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ORIGINAL PAPERS

Improving human sperm motility via red and near-infrared laser irradiation: in-vitro study

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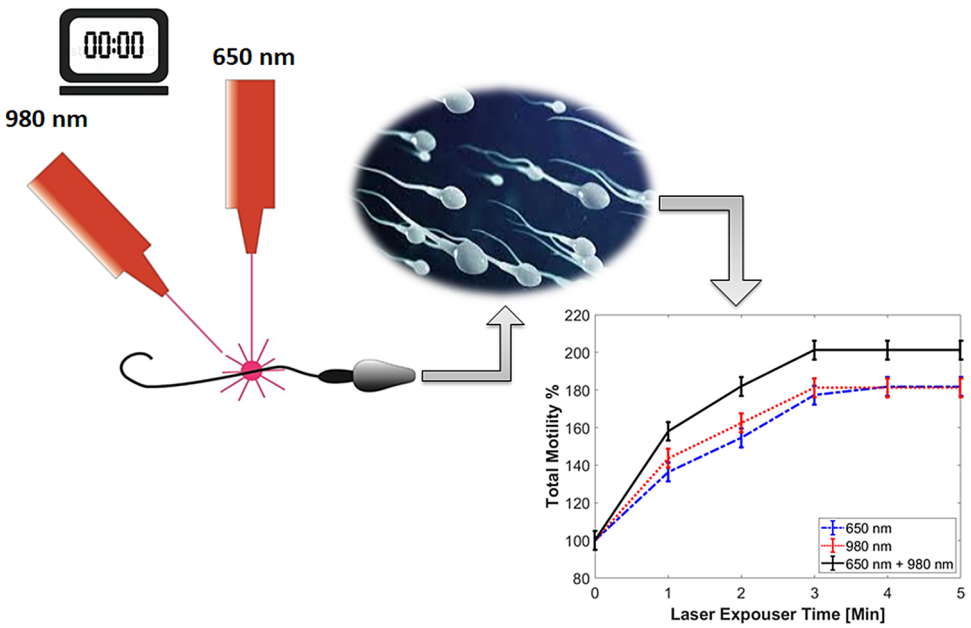
Abstract

Improved sperm motility is necessary for successful sperm passage through the female genital system, efficacious fertilization, and a greater probability of pregnancy. By stimulating the mitochondrial respiratory chain, low-level laser photobiomodulation has been shown to increase sperm motility and velocity. The respiratory chain in mitochondria is the primary site of action for cytochrome c oxidase because it can absorb light in the visible and infrared ranges. The present study aimed to investigate the effects of red laser 650 nm, near infrared laser (NIR) 980 nm, and combination of both on human spermatozoa motility and DNA integrity at different doses. An in-vitro controlled trial was performed in Al Zahraa university hospital laboratory using thirty fresh human semen specimens. Samples were exposed to red laser 650 nm, near infrared laser (NIR) 980 nm, and combination of both for various irradiation times. Sperm motility for the test and control aliquots was assessed as recommended in the manual of WHO-2021. Sperm chromatin integrity was evaluated using the Sperm Chromatin Structure Assay. Results revealed almost 70%, 80% and 100% increase in the total motility after 3 min of the 650-nm, 980-nm and the combined laser irradiation, respectively. Additionally, the Sperm Chromatin Dispersion assay was carried out on sperm heads utilizing human sperm DNA fragmentation, demonstrating that none of the three laser types had any discernible effects.

Graphical abstract



Keywords Photobiomodulation · Low-level lasers · Sperm motility

Extended author information available on the last page of the article

1 Introduction

One in six couples experience infertility globally [1]. About half of all occurrences of infertility are caused by male factors. While the underlying causes are still unknown in 25% of instances, known causes include low sperm counts, abnormal sperm morphology, and decreased sperm motility. Therefore, increasing sperm motility could lead to assisted/artificial reproductive technology (ART) techniques that are less taxing on the healthy female partner, such as intrauterine insemination (IUI) rather than intracytoplasmatic sperm injection (ICSI), which could increase the likelihood of natural conception or at least result in sperm that are more likely to move from one sperm to another more quickly [2–4].

