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Research article

Evaluation of the Detrimental Impact of Low-Intensity Laser Radiation on the Characteristics of Sperm Movement, Motion, and DNA Damage

Zahra Al Timimi*

Laser Physics Department, College of Science for Women, University of Babylon, Hillah, Iraq

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Abstract

It is generally accepted that low-level laser therapy promotes cellular energy production and increases the synthesis of ATP by triggering the mitochondrial electron transport chain's photosensitive cytochrome c oxidase complex. Nevertheless, this investigation aimed to examine the possible adverse impact on sperm function and DNA integrity of low-intensity laser radiation with a wavelength of 980 nm. Following standard analysis, forty semen samples were collected and categorized as either asthenospermic, oligospermic, or normospermic. The remaining semen was subjected to standard semen analysis, and the aliquots were divided into treatment and control groups. A continuous-wave 980 nm laser with an output power of 100 mW and an energy density of 10 J/cm^2 was applied to the treated samples for 30 s. Divided samples underwent incubation at 37^{o}C, and semen analysis was used to assess aliquots at 30 s and 2 h intervals. Following the incubation time, each sample was frozen in 250 mL at -80^{o}C until DNA fragmentation was examined using flow cytometry. Thirty minutes after treatment, there was a noticeable increase in sperm movement; the rise was largest in oligospermic and asthenospermic samples (88%) while samples of normal sperm displayed the least increment (7.5%). There was no discernible increase in DNA damage in the treatment samples compared to the control samples. The treatment samples had 21.8% and the control samples had 25.3% of the DNA fragmentation index. The dynamics of sperm motion were noticeably altered. Short-term exposure to low-level laser radiation appeared to improve the motion and movement of treated sperm, and after 2 h, there was no increase in DNA damage. Asthenospermia and mitochondrial dysfunction may be related in some circumstances. To fully understand the ramifications of these results for possible medicinal applications, more research is needed. Our research's conclusions suggest that low-level laser stimulation is a useful and safe technique for identifying viable immotile spermatozoa in a particular sample. It may also cause these spermatozoa to move about, which could have positive therapeutic effects.

Keywords: sperm; mitochondrial; DNA damage; laser radiation; energy density; asthenospermia

*Corresponding author: E-mail: dr.altimimizahra@gmail.com
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1. Introduction

In reaction to nonionizing electromagnetic radiation, photochemical and photophysical processes produce photobiological side effects. Specifically, laser light energy absorption produces a primary photo signal, whose transduction is then amplified in the cell (Baratto et al., 2011; Houreld, 2016). The coherence of light and the coherence of light's interaction with matter are the two characteristics of laser interaction with biological cells that need to be distinguished in this topic.

At low irradiance levels, one-way laser light in medicine produces a photochemical action. This effect happens when a photon is absorbed by a chromophore, which transfers extra energy to start a biological reaction. Karu (2003 & 2013) discovered that different wavelengths were absorbed at the level of the mitochondrial respiratory chain, influencing distinct chromophores and raising electrical energy levels to improve ATP generation. Biological reactions to laser light and intracellular signal pathways have also been covered by R. Lubart and other writers (Friedmann & Lubart, 1996; Lubart et al., 1997; Amaroli et al., 2016).

Infertility caused by male factors is the most prevalent cause of infertility, accounting for 40% to 50% of cases (Brugh & Lipshultz, 2004; Cooper et al., 2010). Low-quality sperm that falls into one of three categories—oligospermia (less than 20 million sperm /mL), asthenospermic (less than 50% motility), or teratospermia (less than 30% normal morphology)—is the primary cause of infertility related to male factors. Males with azoospermia, a disorder that leaves no sperm in the semen, rarely cause conception naturally, while those with teratospermia, asthenospermia, and oligozoospermia may have a lower probability of producing pregnancy (Evenson et al., 2002; Cousineau & Domar, 2007; Inhorn & Patrizio, 2015). There are currently a few effective treatments for male factor infertility, such as varicocelectomy surgery or pharmaceutical therapy like clomiphene citrate and aromatase inhibitors. More recently, it has been shown that the supplement coenzyme Q10, which is present in mitochondria, increases the motion and movement of sperm. The effectiveness of various treatments varies, and it takes three to six months for any noticeable changes to occur (Evenson et al., 2002; Nowicka-Bauer & Nixon, 2020). More often than not, treatments entail a variety of high-tech IVF techniques, including intracytoplasmic sperm injection, in vitro fertilization, and motile sperm selection. The sperm preparation method used in all of these therapies usually aims to increase sperm motion, movement, and functional capacity for successful fertilization (Sharbatoghli et al., 2015; Bozorgmehr et al., 2020). A popular chemical used to attain this kind of improvement is pentoxifylline, a methylxanthine derivative that functions as a nonspecific inhibitor.

