - Production of ROS
Iaffaldano et al, 2010[66]	Rabbit	He–Ne	632.8 nm	3.96, 6.12, and 9.00 J/cm²	Motility, viability, and acrosome integrity are mostly preserved with an energy dose of 6.12 J/cm² in time 0,24 and 48 after irradiation.
Siqueira et al, 2016[67]	Bull	He-Ne	630nm	5, 7.5 and 10 mW during 5 and 10 min in all powers	- 5, 7.5, and 10 mW applied during 10 min improved sperm function - The positive effect of 5 and 10 mW on motility parameters using a 10 min exposure

Abbreviation: GaAlAs, gallium-aluminum-arsenide; DNA, deoxyribonucleic acid; LED, light-emitting diode; ATP, adenosine triphosphate; ROS, reactive oxygen species; IL1-α, Interleukin 1 alpha; IL6, Interleukin 6; TNF-α, tumor necrosis factor; CD46, cluster of differentiation 46; NIR, near-infrared; He-Ne, helium-neon; AlGaInP, aluminium gallium indium phosphide.

of 0.6 J/cm^2 and after 60 minutes of exposure created the best PSM result. In addition, the DNA fragmentation index (DFI) did not increase by NIR.[63] Based on various dose responses, it seems that shorter radiation of PMB with NIR compared to red light has more useful effects on the motility of sperm with no DNA damage in the sample of fresh and normal semen.

One study assessed the fresh samples of human semen in asthenozoospermic patients and stated that laser energy doses of 4 and 6 J/cm^2 with the exposure time of 0, 0.5, 1, 1.5, and 2 hours of radiation increased the motility and velocity of sperm. PBM showed a non-significant effect on the expression of CD46, a biomarker of acrosome integrity, and the fragmentation level of DNA.[32] In another study, an improvement in sperm motility of asthenozoospermia patients was observed after using PBM. They applied 4 treatments with 3 minutes. of PBM protocols including (a) 850 nm with 2.16 mW/cm^2, (b) 625, 660, and 850 nm with 3.92 mW/cm^2, (c) 470 nm with 5.06 mW/cm^2 and, (d) 470, 625, and 660 nm with 8.23 mW/cm^2. They showed that these protocols significantly increase the proportion of quick progressive sperm and decrease the immotile sperm ratio.[58] In preliminary studies, Firestone et al showed that utilizing an infrared laser pulse for irradiation (905 nm, duration of 30 seconds, and 50 mW/cm^2) reaches a motility increase of 85% in asthenospermic and oligospermia human groups after 30 minutes of exposure. After 2 hours, DNA damage showed a non-significant increase compared to the control group. They suggested that PBM does not enhance DNA damage and has a positive short-time effect on the motility of treated spermatozoa.[62]

As mentioned above, the protective effects of light-emitting diodes (LEDs) and lasers increase the ability to fertilize, the motility of sperm, the reaction of acrosome,[68] and the defenses of antioxidants[69] in different conditions (e.g. asthenospermia). On this subject, Salama et al, in their studies, showed that a light LED (636.6 nm, duration of 2, 5, and 10 minutes for 0.496, 1.241, and 2.482 J/cm^2 respectively) in normal and asthenospermic men increased the sperm motility at different doses, particularly at 5 minutes, and the progressive motility especially decreased over time from 5 to 10 minutes. Following the application of light, an increase in sperm motility was seen in both types. Nevertheless, the increased level of progressive motility was significantly lower only at the 2-minute point of the asthenospermia group than in the control semen group.[60] In general, results show that PBM, regardless of its wavelength, increases the motility of sperm in asthenozoospermia.

Also, Hasani et al showed that photobiomodulation (890 nm, 0.03 J/cm^2, 30 seconds for each testis) improves the motility of sperm and decreases oligospermia caused by scrotal hyperthermia in a mouse model.[59] In general, results show that PBM, regardless of its wavelength, increases the motility of sperm in asthenozoospermia. Thawing and freezing of sperm cause the production of ROS at destructive levels.[70]

ROS elevated levels impair the cellular plasma membrane of sperms via the peroxidation of lipids and the formation of stable products (e.g. malondialdehyde in cells) [71]. In this regard, Peerce et al, in some interesting studies, assessed the effect of red light irradiation (633 nm, 5.66 mW/cm^2 with 35 minutes' duration) on frozen healthy human sperm samples, and they observed a significant increase in swimming speed with little or no production of DNA damage in sperm cells, but the levels of ROS production were not high enough to create significant DNA harm.[52]