The mitochondria found in the spermatozoon's midpiece act as the source of energy for the flagellar movement [5]. Therefore, stimulation of mitochondria will lead to an increase in sperm motility [2]. Additionally, the activation of light-sensitive calcium ion channels and opsin chromophores may cause the mobilization of the calcium store located in the spermatozoon's neck region, which in turn causes flagellar activity [6]. Since the invention of early laser systems, which mostly changed when sperm motility changed, the idea of a light-sensitive signaling system in spermatozoa has been studied. Unexpectedly there does not appear to be a physiological basis for this theory, as light effects during sexual activity and conception are not yet known to be significant.

Animal models have mostly been used to evaluate the precise effects of photobiomodulation or low-level laser therapy (LLLT) on sperm. There are few helpful studies on the effects observed in human spermatozoa [2]. The results from the animal models were extrapolated to get insight into the effects of LLLT on human sperm and potential ramifications for increasing fertility through increased sperm motility. The LLLT effect on improved calcium transport and binding by sperm plasma membranes [7], the induction of the acrosome reaction (8), an increase in the longevity of motility [9], and an improvement in the ability of frozen animal sperm to survive under liquid storage conditions [10] are a few examples of these.

Nowadays, photobiomodulation (PBM) therapy is considered a viable noninvasive method for enhancing brain healing and stimulating neuronal function is. However, the PBM parameter optimization is crucial to achieve the highest levels of effectiveness and tolerability [11]. Consequently, visible to near-infrared (NIR) light penetration through different animal and human tissues has been documented in a number of investigations [12]. On the other hand, laser irradiation has been shown to improve sperm motility in canine [13], bovine [14, 15], and human sperm [2]. Following that, other research looked into the use of laser as an alternative therapy option for male factor infertility caused by asthenozoospermia [16]. However, there are currently no standardized techniques for determining the appropriate energy dose and irradiation time.

Salman Yazdi et al. [17] investigated the effect of various doses low-level laser (830 nm GaAlAs) irradiation on the sperm motility of fresh human semen specimens from asthenospermic patients. Computer-aided sperm analysis was used to evaluate sperm motilities after irradiation. Additionally, the control and high-irradiation groups underwent two tests: the sperm chromatin dispersion (SCD) test and the Hypo-Osmotic Swelling (HOS) test. Their findings demonstrated that, following 30, 45, and 60 min of radiation, the sperm motility of the control groups dramatically dropped, but that of the exposed groups either stayed constant or slightly increased over time depending on the laser density and post-exposure duration [18].

In Safian et al. [19], study, 30 normal human semen samples divided into 3 different PBM protocols (red "630 nm", NIR "810 nm", and red + NIR lasers). Each protocol was adjusted to three energy densities (0.6, 1.2, and 2.4 J/cm^2). Their results revealed that the NIR and red + NIR lasers at 2.4 J/cm^2 energy density significantly increased progressive sperm motility after 60 min compared with the control groups. When compared to the control group, samples exposed to the red laser at 0.6 J/cm^2 exhibited considerably lower viability. While samples exposed to the red + NIR lasers exhibited significantly lower survivability at energy levels of 0.6 J/cm^2, 1.2 J/cm^2, and 2.4 J/cm^2. Between the experimental and control groups, there was no discernible difference in sperm viability as a result of the NIR laser. Treatment with the red + NIR and red lasers at 2.4 J/cm^2 significantly enhanced DFI in comparison to the control groups 120 min after exposure.

For PBM procedure to be effective, endogenous chromophores, also known as photoacceptors, must be able to absorb light of various wavelengths. Since cytochrome c-oxidase (Ccox) can absorb light in the range of visible and infrared wavelengths, the respiratory chain within mitochondria is the primary site of action [10, 20, 21]. Ccox exhibits changes in its redox status following PBM in in vitro tests, and by doing so, satisfies the requirements of a chromophore [22]. Fully oxidized, reduced, or in a semivalent redox state, the copper centers and binuclear heme of Ccox can exist. As a result, they exhibit distinct absorption spectra in the vicinity of near-infrared light up to 905 nm [6]. The enzyme's turnover rate is the only plausible constraint for Ccox. The efficiency of the enzyme activity and the amount of reduced oxygen that can be given as a substrate for ATPase, the enzyme that catalyzes the creation of ATP, increases with improved electron transport between its four active metal centers [21].

