ISSN 2664-8075 (Print) & ISSN 2706-5774 (Online)
South Asian Research Journal of Applied Medical Sciences
Abbreviated Key Title: South Asian Res J App Med Sci

| Volume-7 | Issue-4 | Jul-Aug- 2025 | **DOI: https://doi.org/10.36346/sarjams.2025.v07i04.014**

Original Research Article

Effect of Green Laser on Human Sperm Asthenospermia

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Article History
Received: 02.06.2025
Accepted: 04.08.2025
Published: 13.08.2025

Abstract: Infertility being a challenge that many scholars and other individuals in reproductive field are trying to counter, many designs are usually applied in addressing it. And one of the most important is the laser in the 1980s and 1990s, lasers were employed to treat infertility. Laser irradiation was thought to enhance human sperm motility or make them slender and faster. The received semen was examined physically and morphologically immediately after rapid sample processing. The sperms were subjected to a laser having wavelength 532 nm and output 54 mW. The size of the ejaculate was 5.0 mL and above and the concentration of semen samples used was 20 x 10 3/mL and above. The acquired information shows that primary photo-acceptors are connected with respiratory chains and oxygen metabolism. It is important to consider that the standard procedure was less efficient than the activation using the diode laser because this laser light could not trigger the production of ATP on a comparable level and this had an optimizing effect on sperm motility. Therefore, the laser light was better at energising the sperm when compared to the other method. A comparison with other studies was done. We wanted to study infertility by enhancing sperm motility and to assess the possible advantages of the use of laser in making infertile people able to conceive.

Keywords: Green Laser, Human, Sperm Asthenospermia.

INTRODUCTION

Laser is a term used to refer to an optical amplifier based on the stimulated emission and hence gives light. The phrase, Light Amplification by Stimulated Emission Radiation, was used to coin the term laser (Al-Marayaty, *et al.,* 2017). The energy of the photon that is emitted (DeltaE) must be nothing but the difference between the two states. It is true based on Haken (1985). The National Institutes of Health (2017) has various listed medical applications of lasers, namely, laser surgery, photo-biomodulation therapy, ophthalmoscopy, kidney stones treatment, and cellulite, striae reduction, and hair removal cosmetic skin therapy. According to Fauzi *et al.,* (2018), the mechanism of the interaction of the photons released by the laser with cells and the subsequent excitation of the latter or the improvement of biochemical changes is associated with low-level laser treatment. Infertility is described as the inability to bear or conceive a clinical pregnancy despite efforts being made to indulge in all the medical requirements or even involve in sexual intercourse at least twice or thrice in a week with or without protection. (Schönefeld, 2021). Some of the causes of infertility in men are that their sperm is too slow to reach an egg. Consequently, it is important that sperm be able to move so that it can fertilise (Curi *et al.,* 2003). It is not surprising but known how sperm can move or exhibit motility movement; sperm that may be moving slowly or not at all, or in an inaccurate direction or a circular motion will never be able to penetrate up through the hard outer shell of the oocyte or get through the cervical mucus. The abnormalities of the ultrastructure of the axoneme, defective mitochondria, an imbalanced fibrous sheath, a partial absence of the dynein arms, thickened outer fibre with drooping tails, autoimmunity of sperm and other spermopathies may result in severe asthenozoospermia. What causes additional problems of motility at moderate level is unknown. Buckett and Sierra conducted a study in 2019. Low-level laser therapeutically is

referred to as laser biostimulating, and it is believed that low-level laser therapy induces a change in mitochondrial functioning and increases ATP production. Perhaps, it can lead to asthenozoospermic samples having numerous motile sperm since the light exposure and therefore asthenozoospermic samples may have more motile sperm by triggering the light-to-dark transition resetting (Mohammed *et al.,* 2021).

MATERIALS AND METHODS

In this research, the authors utilized 50 infertile males who were admitted to diagnose infertility in the High Institute between December 2021 and April 2022 and tested in the assisted reproductive technologies laboratory. They had their mean ages between 20 to 40 years old with a mean of 25 + 5 years.

Sperm Fluid Analysis

All the samples were collected after a three-to-five-day abstinence, in a room near the laboratory, by masturbating in a wide mouth sterile box. We conducted testing of the sperm samples according to the guidelines presented in the manuals of the World Health Organization in 2010 and 2021. Part of the samples was treated as a control and the other part was exposed to an experimental laser light of 532 nm wavelength at 10-, 20-, and 30-minutes pulsed intervals.