Pentoxifylline may improve motion and movement because it raises intracellular cyclic adenosine monophosphate (cAMP) as well as cAMP-dependent kinase levels. Although pentoxifylline has been shown to have a positive impact on sperm motion, movement, and kinetics, its usage is still debatable due to the possibility of embryotoxicity and early acrosome responses in sperm (Chen et al., 2019; Coluzzi et al., 2021; Al-Timimi et al., 2024).

For procedures such as intracytoplasmic sperm injection, a viable spermatozoon is necessary; motion and movement are not requirements. As a result, some sperm in an immotile sample may still be useful to a couple attempting to conceive. Currently, the hypo-osmotic swelling test is the sole way to distinguish between immobile, living sperm and dead sperms while maintaining the capacity of the sperm to fertilize an oocyte through intracytoplasmic sperm injection. One indicator of sperm viability is functional sperm

membrane integrity, which is measured by the hypo-osmotic swelling test (Gnoth et al., 2005; Darszon et al., 2011).

It is thought that low-level laser therapy causes mitochondria to produce more adenosine triphosphate (ATP) (Zahra, 2021; Abdel-Salam & Harith, 2015). By means of activation of photosensitive Complex IV within the mitochondrial electron transport chain, the treatment at wavelengths between 600 and 1000 nm boosts the production of ATP. It is believed that more ATP will accelerate healing by enabling faster cell division (Hawkins & Abrahamse, 2006; Moskvin & Apolikhin, 2018; Behtaj & Weber, 2019).

Data regarding DNA damage and potential negative effects on cells of laser exposure have been documented for both prokaryotic and eukaryotic cells, and for lasers with varying power, wavelengths, and fluencies (Preece et al., 2017; Mussttaf et al., 2019). Furthermore, other DNA repair mechanisms, such as error-free homologous and nonhomologous recombination repair, light-dependent and dark DNA repair processes, and nucleotide excision repair, may be able to heal damages caused by laser exposure under certain circumstances (Kültz, 2005; Zahra, 2020). Nonetheless, various exposure regimes are employed, and therapies based on low-level lasers are thought to be safe (Boegheim et al. 1987; Firestone et al., 2012; Kong et al., 2018).

The spermatozoon's tail movement is propelled by a tightly coiled a mitochondrial sheath located in its midpiece. Therefore, an increase in the sperm’s motion and movement would be expected if the midpiece's mitochondrial activity were increased. We postulated that in low-quality semen samples, exposure to low levels of laser radiation might boost sperm motion and movement energy.

2. Materials and Methods

Samples from 40 patients undergoing intracytoplasmic sperm injection, in vitro fertilization, and infertility treatment at the Taiba Center under Project No. 1230-2023 (from 01-09-2023 to 01-12-2024) were hydrolyzed for 30 min immediately after collection, and then conventional analysis was conducted. If a residual volume of I mL or more remained after the standard analysis, it was also included in the study.

On a written consent form, patients gave Babylon Women's and Children's Hospital permission to use their leftover semen samples for this study. Based on World Health Organization criteria, the 40 semen samples were classified as 12 normospermic, 13 as asthenospermic, and 15 as both asthenospermic and oligospermic.

The remaining semen samples were divided into two equal portions and put in 10 mL tapered tubes, designated as treatment and control, after standard analysis. The treatment-marked samples were exposed to 980 nm laser radiation for 30 s at a power of 100 mW and an energy density of 10 J/cm^2, while maintaining the shortest possible distance between the laser emitter and the conical tube.

Semen samples were subjected to two distinct analytical phases after being incubated at 37°C. The initial analysis occurred after a 30-min incubation period, allowing for the evaluation of sperm motility and other parameters shortly after collection. This early assessment is crucial as it reflects the immediate post-ejaculatory state of sperm, which can be influenced by factors such as seminal plasma composition and environmental conditions.

Following this initial analysis, the samples were reevaluated after an additional 90 min at the same temperature. This second assessment is particularly important as it provides insights into how sperm motility and viability may change over time in vitro. Factors such as temperature stability, pH levels, and osmotic pressure can significantly

affect sperm function during this incubation period. By comparing results from both time points, valuable information regarding sperm behavior and potential fertility implications can be obtained.