The objective of the current study is to examine, in an in-vitro controlled trial, the effects of using separate and combined red and NIR lasers on the motility of human spermatozoa and the corresponding DNA integrity. For varying exposure times, samples were subjected to red lasers at 650 nm, near infrared lasers (NIR) at 980 nm, and a combination of the two wavelengths. Results were statistically evaluated using one-way ANOVA test and Receiver Operating Characteristics (ROC) curve.

2 Methods

2.1 Semen samples

Thirty human semen samples with impaired sperm motility (Asthenzoospermia) were collected in a room near the laboratory by masturbation into a wide mouthed sterile specimen container after an abstinence period of 4–6 days from healthy males who were referred to urology clinic of Al Zahraa university hospital for fertility diagnostic and treatment. Participants range in age from 28 to 40 and do not have any chronic health issues. Those with a history of alcoholism or endocrine diseases including diabetes were among the exclusion criteria. Since weighing patients is not a regular procedure in our hospital, the patient's weight was not assessed. Written informed consent was provided by each subject for the use of their semen samples in academic studies.

Each semen sample was analyzed as recommended in the manual of "WHO 2010 and 2021" [23, 24] as follows; the specimen container is placed in the incubator (37 °C) while the semen liquefies. Initial macroscopic examination by simple inspection after liquefaction at 30 min was done followed by initial microscopic investigation using (OLYMPUS CX23) with a magnification of X40 objective lens after thorough mixing of semen. A standard volume of semen (10 µl) was placed onto a clean glass slide and covered with coverslip. These steps are summarized in Fig. 1.

Additionally, each spermatozoon's motility is rated according to the following criteria: Spermatozoa that actively move in either a big circle or a linear motion, regardless of speed, are considered to have progressive motility (PR). Non-progressive motility (NP) refers to any other forms of movement that lack progression, such as swimming in short circles, having a flagellar force that rarely moves the head, or observation of only the beat of the flagella. Immobility, the absence of motion, was denoted as IM. Progressively motile spermatozoa were categorized as rapid or slow. Azolospermia, sperm morphological abnormalities, leukocytospermia, and patients' missing consent were the exclusion criteria. Each sample was divided into four equal portions, three of which were exposed to laser radiation with (red, NIR, and combined red + NIR) for 1, 2, 3, 4, and 5 min, respectively. One portion served as a control and received no laser treatment.

2.2 Laser exposure protocol

Semen samples have been exposed to three laser irradiation protocol. The first one using red laser at 650 nm and the second was using 980 nm. Furthermore, the sample was exposed to a combination between the two wavelengths. The measurements have been obtained via two CW laser sources (PGL-DF, Changchun New Industries Optoelectronics Tech. Co., Ltd, China). The red laser source at 650 nm produces a near TEM_{00} beam with 130-mW power and 3.5-mm diameter at the aperture. While the NIR laser beam (980 nm) is a multimode beam with 108-mW power and 3-mm beam diameter at the aperture. The beam divergence (full angle) was 0.2 mrad for both laser sources.

The semen samples have been exposed to laser irradiation for five time periods (1, 2, 3, 4, 5 min) for each utilized wavelength separately and combined. The studied samples were exposed to the low-level laser sources at a distance of 5 cm after expanding the incident laser beam via diverging lens (N-BK7 plano-concave lens, $f = -150$ mm, Thorlabs, Inc., USA). The distance between the source and the samples

