Effect of Green Laser on Human Sperm Agglutination

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Abstract
Infertility is a problem need to solve, many researchers and fertility doctors work hard for this purpose, and for this reason many different techniques tend to use to solve it. One of the most important one is laser. Lasers began to be used in the treatment of infertility between the 1980s and 1990; the first studies clearly indicate that human sperm motility as well as velocity can be improved by laser irradiation. After collection, the semen was taken immediately to the laboratory for sample preparation, physical and morphological analysis. Semen samples were irradiated by using laser light of (532nm) and power (54mW). The samples of semen used were of a concentration greater than 20 × 106/mL, and the ejaculate volume was greater than 5.0 mL. The gathered data suggests that primary photoacceptors are connected with oxygen metabolism and, in particular, with respiratory chains. It is important to recall that the activation by using diode laser was more effective than when we used ordinary technique, because the activation by laser light was more effective at activating the ATP production and, as a result, increased sperm motility, so laser light energized the sperm more than the other technique. We compared it with other studies. The aim of our study was to enhance infertility by enhancing sperm motility, and also to evaluate the role of laser activity in solving infertility problems.
Keywords: Green Laser, Effect, Sperm, Agglutination

1. Introduction

Laser is a device that emits light through a process of optical amplification based on the stimulated emission of electromagnetic radiation. The term "laser" originated as an acronym for "Light Amplification by Stimulated Emission of Radiation" 1. The emitted energy (ΔE) (photon) must equal to the energy difference between the two states 2.
Lasers have many uses in medicine, including laser surgery, laser healing (photobiomodulation therapy), kidney stone treatment, ophthalmoscopy, and cosmetic skin treatments, cellulite, striae reduction, and hair removal. 3. Low-level laser therapy works by a photochemical mechanism in which photons from the laser source interact with cells that result in stimulation of the cells or biochemical changes 4.
Infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after suitable medical time or more of regular (2-3 times per week) unprotected sexual intercourse 5.
Poor sperm motility is a common cause of male infertility in men since the sperm must move a long way to reach and fertilize the ovum; therefore, the motility of the sperm is a necessary condition for proper fertilization (Al-Zoubi et al.,2021).
It is postulated that laser bio stimulation or low-level laser therapy has a positive effect on the mitochondria and leads to an increase in the synthesis of ATP 6, which may stimulate the sperm motility of asthenozoospermic samples.

2. Materials and Methods

In this study, 50 semen samples were selected of infertile patients referred to High Institute for infertility Diagnosis and Assisted Reproductive Technologies Laboratory from different infertile men through the period from December 2021 to April 2022. Their mean ages were 25 ±5 years average 20-40 years old.

Semen fluid analysis

All samples were collected in a room near the laboratory by masturbation into a wide mouthed sterile specimen container after an abstinence period of 3 to 5 days. Each sperm sample were analyzed was done for each sample as recommended in the manual of WHO 2010 and 2021.
The samples were divided into two portions, one was used as a control part, and one were exposed to different laser doses (10,20 and 30min) with wavelength of (532 nm green).

Liquefaction time

Normal semen sample liquefies within 30 minutes at 37℃, although this usually occurs in a period less than this time. Some samples were induced to liquefy by mechanical mixing or the addition of a volume of culture medium followed by repeated pipetting. The sample was well mixed in the original container before microscopic examination 7.

doi.org/10.31838/hiv22.02.697 Received: 27. 06.22, Revised: 16.7.22, Accepted: 14.08.22

Semen viscosity

Viscosity of the semen sample was estimated by a gentle aspiration into Pasture pipette. The sample was considered normal when the semen leaves the pipette as small drop by drop. Semen sample with abnormal viscosity can be detected when the drops form a thread more than 2 cm length. If the drops were not formed and the semen cannot easily draw up into pipette, this indicates high viscosity of semen sample WHO, 2021.

Sperm Concentration:

Sperm concentration was measured from the mean number of sperms in five high power fields (HPF) under magnification of X 40 objective lens. This number was multiplied by a factor of one million 8. The total sperm count was obtained by multiplying the sperm concentration with sample volume.

Sperm concentration (million/ml) = number of sperm in HPF×106

Total sperm count (million/ejaculate) =sperm concentration × volume.

Sperm concentration is considered normal if it equal or more than 15×106 sperm/ml according to (WHO 2010).

Sperm Motility

The prepared slide was examined for the determination of sperm motility. It was examined immediately in order to prevent the effect of heat of microscope light source on the results. The number of motile sperms in five randomly selected fields away from the cover slide edge was counted. At least one hundred spermatozoa were counted, and the number of progressively motile and immotile sperms was counted, then sperms were counted in four categories 8.

Sperm agglutination

Agglutination of spermatozoa means that motile spermatozoa stick in to each other head-to-head, tail-to-tail or in a mixed way, e.g. head to tail. The adherence either of immotile spermatozoa to each other or of motile spermatozoa agglutination and to mucous strands. Non-sperm cell or debris is considered non-specific aggregation rather than it was recorded Sissako et al., 2017.

Laser source

The laser light source used in this study, continuous type, with an average output power of 54 mW, wavelength 532 nm, and with a beam spot of 4 mm in diameter, as shown in figure (1).

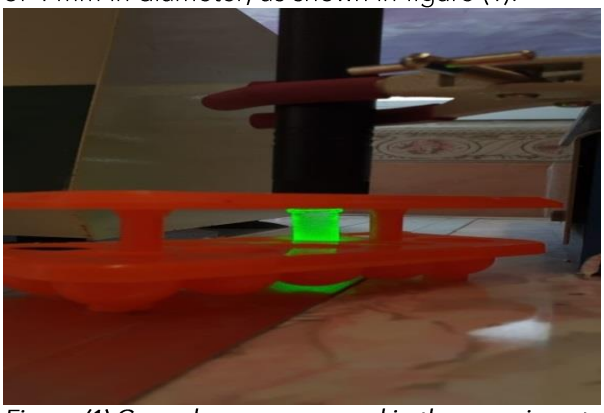

Figure (1) Green laser source used in the experiment.