The use of computer-assisted integrated visual optical system technology allows for high-throughput analysis with enhanced precision compared to manual methods. It employs sophisticated algorithms to track individual sperm movements through video microscopy and analyze their trajectories in real-time. This level of detail not only improves diagnostic accuracy but also aids in identifying specific abnormalities that may contribute to male infertility.

Overall, the integration of computer-assisted systems into semen analysis represents a paradigm shift towards more reliable and reproducible assessments in reproductive health research.

2.1 Evaluation of semen properties

As part of the standard semen analysis, the following parameters were measured: volume, concentration, vitality, movement and motion, path and curved velocity, line velocity, linearity, lateral head movement as described by Agarwal et al. (2003b), and mucus penetration ability as stated by Mortimer (2000) and Ola et al. (2003). Semen samples were introduced into 20 mm microcells chambers in 5 mL aliquots. For each parameter, at least 100 sperm were analyzed across ten distinct fields.

2.2 Analysis of sperm DNA fragmentation

Aliquots of raw semen containing one to two million sperm were frozen at -80°C for the analysis of DNA, which was done in the same manner as previously reported for control and laser-treated samples (Barroso et al., 2000; Evenson & Wixon, 2006). The analysis was completed within 4 h of ejaculation. Breifly, the samples after thawing on ice were diluted to 1-2 x 10^6 cells/mL using lysis buffer. After staining in a McIlvaine buffer (pH 6.0) with 1 mL of 5 mg/mL acridine orange, 200 mL of each diluted sample was combined in portions with 300 mL of detergent solution (pH 1.2) for 30 s. The cells were examined using an air-cooled argon laser-equipped BD FACSCalibur™ three minutes after the staining process began. Two aliquots of each semen specimen were studied, and evaluations were made twice on 5000 cells per sample at a rate of fewer than 300 cells per second for sperm flow. The flow cytometric data were analyzed using Flow Cytometry Standard Express.

2.3 Statistical analysis

We utilized SPSS to do a 2-tailed paired (t-test) taking into account that the distribution of all the outcomes was normal. $P < 0.005$ was designated as the statistical significance threshold.

3. Results and Discussion

For analysis, forty semen samples from male individuals were taken and exposed to low laser light levels. Sperm concentration (the quantity of sperm per milliliter) and motility (the capacity of sperm to move) might differ greatly between these samples. The initial measurements made before any treatment or exposure may be referred to as baseline concentrations. After 30 min, sperm motion and movement were evaluated, and 2 h later,

both DNA damage and motion and movement were evaluated again. Thirty minutes after exposure, computer-assisted semen analytical tests showed a statistically significant increase in motion and movement (18.5%) in the samples that were treated as compared to the untreated samples (Table 1). Two hours after exposure, this difference was not noticed. Regarding the various WHO classifications of the 40 samples under investigation, the size of the treatment effect varied greatly as well (Table 2). Following treatment, the samples that were considered typical sperm (motion and movement of ≥ 50%, concentration of ≥ 20 M/mL) showed a little but noteworthy rise in motion and movement of 7.5%. After therapy, the relative increase in motion and movement was mild at 11.8% in those categorized as asthenospermic (motility, < 50%). Significantly, the samples with < 50% motion and movement and at a concentration < 20 M/mL, i.e both oligospermic and asthenospermic, showed the highest increase in motion and movement (88.5%) after treatment (Table 2).

Table 1. Percentage of motion and movement±SEM of both groups' semen samples

	Untreated Group (n = 40)	**Treated Group (n = 40)**
After 30 min, Percentage of Motion and Movement±SEM	35.2±4.51%	40.9±2.55% (P ≤ 0.002)
After 2 h, Percentage of Motion and Movement±SEM	27.9±3.26%	29.8±3.04% (P ≥ 0.005)

Table 2. Percentage of motion and movement change in a sample using the WHO classification

Sample Using the WHO Classification	**Normal Sperm (n = 10)**	**Asthenospermic (n = 12)**	**Oligospermic and Asthenospermic (n = 11)**
Percentage of motion and movement change	7.5% (P = 0.005)	12.8% (P ≤ 0.001)	88.5% (P ≤ 0.001)

After 2 h, the treatment samples had 21.8±0.053% and the control samples had 25.3%±0.024% of the DNA fragmentation index, as determined by flow cytometry, according to the mean (±SEM) values (Figure 1). There was no appreciable difference in the sample rate of DNA damage between the treated and the untreated samples two hours after laser irradiation.

Thirty minutes after treatment, the oligospermic and asthenospermic samples showed significant improvements in straight-line velocity and linearity, as shown in Figures 2 and 3. In the oligospermic and asthenospermic samples, as presented in Table 3, the straight-line velocity and linearity considerably increased 30 min after the treatment, whereas the amplitude of lateral head displacement, route velocity, and curvilinear velocity were not affected.