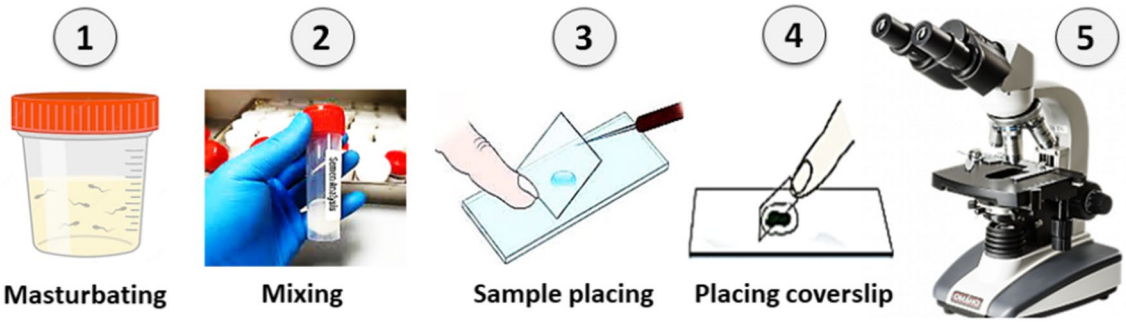


Fig. 1 The different steps for sample collection, preparation and examination

was specified to guarantee that the sample's whole surface area (1 cm^2) is adequately exposed. The resultant irradiance (i.e., power density) at target sample was 0.138 W/cm^2 and 0.108 W/cm^2 for the red and NIR lasers, respectively. The angle between the two sources in case of combination was 45°. Schematics for the implemented optical setup are illustrated in Fig. 2.

2.3 Sperm chromatin dispersion (SCD) assay

The evaluation of sperm DNA fragmentation has grown in significance as a method for offering important details about sperm quality and for its ability to independently forecast sperm fertilization ability. As an alternative method for identifying sperm DNA damage, the sperm chromatin dispersion (SCD) assay yields results that are on par with those of the gold standard, the sperm chromatin structure assay. The SCD test is predicated on the idea that after acid denaturation and nuclear protein removal, sperm with fragmented DNA are unable to generate the distinctive halo of scattered DNA loops seen in sperm with intact DNA [25].

The first step in the SCD procedure is the chemical denaturation of sperm cells, which results in the creation of single-stranded (ss) DNA patterns from the ends of pre-existing DNA breakage sites. The nuclear and cell membranes of the sperm cells are then deproteinized. The resulting "sperm nucleoid," which is the sperm nucleus encircled by a halo of relaxed DNA loops, is examined under a microscope to determine whether DNA damage has occurred [26]. In the present investigation, SCD assay was performed on sperm heads using Human Sperm DNA Fragmentation (HSDF-ZD) kit by ZEUS Diagnostic, USA. Values of DNA defragmentation above 25% were considered pathological. On a prepared slide, sperm are submerged in an inert agarose gel. The DNA in those sperm cells that have fragmented DNA is denatured by an initial acid treatment. Once the majority of the nuclear proteins have been eliminated by the lysing solution, nucleoids with sizable halos of spreading DNA loops emerge from central core and, in the absence of significant DNA breaking, are produced. On the other hand, the dispersion halo is either absent or very faint in the nucleoids from spermatozoa containing fragmented DNA.

3 Results and discussion

3.1 Motility improvement via laser exposure

All the treated samples have impaired sperm motility (i.e., asthenzoospermia) with less than 40% initial total sperm's motility based on the WHO's 2010 and 2021 criteria [23, 24]. Healthy normal samples (i.e., those with > 40% motility) were not subjected to laser irradiation in the current investigation as they had sufficient motility and didn't require improvement. Consequently, impaired non-irradiated samples were used for comparison.

As can be observed in Fig. 3, sperm motility was markedly improved by laser treatment during various exposure times.

Following the initial exposure period, which lasted one minute, the total motility increased for the 650-nm and 980-nm lasers, respectively, by 35% and 40%. Moreover, the combination between the two laser sources provided about 60% increase in the motility. After 2-min irradiation time, the total motility increased by 40%, 50% and 80% for 650-nm, 980-nm and the combination respectively.

A steep rise of total motility was observed after irradiation for 1, 2, 3 min by all wavelengths with a significant increase regarding PBM by the combined wavelengths compared to each separate one. After 4-min exposure, a plateau