Safian et al studied the quality of human sperm characteristics via cryopreservation. They observed that applying PBM preconditioning (810 nm) before thawing and freezing procedures of sperm can maintain human spermatozoa versus peroxidation of lipids and increase ROS levels and PSM of cryopreserved human sperm after thawing.[61]

In a notable series of investigations, Iaffaldano et al estimated the helium-neon laser irradiation (632.8 at 3.96, 6.12, and 9 J/cm^2), and they showed that cryopreserved ram sperm increases mass sperm and progressive motility, viability, DNA and Acrosome integrity, and content of ATP.[49] In another study, the same authors assessed the He–Ne irradiation (632.8 nm with energy doses of 3.96, 6.12, and 9.00 J/cm^2) on rabbits' sperm parameters. Viability, motility, and acrosome integrity are mainly protected with an energy dose of 6.12 J/cm^2 in time. There was a slight increase in the motility of irradiation samples and integrity of sperm compared to the control group at the time zero, as well as a particularly significant increase in an energy dose of 6.12 J/cm^2 after 24 and 48 hours of irradiation.[66] AlGaInP irradiation (660 nm in two doses of 4 and 6 J) before cryopreservation caused statistical differences in the percentage of cell motility with an increase of 4 J in the proportion of acrosome integrity and live sperm cells in connection with control cells when subjected to low-power laser irradiation in two disparate doses.[64] Likewise, Salman Yazdi et al showed that the GaAlAs laser (830 nm, 200 m W and 4, 6, 10 J/cm^2 for 30, 45, and 60 minutes) significantly increased the progressive motility in all three doses. The highly irradiated group indicated better motility than the other groups, and no significant increase was observed in the level of DNA fragmentation in sperm cells.[57]

Siqueira et al investigated the effect of the He-Ne laser (633 nm) on the functions of frozen bovine sperm and simultaneously assessed the effect of various times and

output powers of irradiation. Their results showed significant effects related to power while applying 10-minute irradiation on the parameters of motility and the potential of mitochondria. Thus, it seems that the

effect of PBMT depends on not only fluency or doses but also irradiation power and exposure duration.[67] The transcription factors (e.g. NF-κB) can be activated by involved photons, which simulate more ATP and produce less ROS. The enhancement of PBM effects seen in clinics is caused by gene products.[72] It is possible to hypothesize that the mild oxidative stress stimulation of the sperm cells before freezing may be responsible for the beneficial effects of PBM preconditioning.

Conclusion

Since sperm contains large quantities of mitochondria, the application of red to NIR lights (600-1100) for the treatment of low-quality semen is very attractive. The main problem until now has been to get enough light in the sperm to achieve the beneficial effects. The activation of intracellular signaling and transcription factors (e.g. NF-κB) simulate more ATP and produce less ROS. PBMT has the most important effects on improving sperm motility, stimulating antioxidant defenses, and increasing fertility sperm motility deficits associated with many male infertility disorders. The overall results from extensive preclinical and clinical studies in the sperm PBM field suggest that the modest levels of red and NIR light show stimulatory effects without significant DNA damage, decrease ROS levels, and could be activated by involved photons. The transcribed products of genes are responsible for improving the effects of PBM observed in clinics.

Conflict of Interests

The authors declare that there is no conflict of interest.

Ethical Considerations

Not applicable.