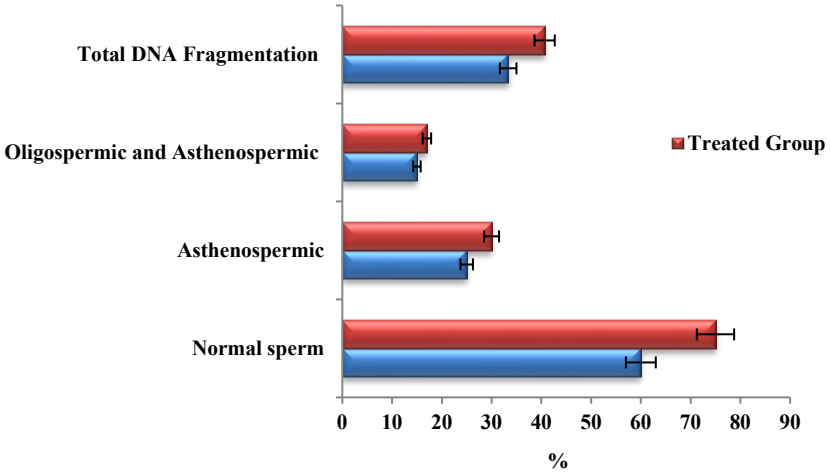

Figure 1. DNA fragmentation for both treated and untreated groups

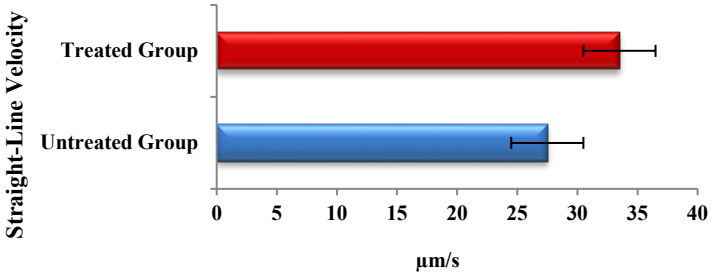

Figure 2. The variations in straight-line velocity for both treated and untreated groups

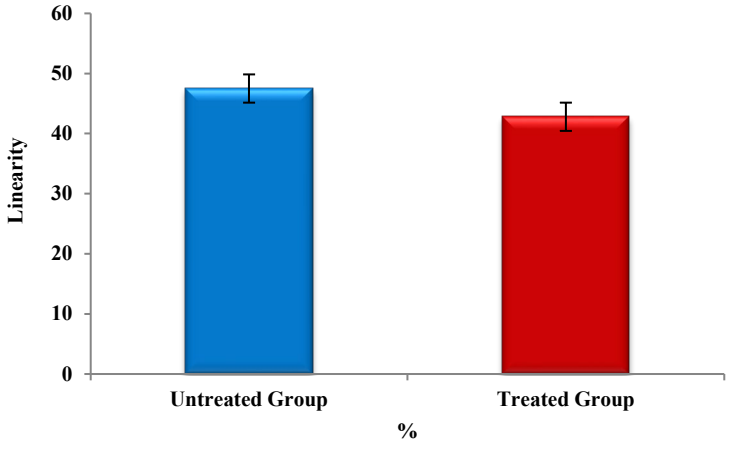

Figure 3. The variations in linearity for both treated and untreated groups

Table 3. The changes of the sperm kinetic characteristics for both groups

	Untreated Group	Treated Group After 30 min	P Value
(Normal sperm)			
Path Velocity (μm/sec)	54.64	56.38	0.79
Line Velocity (μm/sec)	43.68	42.83	0.11
Curvilinear Velocity (μm/sec)	83.16	83.36	0.93
Lateral Head Displacement (μm)	4.01	4	0.93
Linearity (%)	51.8	52	0.43
Penetration of Mucus (%)	36	37.1	0.11
(Asthenospermic)			
Path Velocity (μm/sec)	45.3	49.7	0.62
Line Velocity (μm/sec)	34.7	32.7	0.94
Curvilinear Velocity (μm/sec)	82	73.7	0.62
Lateral Head Displacement (μm)	3.7	3.9	0.33
Linearity (%)	50.3	48.8	0.18
Penetration of Mucus (%)	24.2	34.6	0.79
(Oligospermic/asthenospermic)			
Path Velocity (μm/sec)	35.81	46.12	0.07
Line Velocity (μm/sec)	27.17	33.11	0.02
Curvilinear Velocity (μm/sec)	62.26	69.44	0.35
Lateral Head Displacement (μm)	2.91	3.95	0.24
Linearity (%)	42.28	47.18	0.03
Penetration of Mucus (%)	24.18	28.45	0.31

Forty semen samples were used in this study, and each one was exposed to 30 s of low-level laser radiation at a wavelength of 980 nm with an energy density of 10 J/cm^2. Within 30 min of the exposure, the treated samples' motion and movement rose by an average of 18.5% as compared to the untreated samples; however, 2 h later, there was no discernible increase in the motion and movement of the treated samples.