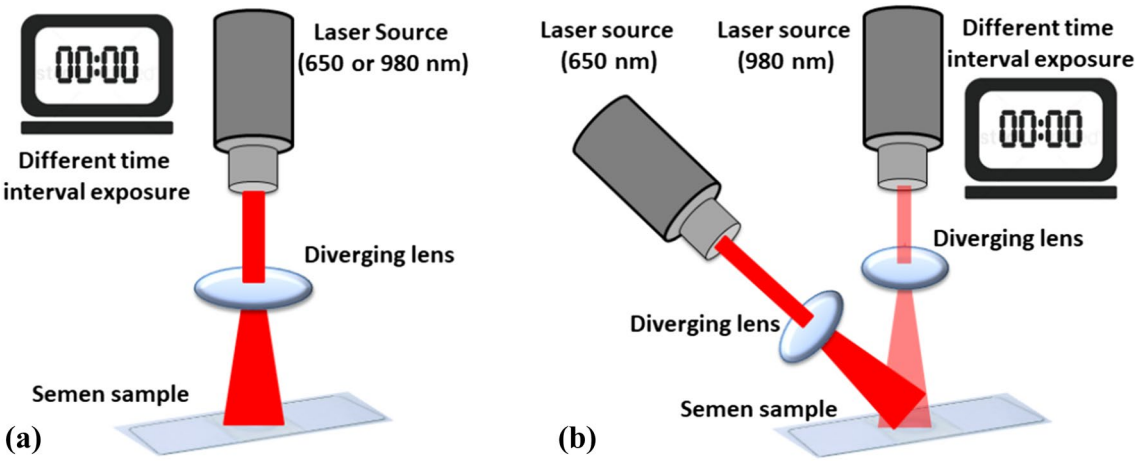


Fig. 2 Schematic of the laser exposure procedures, **a** using single laser source, **b** using two laser sources

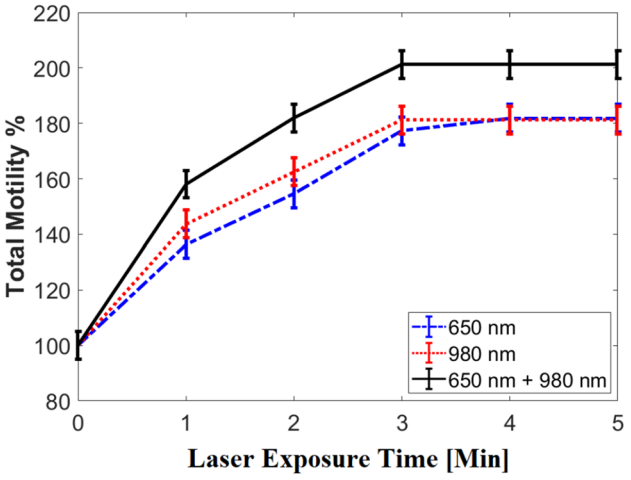


Fig. 3 Change of motility with laser exposure time for the different irradiation protocols

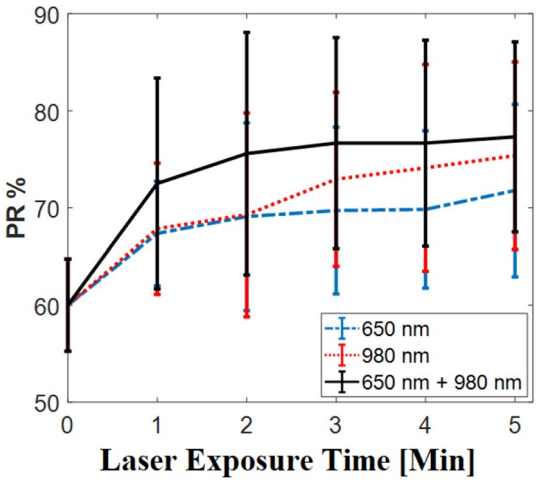


Fig. 4 Progressive at every laser exposure time for the different irradiation protocols

was observed with the same effect achieved at the 3 min for each wavelength that was 70%, 80% and 100% increase in the total motility using 650-nm, 980-nm and the combination irradiation, respectively. Stability or a very little attenuation of sperm motility was observed after irradiation for 5 min for each wavelength though it still a highly significant compared to initial point.

Progressive motility was significantly increased after each irradiation protocol for every exposure period (with a p-value < 0.001 (HS)) as presented in Fig. 4. In contrast, a high significant decrease of the non-progressive motile sperms was observed after PBM by combining the 650 nm and 980 nm wavelengths for the all exposure times.