References

1. Moghbelinejad S, Mozdarani H, Ghoraeian P, Asadi R. Basic and clinical genetic studies on male infertility in Iran during 2000-2016: a review. Int J Reprod Biomed. 2018;16(3):131-48.
2. Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: role of genetic background. Reprod Biomed Online. 2007;14(6):734-45. doi: 10.1016/s1472-6483(10)60677-3.
3. Chemes HE, Rawe VY. The making of abnormal spermatozoa: cellular and molecular mechanisms underlying pathological spermiogenesis. Cell Tissue Res. 2010;341(3):349-57. doi: 10.1007/s00441-010-1007-3.
4. Silva JV, Cruz D, Gomes M, Correia BR, Freitas MJ, Sousa L, et al. Study on the short-term effects of increased alcohol and cigarette consumption in healthy young men's seminal quality. Sci Rep. 2017;7:45457. doi: 10.1038/srep45457.
5. Ding J, Shang X, Zhang Z, Jing H, Shao J, Fei Q, et al. FDA-approved medications that impair human spermatogenesis. Oncotarget. 2017;8(6):10714-25. doi: 10.18632/oncotarget.12956.
6. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. J Hum Reprod Sci. 2015;8(4):191-6. doi: 10.4103/0974-1208.170370.
7. Poorhassan M, Navae F, Mahakizadeh S, Bazrafkan M, Nikmehr B, Abolhassani F, et al. Flaxseed can reduce hypoxia-induced damages in rat testes. Int J Fertil Steril. 2018;12(3):235-41. doi: 10.22074/ijfs.2018.5298.
8. Faraj K, Dave C, Bennett RC, Vakharia P. Male Infertility. eMedicine Specialities: Urology, 2016. https://emedicine.medscape.com/article/436829-overview. Accessed September 7, 2016.
9. Leaver RB. Male infertility: an overview of causes and treatment options. Br J Nurs. 2016;25(18):S35-S40. doi: 10.12968/bjon.2016.25.18.S35.
10. Saez F, Motta C, Boucher D, Grizard G. Antioxidant capacity of prostasomes in human semen. Mol Hum Reprod. 1998;4(7):667-72. doi: 10.1093/molehr/4.7.667.
11. Poor Hassan M, Abdollahifar MA, Aliaghaei A, Tabeie F, Vafaei-Nezhad S, Norouzian M, et al. Photobiomodulation therapy improved functional recovery and overexpression of interleukins-10 after contusion spinal cord injury in rats. J Chem Neuroanat. 2021;117:102010. doi: 10.1016/j.jchemneu.2021.102010.
12. Tsai SR, Hamblin MR. Biological effects and medical applications of infrared radiation. J Photochem Photobiol B. 2017;170:197-207. doi: 10.1016/j.jphotobiol.2017.04.014.
13. Hamblin MR. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. AIMS Biophys. 2017;4(3):337-61. doi: 10.3934/biophy.2017.3.337.
14. Yu W, Naim JO, McGowan M, Ippolito K, Lanzafame RJ. Photomodulation of oxidative metabolism and electron chain enzymes in rat liver mitochondria. Photochem Photobiol. 1997;66(6):866-71. doi: 10.1111/j.1751-1097.1997.tb03239.x.
15. Mostafavinia A, Ahmadi H, Amini A, Roudafshani Z, Hamblin MR, Chien S, et al. The effect of photobiomodulation therapy on antioxidants and oxidative stress profiles of adipose derived mesenchymal stem cells in diabetic rats. Spectrochim Acta A Mol Biomol Spectrosc. 2021;262:120157. doi: 10.1016/j.saa.2021.120157.
16. Cardullo RA, Baltz JM. Metabolic regulation in mammalian sperm: mitochondrial volume determines sperm length and flagellar beat frequency. Cell Motil Cytoskeleton. 1991;19(3):180-8. doi: 10.1002/cm.970190306.
17. du Plessis SS, Agarwal A, Mohanty G, van der Linde M. Oxidative phosphorylation versus glycolysis: what fuel do spermatozoa use? Asian J Androl. 2015;17(2):230-5. doi: 10.4103/1008-682x.135123.
18. Bracke A, Peeters K, Punjabi U, Hoogewijs D, Dewilde S. A search for molecular mechanisms underlying male idiopathic infertility. Reprod Biomed Online. 2018;36(3):327-39. doi: 10.1016/j.rbmo.2017.12.005.
19. Birkner A, Tischbirek CH, Konnerth A. Improved deep two-photon calcium imaging in vivo. Cell Calcium. 2017;64:29-35. doi: 10.1016/j.ceca.2016.12.005.
20. Shahrokhi SZ, Salehi P, Alyasin A, Taghiyar S, Deemeh MR. Asthenozoospermia: cellular and molecular contributing factors and treatment strategies. Andrologia. 2020;52(2):e13463. doi: 10.1111/and.13463.
21. Lestari SW, Larasati MD, Mansur IG, Margiana R. Sperm Na+,K+-ATPase and Ca2+-ATPase activities: a potential predictive parameter of sperm motility disorder in infertile men. Biomed Pharmacol J. 2018;11(1):411-6. doi: 10.13005/bpj/1388.
22. Boczek T, Lisek M, Ferenc B, Kowalski A, Stepinski D, Wiktorska M, et al. Plasma membrane Ca2+-ATPase isoforms composition regulates cellular pH homeostasis in differentiating PC12 cells in a manner dependent on cytosolic Ca2+elevations. PLoS One. 2014;9(7):e102352. doi:

10.1371/journal.pone.0102352.
23. Frank SA, Hurst LD. Mitochondria and male disease. Nature. 1996;383(6597):224. doi: 10.1038/383224a0https://doi.org/10.1038/383224a0.
24. Cummins JM, Jequier AM, Kan R. Molecular biology of human male infertility: links with aging, mitochondrial genetics, and oxidative stress? Mol Reprod Dev. 1994;37(3):345-62. doi: 10.1002/mrd.1080370314.
25. Spiropoulos J, Turnbull DM, Chinnery PF. Can mitochondrial DNA mutations cause sperm dysfunction? Mol Hum Reprod. 2002;8(8):719-21. doi: 10.1093/molehr/8.8.719.
26. Ishikawa T. Axoneme structure from motile cilia. Cold Spring Harb Perspect Biol. 2017;9(1):a028076. doi: 10.1101/cshperspect.a028076.
27. Amaroli A, Benedicenti A, Ferrando S, Parker S, Selting W, Gallus L, et al. Photobiomodulation by infrared diode laser: effects on intracellular calcium concentration and nitric oxide production of *Paramecium*. Photochem Photobiol. 2016;92(6):854-62. doi: 10.1111/php.12644.
28. Thompson A, Agarwal A, du Plessis SS. Physiological role of reactive oxygen species in sperm function: a review. In: Parekattil SJ, Agarwal A, eds. Antioxidants in Male Infertility: A Guide for Clinicians and Researchers. New York, USA: Springer Science and Business Media; 2013. p. 69-89.
29. Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J. Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. Fertil Steril. 2008;89(1):124-8. doi: 10.1016/j.fertnstert.2007.01.166.
30. Aitken RJ, Gibb Z, Baker MA, Drevet J, Gharagozloo P. Causes and consequences of oxidative stress in spermatozoa. Reprod Fertil Dev. 2016;28(1-2):1-10. doi: 10.1071/rd15325.
31. Moazamian R, Polhemus A, Connaughton H, Fraser B, Whiting S, Gharagozloo P, et al. Oxidative stress and human spermatozoa: diagnostic and functional significance of aldehydes generated as a result of lipid peroxidation. Mol Hum Reprod. 2015;21(6):502-15. doi: 10.1093/molehr/gav014.
32. Espey BT, Kielwein K, van der Ven H, Steger K, Allam JP, Paradowska-Dogan A, et al. Effects of pulsed-wave photobiomodulation therapy on human spermatozoa. Lasers Surg Med. 2022;54(4):540-53. doi: 10.1002/lsm.23399.
33. Henkel RR. Leukocytes and oxidative stress: dilemma for sperm function and male fertility. Asian J Androl. 2011;13(1):43-52. doi: 10.1038/aja.2010.76.
34. Griveau JF, Le Lannou D. Reactive oxygen species and human spermatozoa: physiology and pathology. Int J Androl. 1997;20(2):61-9. doi: 10.1046/j.1365-2605.1997.00044.x.
35. Ryan MT, Hoogenraad NJ. Mitochondrial-nuclear communications. Annu Rev Biochem. 2007;76:701-22. doi: 10.1146/annurev.biochem.76.052305.091720.
36. Mostafavinia A, Amini A, Sajadi E, Ahmadi H, Rezaei F, Ghoreishi SK, et al. Photobiomodulation therapy was more effective than photobiomodulation plus arginine on accelerating wound healing in an animal model of delayed healing wound. Lasers Med Sci. 2022;37(1):403-15. doi: 10.1007/s10103-021-03271-8.
37. Vo-Dinh T. Low-power laser therapy. In: Biomedical Photonics Handbook. CRC Press; 2003. p. 1265-90.
38. Butow RA, Avadhani NG. Mitochondrial signaling: the retrograde response. Mol Cell. 2004;14(1):1-15. doi: 10.1016/s1097-2765(04)00179-0.
39. Isobe N, Yoshimura Y. Deficient proliferation and apoptosis in the granulosa and theca interna cells of the bovine cystic follicle. J Reprod Dev. 2007;53(5):1119-24. doi: 10.1262/jrd.19041.