Liquefaction Period

The normal duration of a semen sample to become liquid at 37C is not more than 30 minutes but on average it happens in less time. Some were dissolved by either mechanically mixing them or by adding some volume of culture media into them and pipetting repeatedly. Before using a microscope, the sample was thoroughly diluted into its initial container (Serra *et al.,* 2018). Through a soft aspiration together with a Pasture pipette, we could find the viscosity of the semen sample. The slowly dripping out of the pipette semen was considered a typical sample. Droplets of semen may form threads longer than 2 cm and this is also a sign of an abnormally thick specimen. The World Health Organisation (2021) shows that the presence of the high viscosity semen sample corresponds to the situation where the drops are not formed, and the semen is hard to pipette. An average number of five high power fields (HPF) viewed using an X 40 objective lens was used to calculate a concentration of sperm. This figure was multiplied one million times over (Al-Dujaily, 2006). we added sperm concentration and sample volume, and thus the overall sperm count was defined. Sperm concentration can be calculated as the sperms in the HPF x 106 in millilitres. Sperm concentration and sperm volume multiplied gives the formula of the total number of sperm, written in millions per ejaculate. According to the World Health Organisation (2010) concentrations of sperm exceeding or equaling 15x106perml are considered normal.

Sperm Fertility

In order to identify the motility of the sperm, a look at the prepared slide was viewed. The heat of the microscope light source did not affect the results since it was studied immediately. The number of viable sperm in five randomly selected fields outside of the edge of the cover slide was counted. It involved at least 100 spermatozoa being counted, spermatozoa being categorized into those with and without motility and finally classification of sperm into four groups (Magdanz *et al.,* 2019; WHO, 2021).

The Agglutination of Sperm

Agglutination of spermatozoa is when this motile sperm clumps together in many different ways such as head-to-head, tail-to-tail, or a mix of these two. The adhesion occurs as the mucous strands that stick to immotile spermatozoa or the spermatozoa sticking to one another. Sissako *et al.,* (2017) believe that any other aggregation that does not include sperm cells or detritus is called non-specific aggregation.

Laser Focusing Concentrator of the Light

The details of the continuous-type laser light source that was used in this study were wavelength of 532 nm, beam spot width of 4 mm and average output power of 54 mW (1).

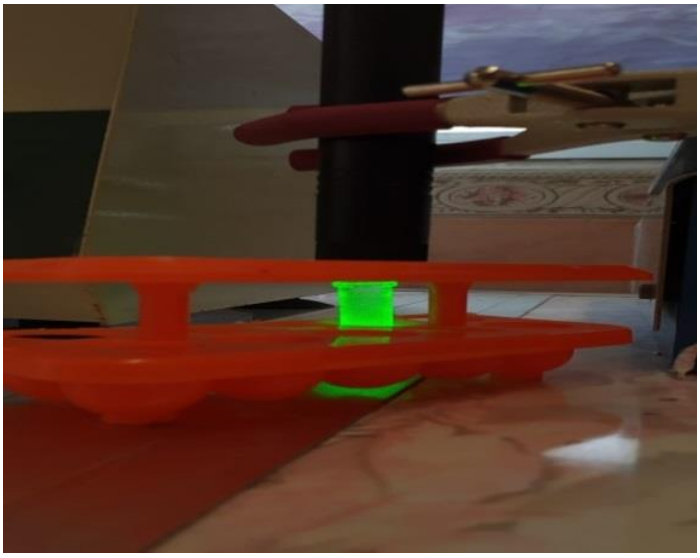

Figure 1: Green laser source used in the experiment

First part: Not treated, 1 mL in 1 volume used as the control.
Second part: In a similar way, it produced 54 mW of electricity subjected to radiation of 10, 20 and 30 minutes.
Exposure time (t) = 30 min = 1800 s
Power (P) = 0.85 mW = 0.85×10^{-3} W
Energy = Pt = $0.85 \times 10^{-3} \times 1800 = 1.53$ J
Energy per unit area = 1.53/0.125 = 12 J/cm$^{2.}$

We used a microscope to look at both the treated and untreated samples after we prepared them with a green laser with a wavelength of 530 nm.

RESULTS

Classification of the group of study

In this cross-sectional comparative study done on 50 infertile couples, 18 males showed normozoospermia, 15 had asthenozoospermia and 10 had oligozoospermia. 7 males with haemolytic anaemia.