First part: One that was untreated and used as a control volume of (1 mL).

Second part: That was irradiated for (10,20,30) min in the same manner produce power of (54 mW).

Since diameter of the laser spot (D) = 2 mm = 0.2 cm

Radius (r) = 0.1 cm2

Area (A) = πr2 = 3.14 × (0.1)2 = 0.0314 cm2

The power density= power W/Area cm2

Power density= 0.0.54 W / 0.0314 cm2

Power density = 1.719 W/cm2

After preparation laser (Green laser wavelength530) treated and untreated samples, samples were examined using Microscope.

3. Results

Classification of the study groups

Total number of 50 infertile couples were enrolled in this cross-sectional comparative study, 18 men were diagnosed with normozoospermia and 15 patients with asthenozoospermia, 10 men with oligozoospermia 7 men with abnormal agglutination.

Table .1 Rule of green laser exposure on certain sperm function parameters of abnormal agglutination semen.

Certain sperm parameters		Before activation	Green laser exposure (532 nm)			P value
			10 min	20 min	30min	
Sperm concentration (million/ml)		47.57 ±6.94 A	51.00 ±5.97 AB	52.57 ±4.75 AB	55.28 ±3.46 B	0.05 *
Sperm motility (%)	Progressive	29.85 ±2.08 A	36.14 ±3.53 B	39.28 ±6.83 B	39.71 ±6.65 B	0.051*
	Non-progressive	40.42 ±2.19 A	40.28 ±3.13 A	37.00 ±4.02 A	34.57 ±5.02 A	0.641 NS
	Immotile	29.71±1.75 A	26.71 ±4.03 A	22.57 ±2.44 A	25.28 ±2.77 A	0.450 NS
Morphologically normal sperm (%)		51.42 ±6.70 A	51.43 ±6.70 A	51.43 ±6.70 A	55.71 ±6.85 A	0.958 NS
Agglutination (%)		24.28 ±3.52 A	12.28 ±3.16 B	5.85 ±1.10BC	4.43 ±0.99 C	0.0001 **

Table 1 shows a significant difference in sperm concentration between before and after sperm expose to (10, 20, 30) min of wavelength (532 nm) laser. And this difference was positive which mean that there was an increase in concentration after this laser exposure, with p <0.05. The effect on sperm motility was higher when the time of exposure 30 min (p=0.051), means that an increase in laser dose cause

an increase in progressive sperm motility. The table also represent a comparison between 10,20- and 30-min laser exposure results and shows a non-significant difference with (p=0.641) for non-progressive sperm. Moreover, there was no significant difference in immotile sperm (p=0.450). The percentage of morphologically normal sperm did not change after exposure for different times of irradiation(p=0.958). A High statistical significant (p=0.0001) difference of agglutination percentage was recorded after green laser exposure, shown after10,20 and 30mins.

4. Discussion

Limited heating that may induce energy supply determines how fast and how far a spermatozoon moves. Naturally, when the sperm facing the environment for fertilization of the ovum, required capacitation and acrosome reaction processes include energy supply by increased adenosine-5'-triphosphate (ATP) generation, thus sperm only activate and become motile after ejaculation 13. Therefore, laser exposure will increase the ATP production, leading to an increase in the Ca+2 influx and, in turn, the motility of sperm 14. It is well known that photon energy can be absorbed at several cellular levels and that the cell organelle with the greatest concentration of chromophores is the mitochondrial membrane, specifically, at the cytochromes of the electronic transport chain. It has been pointed out that the cytochrome is the one that particularly absorbs photons of the laser light wavelength (532nm). It seems that absorption at the end of the breathing chain eases the synthesis of ATP 15. It has been proven that ATP plays an important role in the response of cells to light and increasing energy bioavailability (Marín et al.,2003). This proves that a cell has different ways to absorb and transform photon energy into useful energy for the cell.

The effect of the specific light wavelength used in the present study was designed to increase electron transport chain activity, resulting in an increase in ATP production this leads to increasing in sperm motility (activation).

The current study reported abnormal percentage of sperm agglutination which means that motile spermatozoa stick to each other in the ejaculate. It can be due to infection or antibodies that react with sperm 11.

True or specific agglutination of spermatozoa means that motile spermatozoa stick to each other head-to-head, tail-to-tail or in a mixed way, e.g., head to tail. Adherence of immotile spermatozoa to immotile spermatozoa or motile spermatozoa to mucous strands non-sperm cells or debris are considered non-specific aggregation rather than being recorded Sissako et al., 2017. Sperm agglutination has a negative impact on fertility due to impedance of sperm function parameters, inhibition of fertilization and implantation 17. Antisperm antibodies (ASA) cause sperm agglutination and affect sperm motility, viability and sperm migration in the female reproductive tract. As a result of our study, agglutination decreased significantly, as shown in table (1). Resulting in an increase in the concentration (number of sperms), which means more chances for fertility. The turn of the laser in this process was to decrease the antibodies.

The absorption of green laser was high because of the high penetration depth of it, this leads to decrease the loss of scattering of laser beam and leads to decreased the energy deposit in the sperm. This process of activation lead to activate ATPs secretion by the mitochondria, thus the study concluded that green laser effects enhance the results of certain sperm parameters.

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