40. Ahmadi H, Bayat M, Amini A, Mostafavinia A, Ebrahimpour-Malekshah R, Gazor R, et al. Impact of preconditioned diabetic stem cells and photobiomodulation on quantity and degranulation of mast cells in a delayed healing wound simulation in type one diabetic rats. Lasers Med Sci. 2022;37(3):1593-604. doi: 10.1007/s10103-021-03408-9.
41. Durairajanayagam D, Singh D, Agarwal A, Henkel R. Causes and consequences of sperm mitochondrial dysfunction. Andrologia. 2021;53(1):e13666. doi: 10.1111/and.13666.
42. Brown GC. Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase. Biochim Biophys Acta. 2001;1504(1):46-57. doi: 10.1016/s0005-2728(00)00238-3.
43. Karoussis IK, Kyriakidou K, Psarros C, Koutsilieris M, Vrotsos JA. Effects and action mechanism of low level laser therapy (LLLT): applications in periodontology. Dentistry. 2018;8(9):1000514. doi: 10.4172/2161-1122.1000514.
44. Shiva S, Gladwin MT. Shining a light on tissue NO stores: near infrared release of NO from nitrite and nitrosylated hemes. J Mol Cell Cardiol. 2009;46(1):1-3. doi: 10.1016/j.yjmcc.2008.10.005.
45. Amaral A, Paiva C, Attardo Parrinello C, Estanyol JM, Ballescà JL, Ramalho-Santos J, et al. Identification of proteins involved in human sperm motility using high-throughput differential proteomics. J Proteome Res. 2014;13(12):5670-84. doi: 10.1021/pr500652y.
46. Karu TI. Mitochondrial signaling in mammalian cells activated by red and near-IR radiation. Photochem Photobiol. 2008;84(5):1091-9. doi: 10.1111/j.1751-1097.2008.00394.x.
47. Lubart R, Breitbart H. Biostimulative effects of low-energy lasers and their implications for medicine. Drug Dev Res. 2000;50(3-4):471-5. doi: 10.1002/1098-2299(200007/08)50:3/4<471::aid-ddr30>3.0.co;2-e.
48. Gao X, Xing D. Molecular mechanisms of cell proliferation induced by low power laser irradiation. J Biomed Sci. 2009;16(1):4. doi: 10.1186/1423-0127-16-4.
49. Iaffaldano N, Paventi G, Pizzuto R, Di Iorio M, Bailey JL, Manchisi A, et al. Helium-neon laser irradiation of cryopreserved ram sperm enhances cytochrome c oxidase activity and ATP levels improving semen quality. Theriogenology. 2016;86(3):778-84. doi: 10.1016/j.theriogenology.2016.02.031.
50. Florman HM, Jungnickel MK, Sutton KA. Regulating the acrosome reaction. Int J Dev Biol. 2008;52(5-6):503-10. doi: 10.1387/ijdb.082696hf.
51. Gabel CP, Carroll J, Harrison K. Sperm motility is enhanced by low level laser and light emitting diode photobiomodulation with a dose-dependent response and differential effects in fresh and frozen samples. Laser Ther. 2018;27(2):131-6. doi: 10.5978/islsm.18-OR-13.
52. Preece D, Chow KW, Gomez-Godinez V, Gustafson K, Esener S, Ravida N, et al. Red light improves spermatozoa motility and does not induce oxidative DNA damage. Sci Rep. 2017;7:46480. doi: 10.1038/srep46480.
53. Sato H, Landthaler M, Haina D, Schill WB. The effects of laser light on sperm motility and velocity in vitro. Andrologia. 1984;16(1):23-5. doi: 10.1111/j.1439-0272.1984.tb00229.x.
54. Wechalekar H, Setchell BP, Peirce EJ, Ricci M, Leigh C, Breed WG. Whole-body heat exposure induces membrane changes in spermatozoa from the cauda epididymidis of laboratory mice. Asian J Androl. 2010;12(4):591-8. doi: 10.1038/aja.2010.41.
55. Chang CM, Lin YH, Srivastava AK, Chigrinov VG. An optical system via liquid crystal photonic devices for photobiomodulation. Sci Rep. 2018;8(1):4251. doi: 10.1038/s41598-018-22634-w.
56. Lenzi A, Claroni F, Gandini L, Lombardo F, Barbieri C,

