

## Immediate and Late Effect of in Vitro Low Level Helium-Neon Laser Irradiation on Human Sperm Activity

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### ABSTRACT:

#### BACKGROUND:

Sperm motility is essential in fertilization because non active or mal active sperms never reach the ovum, but the progress in the assisted reproductive techniques makes it easy for doctors to select viable sperm to be used in intracytoplasmic sperm injection thus the differentiation between viable and non-viable sperms is very important especially in sever asthenospermic samples.

#### OBJECTIVE:

To evaluate low level Helium Neon laser irradiation of the whole seminal sample as a method to activate mal or non-active sperms and to study and follow up the late effect of laser irradiation on the seminal samples.

#### MATERIALS AND METHODS:

100 samples were included in this study from patients attending seminal fluid examination lab. After digital seminal fluid examination, samples were divided in 2 parts, the first was incubated for 120min at 37C and the other was irradiated with 623.8 wave length Helium Neon laser and an output power of 2mW at 1cm distance for 10min and then examined digitally after irradiation then incubated with the first part to 120min also to be examined again with the first part.

#### RESULTS:

It was found that there is a significant increase in the progressive motile sperm percentage in the laser irradiated samples from about 33.6% to 41.6% ( $p<0.005$ ) and also decrease in the percentage of the immotile sperms from about 44.1% to 34.4% ( $p<0.001$ ). While after 120min incubation, active sperms percentage in the irradiated sample was about 43% which is higher than the non-treated part (31.6%;  $p<0.01$ ) that showed also higher percentage of immotile sperms (52.8%) against (39.9%) in the treated part.

#### CONCLUSION:

Helium Neon laser irradiation to the whole seminal sample increase the sperms activity and decrease the percentage of immotile sperms which mean that it stimulates viable but immotile sperm to move. And also, sperms in laser irradiated samples preserved their increased activity for more than 2hours after laser irradiation.

**KEY WORDS:** sperm activity, laser, immotile sperms, sperm viability.

### INTRODUCTION:

In mammals, the reproduction and regeneration is based on the function of spermatozoon which is the smallest cell in the body that has a function to be performed outside the body. The human reproductive system produces more than 100million sperm each day and the natural selection start from the population of sperms in the ejaculated semen; as only active and well shape sperm reach the ovum. Thus the sperm motility is the most important sperm character as it is the way through which sperm reach the ova during natural fertilization <sup>(1)</sup> and without assisted reproductive techniques (ART) a non-motile or

mal-motile sperm never reach the ovum and fertilize <sup>(2)</sup>. Asthenozoospermia is the state of reduced sperm motility or progressive motility or both <sup>(3)</sup>. Low sperm count or oligospermia is the common cause of male subfertility but still asthenospermia is one of the important findings in semen samples of infertile males <sup>(4)</sup>. In rare cases, asthenospermia is due to fine structural abnormalities of the sperm flagellum (tail) that is of genetic factors <sup>(5)</sup>. These 'primary' aberrations tend to be sever and homogeneous and affect all or most of the sperms in the ejaculate; clearly they are irreversible <sup>(6)</sup>. While the secondary cause is the common one; and in which defects in sperm tail and/or mitochondria are unclear. Studies suggested lower urinary tract infection, testicular injury, testicular and/or epididymis

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infection, varicocele, or the presence of anti-sperm antibodies. And these factors may induce multiple ultra-structural changes in the sperm, which are accompanied by a loss of motility and/or DNA damage. In general, these secondary or called "acquired effects" are usually heterogeneous within the sperm sample. In contrast to the genetic defects, these changes may be reversible through treatment of the underlying pathology<sup>(3)</sup>.

Asthenozoospermia might be managed by in vitro sperm activation using different types of stimulators, and then intrauterine insemination (IUI) or in vitro fertilisation (IVF)<sup>(7)</sup>. In these types of assisted reproductive techniques (ART), the progressive motility of the sperm remained an important factor. However, since the development of "intracytoplasmic sperm injection" (ICSI) which is the technique that has been used widely to overcome sperm structural and motility problems and fertilization could be successfully achieved using viable sperm with severe structural tail defects or even immotile<sup>(8)</sup>. But still (IUI) and (IVF) are widely used in infertility treatment because they are cheaper and require less equipment and could be used if the semen sample contains good sperm concentration and high percentage of sperms with progressive motility<sup>(7)</sup>.

It is known that sperm cell consists of a head that contains the genetic material as packed condensed DNA, then a short midpiece containing mitochondria that not only supply energy as ATP to the sperm but regulates the calcium flux which is found to be a key role in the initiation and quality of sperm motility<sup>(11)</sup>.