A significant increase of rapid progressive motile sperms and decrease in the slow progressive ones was observed after

Table 1 The resultant variations in the rapid progressive motile sperms

Procedure	Exposure time (min)				
	1	2	3	4	5
Using 650 nm	63%	67%	80%	80%	80%
Using 980 nm	60%	69%	76%	78%	78%
Using 650 nm + 980 nm	64%	81%	88%	88%	88%

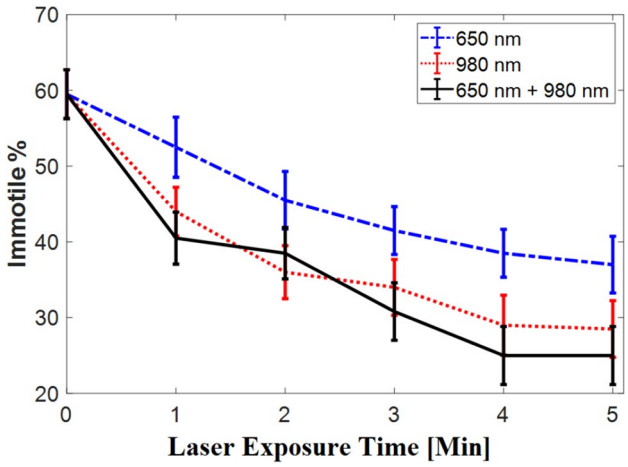


Fig. 5 Variations in the immotile sperms percentage at every laser exposure time for the different irradiation protocols

laser irradiation by both red and NIR laser for each time interval. The main results are summarized quantitatively in Table 1.

A high significant decrease of immotile sperms were obviously noticed after PBM for all radiation times according to our protocol with a p-value 0.003 for the 660-nm laser, and < 0.001 for the NIR laser and the combination of both. The resultant decrease in the immotile sperms percentage at every laser exposure time for the different irradiation protocols is illustrated in Fig. 5.

3.2 Sperm chromatin dispersion (SCD) assay analysis

Due to the wavelength and dose dependence of biological reactions, results from PBM investigations must be interpreted in light of the unique study conditions and parameters. In the current study, Sperm Chromatin Dispersion assay was performed on sperm heads using human sperm DNA fragmentation showing that there was no significant effect regarding three types of lasers as demonstrated in Fig. 6.

Damaged spermatic DNA had no or little halos, but normal spermatic DNA exhibited radiating halos. Sperm with a small or no halo are classified as fragmented. One side of

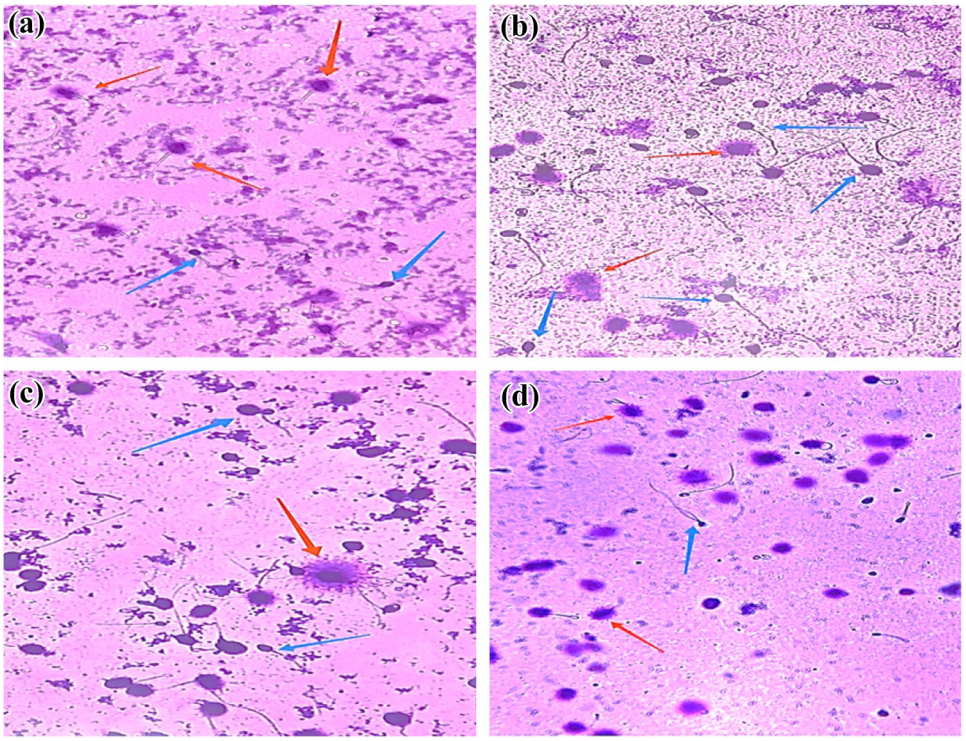


Fig. 6 Microscopic images of the examined sperm samples **a** before, **b** after exposure to laser irradiation using 650 nm, **c** using 980 nm and **d** using 650 + 980 nm. The red arrow is for big halo"-intact DNA and the blue arrow is for no halo-fragmented DNA