Lino A, et al. Laser radiation and motility patterns of human sperm. Arch Androl. 1989;23(3):229-34. doi: 10.3109/01485018908986845.
57. Salman Yazdi R, Bakhshi S, Jannat Alipoor F, Akhoond MR, Borhani S, Farrahi F, et al. Effect of 830 nm diode laser irradiation on human sperm motility. Int J Fertil Steril. 2015;9(Suppl 1):68-9.
58. Ban Frangez H, Frangez I, Verdenik I, Jansa V, Virant Klun I. Photobiomodulation with light-emitting diodes improves sperm motility in men with asthenozoospermia. Lasers Med Sci. 2015;30(1):235-40. doi: 10.1007/s10103-014-1653-x.
59. Hasani A, Khosravi A, Rahimi K, Afshar A, Fadaei-Fathabadi F, Raoofi A, et al. Photobiomodulation restores spermatogenesis in the transient scrotal hyperthermia-induced mice. Life Sci. 2020;254:117767. doi: 10.1016/j.lfs.2020.117767.
60. Salama N, El-Sawy M. Light-emitting diode exposure enhances sperm motility in men with and without asthenospermia: preliminary results. Arch Ital Urol Androl. 2015;87(1):14-9. doi: 10.4081/aiua.2015.1.14.
61. Safian F, Ghaffari Novin M, Nazarian H, Shams Mofarahe Z, Abdollahifar MA, Jajarmi V, et al. Photobiomodulation preconditioned human semen protects sperm cells against detrimental effects of cryopreservation. Cryobiology. 2021;98:239-44. doi: 10.1016/j.cryobiol.2020.09.005.
62. Firestone RS, Esfandiari N, Moskovtsev SI, Burstein E, Videna GT, Librach C, et al. The effects of low-level laser light exposure on sperm motion characteristics and DNA damage. J Androl. 2012;33(3):469-73. doi: 10.2164/jandrol.111.013458.
63. Safian F, Ghaffari Novin M, Karimi M, Kazemi M, Zare F, Ghoreishi SK, et al. Photobiomodulation with 810 nm wavelengths improves human sperms' motility and viability in vitro. Photobiomodul Photomed Laser Surg. 2020;38(4):222-31. doi: 10.1089/photob.2019.4773.
64. Fernandes GH, de Tarso Camillo de Carvalho P, Serra AJ, Crespilho AM, Peron JP, Rossato C, et al. The effect of low-level laser irradiation on sperm motility, and integrity of the plasma membrane and acrosome in cryopreserved bovine sperm. PLoS One. 2015;10(3):e0121487. doi: 10.1371/journal.pone.0121487.
65. Shahar S, Wiser A, Ickowicz D, Lubart R, Shulman A, Breitbart H. Light-mediated activation reveals a key role for protein kinase A and sarcoma protein kinase in the development of sperm hyper-activated motility. Hum Reprod. 2011;26(9):2274-82. doi: 10.1093/humrep/der232.
66. Iaffaldano N, Rosato MP, Paventi G, Pizzuto R, Gambacorta M, Manchisi A, et al. The irradiation of rabbit sperm cells with He-Ne laser prevents their in vitro liquid storage dependent damage. Anim Reprod Sci. 2010;119(1-2):123-9. doi: 10.1016/j.anireprosci.2009.10.005.
67. Siqueira AF, Maria FS, Mendes CM, Hamilton TR, Dalmazzo A, Dreyer TR, et al. Effects of photobiomodulation therapy (PBMT) on bovine sperm function. Lasers Med Sci. 2016;31(6):1245-50. doi: 10.1007/s10103-016-1966-z.
68. Ocaña-Quero JM, Gomez-Villamandos R, Moreno-Millan M, Santisteban-Valenzuela JM. Biological effects of helium-neon (He-Ne) laser irradiation on acrosome reaction in bull sperm cells. J Photochem Photobiol B. 1997;40(3):294-8. doi: 10.1016/s1011-1344(97)00072-9.
69. Cohen N, Lubart R, Rubinstein S, Breitbart H. Light irradiation of mouse spermatozoa: stimulation of in vitro fertilization and calcium signals. Photochem Photobiol. 1998;68(3):407-13.
70. Ko EY, Sabanegh ES Jr, Agarwal A. Male infertility testing: reactive oxygen species and antioxidant capacity. Fertil Steril. 2014;102(6):1518-27. doi: 10.1016/j.fertnstert.2014.10.020.
71. Len JS, Koh WSD, Tan SX. The roles of reactive oxygen species and antioxidants in cryopreservation. Biosci Rep. 2019;39(8):BSR20191601. doi: 10.1042/bsr20191601.
72. Farivar S, Malekshahabi T, Shiari R. Biological effects of low level laser therapy. J Lasers Med Sci. 2014;5(2):58-62.