Table 1: Impact of green laser exposure on certain sperm function parameters of Asthenozoospermic men

Certain Sperm parameters		Before activation	Green laser exposure (532 nm)			P value
			10min	20min	30min	
Sperm concentration (million/ml)		34.20 ±4.16 A	29.00 ±2.23 A	29.13 ±2.74 A	26.47 ±1.99 A	0.304 NS
Sperm motility (%)	Progressive	23.47 ±1.36 A	31.00 ±2.88 B	30.66 ±3.15 B	32.78 ±2.40 B	0. 0356 S
	Non-progressive	43.53 ±1.76 A	40.67 ±2.49 A	41.86 ±2.53 A	38.93 ±2.63 A	0.527 NS
	Immotile	33.00 ±2.77 A	28.33 ±3.52 A	27.66 ±3.79 A	29.29 ±3.88 A	0.870 NS
Morphologically normal sperm (%)		45.66 ±4.72 A	46.34 ±4.72 A	47.36 ±4.72 A	49.68 ±4.72 A	1.00 NS

Table (1): There was no significant difference (p=0.304) between the mean sperm concentration (million/ml) before and after 10, 20 and 30 minutes of laser treatment relative to the non-exposed group. The sperm motility, however, which had increased gradually, showed a markedly elevated level following the exposure (p=0.0356) as compared to the earlier levels. However, all non statistically significant improvements were found in pre-laser exposure values of non progressive motility (p=0.527), intact sperm (p=0.870) or percentage of morphologically normal sperm cells (p=1.00).

DISCUSSION

The speed of movement and the distance covered by a spermatozoon depend on its partial heating, which is able to trigger the process of energy supply. Only upon ejaculation do sperm become mobile due to the provision of energy by the more adenosine-5' - triphosphate (ATP) generation that is needed to accomplish the capacitation and acrosome reaction processes that the sperm then experience in preparation to access ovum in fertilization. In this way, Abdel-Salam *et al.,* (2011) state that the greater ATP synthesis, the more Ca+2 inflow promotes sperm motility. It is through established literature that photon energy can be absorbed at different levels in the cells, and the concentration of chromophores of any cell organelle is highest in the mitochondrial membrane, more so, at the cytochromes of the electron transport chain. It is

observed that actually it is the extra-specific protein (cytochrome) which absorbs the light at the wavelengths of 532 nm light. Iaffaldano *et al.,* (2010) writing that ATP production becomes easier due to absorption at the end of the respiratory chain. To the research of Marin and colleagues (2003), ATP plays a vital role in the cells to respond to light and their ability to enhance the availability of energy. Consequently it is now understood that cells are able to transform light into energy in very many ways.

A given light wavelength has been selected in this work in order to promote sperm motility (activation) by increasing the production of ATP, which then raises the activity of the electron transport chain. Also there was an abnormally great amount of sperm agglutination in this study, which shows that the ejaculate has adherent motile spermatozoa. Two probable reasons are antibodies that react with infections or sperm (Al-Dujaily *et al.,* 2012). Agglutination of sperm be it true or specific is the union of motile sperm by hooking, binding and interweaving of sperm in any way, and this interaction may or may not disrupt the integrity of the sperm. The interference between immotile and motile spermatozoa as also the motile spermatozoa and mucous strands. The existence of cell or detritus not sperm is not noted as this is considered as non-specific aggregation (Sissako *et al.,* 2017). The agglutination of the sperm lowers the fertility since it does not allow the parameters of the sperm activity that then destroys the fertilization and implantation (Salman, 2009). Albarazanzi *et al.,* (2005) postulate that ASAs decrease sperm motility, viability and migration within the female reproductive tract causing sperm to agglutinate.

The results of our experiment reveal a significant decline in agglutination which is depicted in table (1). thereby raising the chances of fertilization due to an increase in the concentration (the number of sperms). In this case, the laser was involved to decrease the level of antibodies (Alkaisy, 2007). Green lasers are the best of all because they penetrate deeply resulting into a high level of absorption having less energy lost in dispersion and less energy stored in sperm. The application of green laser has been found to enhance the performance of certain sperm parameters due to the subsequent activation of the ATP production made by the mitochondria as found by the researchers.

CONCLUSION

Investigators identified that green laser (532 nm) influences sperm parameters of asthenozoospermic men. Although there was no visible impact on sperm concentration after the use of the green laser, the findings showed that progressive sperm motility significantly improved (20 and 30 minutes after exposure). The proportion of sperm motility normal based on the morphology, the number of immotile sperm, and the proportion of sperm with non-progressive motility were, however, not altered significantly. Further studies are required to fully comprehend the underlying processes and benefits of green laser treatment in the long-term, but our findings indicate that potentially those males with asthenozoospermia would obtain some advantage in their sperm motility, especially progressive motility.

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