the halo's thickness was less than one-third the diameter of the thinnest region of the head [27]. The percentage of sperm with fragmented DNA (SDF (%)) was deemed normal when it was less than 25% and equal to the number of sperm with × 100% of fragmented DNA. Using bright field microscopy to count 200 sperm with (large halo + medium halo) and without (small halo + without halo) fragmentation, the DNA fragmentation index represents the percentage of sperm with fragmented DNA. Thus, comparing the DNA fragmentation index of the irradiated samples to the non-irradiated ones, there was no significant difference.

3.3 Statistical evaluation

IBM SPSS statistics-6 software was used to analyze all of the data that was obtained. In SPSS, the "one-way ANOVA test" was used to compare the data in independent groups once the data's linearity had been verified. In general, the analysis of all the data produced significant results (see supplementary material S1). Furthermore, by visualizing the sensitivity vs. 1-specificity of a particular test or collection of tests based on the principles of true positive, true negative, false positive, and false negative, ROC is a graphical representation that is used to analyze diagnostic test accuracy [28, 29]. The ROC analysis was used in our suggested study to assess the sensitivity and accuracy of the experimental data collection for the radiated and non-radiated (i.e., control) samples. According to our ROC curves analysis, each laser exposure regimen had a statistically significant area under curve (AUC) that is 0.83, 0.92 and 0.84 for 650-nm, 980-nm and the combination irradiation, respectively. Moreover, the various ROC-curve measures, such as sensitivity, specificity, and accuracy, are also summarized in the supplementary material S2.

As far as we know, this is the first study that has chosen PBM by 650-nm, 980-nm, and a combination of both wavelengths on sperm motility that resemble those easily available within the clinical context. These chosen wavelengths also help to close a knowledge gap in the field of how different wavelengths affect sperm motility, as only wavelengths of 830 nm [17], 905 nm [2], and 940 nm [30] have been employed in previous researches. The wavelength and other factors employed in earlier investigations that demonstrated how LLLT affected sperm motility have not been rendered clear [31, 32].

In 1984, Sato et al. [32] demonstrated how human spermatozoa with low energy density are affected by laser light stimulation. Some studies have then reported the probability of increasing sperm motility following laser irradiation in the sperm of bovines [33], canines [34], and humans [35]. Various light sources, such as He–Ne lasers, diode lasers, or LEDs, as well as different wavelengths, powers, and exposure times were all used in earlier investigations; however, few of these factors were completely described. To achieve the desired outcome, all LLLT exposure parameters must fall within an optimal range because photobiomodulation on cells is known to be dose dependent [20].

Following the use of 650-nm laser irradiation, sperm motilities were assessed, and chromatin condensation of sperms was identified in a previous publication [36]. The photobiomodulation was carried out in a dark setting at a distance of 10 cm using a 36-cm^2 aperture and 200-mW output power for 30 and 60 min. Their results showed that the sperm motilities of the photobiomodulation groups were significantly higher than those of the controls. Despite employing alternative exposure factors, these results agreed with our own. Although sperm motilities tended to differ between the groups exposed to red light for 30 and 60 min, this difference was not statistically significant. Non-progressive motility sperms showed no discernible difference when the motility grades were assessed. In contrast to the control group, progressive sperms increased in the photobiomodulation groups while immotile sperms considerably decreased.