It is essential to note that while there may be an initial increase in mitochondrial activity following laser irradiation, this effect could be transient, suggesting that after a brief period—such as 30 s of irradiation—the cells might return to baseline metabolic rates once the excitation from amino acids or other substrates ceases. This phenomenon could be attributed to various factors, including photodamage at higher intensities or feedback mechanisms within cellular metabolism. Furthermore, movement improved more in low-quality samples than in normozoospermic samples, indicating that asthenospermia may have an underlying reason related to decreased mitochondrial activity (Azzam et al., 2012; AL-Timimi & Mustafa, 2018; Sommer, 2019; Timimi, 2020).

The objective of radiation wavelength employed in this investigation was to boost ATP generation by enhancing the activity of the electron transport chain. The finding that exposure to a particular wavelength of radiation enhances sperm motion and movement suggests a possible connection between the aberrant sperm motion and movement in the asthenospermic group and reduced mitochondrial function and ATP production. It is impossible to ascertain if the mitochondria had diminished tricarboxylic acid substrates for ATP synthesis, increased mitochondrial DNA mutations impacting ATP generation, or both (Agarwal et al., 2003a; Azzam et al., 2012; Kong et al., 2018).

It was crucial to ascertain whether the sperm viability was negatively impacted by the laser treatment. Additionally, we examined at how the treatment affected the DNA fragmentation index, which is a sign of possible sperm harm. Two hours after treatment, the treated and control samples underwent an assessment of their DNA fragmentation

indices to calculate the total quantity of possible laser-induced DNA breaks. Table 1 demonstrates that there was no discernible increase in DNA damage following the treatment.

An increase in motion and movement in a particular semen sample may raise the likelihood of pregnancy when using in vitro fertilization or intrauterine insemination as assisted reproductive technologies. For patients with asthenospermic sperm, laser therapy may prove to be a useful treatment, particularly if a longer-lasting result can be obtained (Hawkins & Abrahamse, 2006; Dreyer, 2011; de Almeida et al., 2019).

Furthermore, low-level laser irradiation may stimulate viable but immotile sperm to move, offering a non-damaging and clinically relevant way to identify the immotile sperm that are alive in a given sample. In place of the hypo-osmotic swelling test, this method may offer a straightforward and useful way to choose immotile sperm for intracytoplasmic sperm injection (Mustafa et al., 2011; Al-Timimi, 2022).

In addition, infertile couples undergoing advanced reproductive technology for male factor infertility may see a marked improvement in the probability of conception if sperm are treated with laser therapy. To further understand the impact of laser radiation on sperm motion and movement, much more research is required. It is necessary to conduct time course and dose-response trials. Furthermore, it would be advantageous to assess the treatment's functional effect on different sperm parameters by the use of a sperm penetration assay, for instance.

4. Conclusions

The study results showed that thirty seconds of exposure to low-power laser radiation considerably improved the movement of the sperm, especially in samples that were both oligospermic and asthenospermic. According to the current study, activity in the electron transport chain of the mitochondria may have been the reason for the laser's brief effect on sperm movement. Although there do not seem to be any negative effects on the fragmentation of spermatozoon DNA, more research is required to determine the therapeutic effectiveness of this medicine.

The study's findings reveal that low-power laser irradiation at 980 nm significantly increases sperm motility. This work introduces a novel method of laser cell stimulation, emphasizes the biological implications of treatment with lasers, and underscores the correlation between the application of lasers and sperm motility. When reviewing the results, several factors including the method used for the research, the number of samples collected, laser therapy power, irradiation period, technique for laser irradiation, and frequencies must be considered. When thoroughly examining those factors, researchers can gain a better understanding of laser treatment and its impact on sperm motility, perhaps opening up new avenues for future research in this field.

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6. Conflicts of Interest

The author of this article has disclosed no relevant conflicts of interest.

ORCID
Zahra Al Timimi https://orcid.org/0000-0002-3209-553X

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