The bio-stimulation effect of low level laser (LLL) was suggested as an activator of sperms in the seminal samples with different types and dose of laser<sup>(10,11)</sup>. Although use of LLL therapy is increasing in many aspects of patients' managements, the molecular mechanisms of action are still unclear. Molecular targets which could be called "chromophores" those are mitochondrial cytochromes and porphyrins in the cytoplasm<sup>(12)</sup>. The energy of laser radiation is absorbed by chromophores and subsequent intracellular transducers that transform laser energy into a cellular signal<sup>(13)</sup>. Thus photo-signal activation will lead to a cascade of molecular effects, including an increase in nucleic acids<sup>(14)</sup> and ATP<sup>(15)</sup>, as well as gene transcription<sup>(16)</sup>. As a result, there will be increase metabolism, protein secretion, and cellular division after LLL exposure<sup>(15)</sup>. This

mode of activation could be used in increasing wound healing and tendon injury as a cellular biostimulation and/or biomodulation<sup>(17)</sup>. Another theory suggested that LLL can induce free radical generation<sup>(18)</sup> and sub-lethal DNA lesions<sup>(19)</sup>. Additionally, these lasers induce significant responses that could be regarded as DNA repair mechanisms<sup>(12,20)</sup>.

It is documented that low- power laser LLL irradiation of spermatozoa can increase their motility and many publications showed that not only sperm motility but also the velocity can be improved by He-Ne laser irradiation<sup>(10)</sup> as well as decreasing the percentage of immotile sperms by induction motility to some non-motile but viable sperms<sup>(21)</sup>.

The objectives of this are to re-evaluate the immediate effect of 10minute (632.8 Helium Neon) laser-irradiation laser irradiation to seminal fluid sample in addition to a follow up of treated sample 2 hours.

#### PATIENT AND METHODS:

Samples were collected from patients attending Al-Nadhaer lab (department of seminal fluid examination) referred from different Iraqi infertility centres in the period from 1/2/2014 to 1/8/2014. After at least 3 days of sexual abstinence, seminal sample was collected by masturbation in a private room in the same lab in special wide head jar, samples then incubated for about 30 min at 37°C to be liquefied. Samples of very small volume (less than 2ml) or with abnormal colour or long liquefaction (more than 45min) were not included in this study. The standard seminal analysis using light microscope was done first to exclude samples with severe oligospermia (less than 50000sperm/ ml) or azoospermia (no sperm at all) according to the WHO manual 1999 (22) and for more accurate results; Computer Assisted Sperm Analysis (CASA) was used (VIDEO TEST – SPERM 2.1); a digital system designed for automatic analysis of the sperm concentration, motility, and morphology. Then each sample was divided into 2 parts: first part will be treated with 632.8 wave length Helium Neon laser and an output power of 12mW being adjusted to the powers of 2mW at 1cm distance and for 10min and examined by CASA then incubated for 120min to be examined again. While the second part will be examined then incubated at 37°C for 2hours to be examined again. A total 100 sample was included in this study by CASA.



**Figure 1: Seminal sample irradiated by (He-Ne) laser.**

Motility record of sperms was divided into progressive, non-progressive motility, and immotile by the CASA.



**Figure 2: Computer assisted sperm analysis system (CASA). The blue lines represented the motility lines while the red dots showed the immotile sperms.**

Patients informed that laser treatment will be done to their samples and they could take the final results of this treatment. All samples discarded by direct flame at the end of the procedure.

#### **Statistical analysis:**

Statistical package for social science version 20 (SPSS20) was used for both data entry and data analysis. Continuous variable presented as mean  $\pm$  SD. Independent sample t test was used to test

the significance of association of variables. P-value of  $< 0.05$  was considered significant.

#### **RESULTS:**

The count and the character of sperms were not affected by time and laser treatment. In table 1, the progressive motility significantly increase after laser treatment with a significant decrease in the number of immotile sperms while a non-significant activation was observed on the non-progressive sperms.

**Table 1: The effect of 10 min laser exposure on 100 semen samples.**

	Before laser	10 min treated	P value
Progressive motility	33.6 $\pm$ 14.1	41.6 $\pm$ 15.1	0.005
Non progressive motility	21.2 $\pm$ 12.9	23.2 $\pm$ 13.2	0.088
Immotile	44.1 $\pm$ 19.2	34.4 $\pm$ 18.5	0.0001

The untreated part of the sample was incubated for 120 min and after that time the samples showed a significant decrease in sample's motility quality and a significant increase in the

number of immotile sperms although progressive motility decreased was significant and these data were shown in table 2.

**Table 2: The effects of 2 hour time after liquefaction of the non-treated samples.**

	After liquefaction	120 min later	P value
Progressive motility	33.6±14.1	31.6±13.9	0.09
Non progressive motility	21.2±12.9	15.3±11.5	0.01
Immotile	44.1±19.2	52.8±17.1	0.01

In table 3; samples that had been laser treated and incubated for 2 hours showed preservation of the overall motility state represented by a non-

significant increase in progressive sperm percentage and a non-significant increase in the number of the immotile sperms.

**Table 3: The effects of 2 hour time after liquefaction on 10 min laser treated samples.**

	After 10 min laser	120 min later	P value
Progressive motility	41.6±15.1	43.4±12.8	0.18
Non progressive motility	23.2±13.2	16.8±12.4	0.04
Immotile	34.4±18.5	39.9±19.1	0.08

In table 4; a the data of both laser treated and non-treated samples were analysed after 2 hours showing a significant differences in the

progressive motility and the immotile sperms percentages being better in the laser treated samples.