In a different investigation by Espey et al. [37], three aliquots were subjected to a pulsed laser probe with energies of 4, 6, and 10 J/cm^2 (wavelength 655 nm, output power 25 mW/cm^2, impulse duration 200 ns). Semen parameters were evaluated five times: at 0 min (immediately after PBM), 30, 60, 90, and 120 min afterwards. In patients with asthenozoospermia and normozoospermia, sperm motility and velocity were assessed, and the quantity of DNA strand breaks was examined using ligation-mediated quantitative polymerase chain reaction. Their findings showed that sperm motility was significantly affected by laser energy dosages of 4 and 6 J/cm2 in both asthenozoospermic and normozoospermic patients. In the first two minutes following treatment, it showed enhanced PR. This is consistent with our work, in which PBM at 650 nm for two minutes demonstrated a significant increase in total, progressive, and rapid progressive motility compared to the baseline, as well as a significant decrease in both immotile and slow progressive sperms (see Figs. 3, 4, 5 and Table 1).

Philip Gabel et al. used three samples of human sperm in their study: one fresh and two frozen. Sperm were exposed to light from two sources for varying periods of time: a GaAlAs single laser (810 nm 200 mW) and an LED cluster (660 nm and 850 nm combined power 2 W). The Sperm Motility Index and Total Functional Sperm Count were subsequently found to have increased up to four times compared to controls, with inhibitory effects observed at higher doses (longer irradiation periods). Depending on the radiation dose, exposure time, and whether the material was fresh or frozen, the maximal effect changed [38].

Additionally, Hasani et al. [39] demonstrated that photobiomodulation (890 nm, 0.03 J/cm^2, 30 s for each testis) enhances sperm motility and reduces oligospermia brought on by scrotal hyperthermia in a mouse model. The majority of the evidence suggests that PBM, regardless of its wavelength, enhances sperm motility in asthenozoospermia [40]. Siqueira et al. examined the impact of different irradiation times and output levels on the activities of frozen bovine sperm while also examining the influence of the He–Ne laser (633 nm). Their findings demonstrated that the parameters of motility and mitochondrial potential were significantly affected by power during a 10-min irradiation. It appears that fluency, dosages, irradiation power, and exposure length all affect the effectiveness of photobiomodulation therapy (PBMT) [15].

Irradiation with visible light (600 nm, 40 mW/cm^2 in 3 min) increased hyperactivated motility and ROS production [41]. However, prior research primarily examined the impact of PBM in the red to near-infrared region on human sperm that is normal and fresh. They demonstrated that progressive sperm motility (PSM) grows when exposed to all experimental wavelengths [36]. The best PSM result was obtained at the energy density sample with the NIR laser's shortest radiation time (23 s). This study supports our findings, which show a strong, significant increase in total, progressive, and rapidly progressing motility following four minutes of laser irradiation. Table 1 and Figs. 3 and 4 show that five minutes of radiation does not result in any additional improvement in total and progressive motility while simultaneously increasing slow progressive and immotile sperm.

Moreover, the DNA fragmentation index (DFI) also did not rise by NIR [19] which supports our results as well. Based on a variety of dose reactions, it appears that PMB with NIR radiation, which is shorter than red light, has more beneficial effects on sperm motility while causing no DNA damage in the sample of fresh and healthy semen. Congruent with our findings, Mahnaz Poorhassan et al. [42] revealed that the overall findings from comprehensive preclinical and clinical investigations in the field of sperm PBM indicate that small amounts of red and NIR light exhibit stimulatory effects without causing significant DNA damage.

4 Conclusion

In conclusion, we demonstrated that PBM utilizing various wavelengths (650 nm, 980 nm, and combination of both) for various irradiation intervals (1, 2, 3, or 4, 5 min) led to significantly higher sperm motility post-irradiation. Generally, our findings show a highly significant increase in overall motility of the asthenozoospermic individuals following irradiation relative to the baseline for each of the three wavelengths. Along with examining how PBM affects sperm motility in patients with asthenozoospermia, we also examined how DNA fragmentation levels and acrosome integrity were impacted revealing no significant effect due to laser radiation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43630-023-00525-y.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no financial or commercial interests related to this study.

Ethics approval and consent to participate The proposed study was carried out in accordance with the Helsinki Declaration and was approved by the NILES, Cairo University ethics committee (No: NILES-EC-CU 23/1/1). All patients were treated at an academic hospital. All patients were requested to give their general agreement for the scientific examination of disease-specific data in anonymized form upon admission to the hospital. Patients could potentially decline to give their consent.

Consent for publication Not applicable.

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