**Table 4: The data of irradiated and non-irradiated samples after 2hours.**

	Non-treated samples after 2 hours	Laser treated samples after 2 hours	P value
Progressive motility	31.6±13.9	43.4±12.8	0.01
Non progressive motility	15.3±11.5	16.8±12.4	0.92
Immotile	52.8±17.1	39.9±19.1	0.01

## DISCUSSION:

In this study, low level laser therapy to the seminal samples resulted in a significant improve in the total motility and this was in harmony previous studies<sup>(10,15, 21,23)</sup>.

Schindl and his team<sup>(24)</sup> found that some wavelengths of light in visible and near visible spectrum can induce a variety of cellular effects in some non-photosynthetic cells. While Pastore and his team<sup>(25)</sup> studied the basic mechanism of LLL and implicates cytochrome C oxidase as the primary photo-acceptor and once cytochrome C oxidase is stimulated by light, electron transport is accelerated leading to increased ATP production. At the same time, this photo-bio-stimulation is associated with the generation of reactive oxygen species (ROS) that accelerate the metabolism and then participate to provide energy<sup>(26)</sup>. These ROS are intracellular signals that even at low concentrations, they could mediate various intracellular processes and in sperm cells ROS have a pivotal role in cellular physiology and fertilization capability<sup>(27)</sup>.

In the opposite; Stadtman<sup>(28)</sup> found a significant decrease in lipid peroxidation and increase in proteins damage after He-Ne laser irradiation that might lead to a decrease in oxidative stress that might become a threat to cell survival.

The time factor is essential in sperm preparing techniques especially before ART and according to the recommendations of the WHO about seminal fluid examination; the seminal sample should be examined within 2hours of collection as the activity and the viability of the sperms decrease<sup>(22)</sup>.

The activity of sperms is known to be decreased with time<sup>(29)</sup>. In this study, in addition to the initial activation, the laser treated samples showed a sort of preservation of activity to120min and more; as the percentage of active motile sperms was about 43% while in non-treated sample it was less than 31% which was significantly higher.

Many studies showed the benefit or the curing effect of laser on different tissues. El Batanouny and coworkers<sup>(30)</sup> found that low dose of He-Ne laser cause a decrease in percentage of cells damage in addition to promote the cell cycle of lymphocyte cells. Also Taha and team<sup>(31)</sup> found that a 632.8 helium neon laser before a sub-lethal dose of ultra-violet light increase the number of survived lymphocytes. Similar findings were found by Fernandes and co-workers<sup>(32)</sup> in cryopreserved laser irradiated bovine semen

samples that showed higher activity and survival rates. The mechanism of protection by pre-irradiation of Helium Neon laser is not understood. Reactive oxygen species (ROS) was suggested, which can be a production of photosensitization of endogenous chromophores such as cell cytochromes such as flavins/riboflavins that might have a role in this light/tissue interaction<sup>(33)</sup>.

In this study and after laser, not only the percentage of progressive motile sperms increased but also the immotile sperms decreased meaning that laser dose change the motility state of the samples with immediate effects in addition to an effect that preserved and continue to more than 2hours and also 10 minutes laser dose to a semen sample cause immotile but viable sperm to move and regain its activity to a relatively long period.

These findings might give an idea about the non-thermic photo stimulation activation that occurs due to LLL therapy which is differ from the heat stimulation that results in rapid increase in the motility potential of the samples when heated to a level causing no protein denaturation but this type of activation was found to be rapid and fadeout with time and this was called "heat shock" in some reports<sup>(34)</sup> which might be due to an increase in sperm rate of metabolism or due to hostile hot environment affecting sperms<sup>(35)</sup>. The later decrease in sperms activity after heat shock might be due to the depletion of the energy stored in the cells and the seminal plasma or due to the damaging effect of heat on the sperms<sup>(36)</sup>. For fertilization to be successful, it needs viable sperm that is not necessary to be motile in ICSI. Therefore, within a sever oligospermic or asthenospermic samples, doctors had to identify viable sperms to be used in fertilization and some that are classified as immotile but still could be of use to a couple trying to conceive<sup>(37)</sup>. At present, the only way to differentiate dead spermatozoa from living, immotile ones, with preserving sperm capability to fertilize an oocyte by means of ICSI, is the "hypo-osmotic swelling test" (HOST)<sup>(38)</sup>. The HOST measures functional sperm membrane integrity, which is one characteristic of sperm vitality<sup>(39)</sup>. This procedure is complicated and difficult and rarely done in infertility labs and the new methods was suggested such as a microscopic "Nano-laser" shoot on the root of the tail of the immotile sperm that induce a flexion movement of the tail in viable sperm and has no effect in nonviable

one<sup>(40)</sup> but this procedure is difficult and need especial equipment.

Thus.

## CONCLUSION:

That in vitro laser irradiation of the seminal fluid not only activate the sperms but also elongate their activity and survival time and such method could be suggested a future technique for curing the mal active seminal samples in addition, laser might suggested as a method to test sperm viability before ART but further studies are suggested to find laser effect on the genetic material and the fertilization ability of sperms.

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