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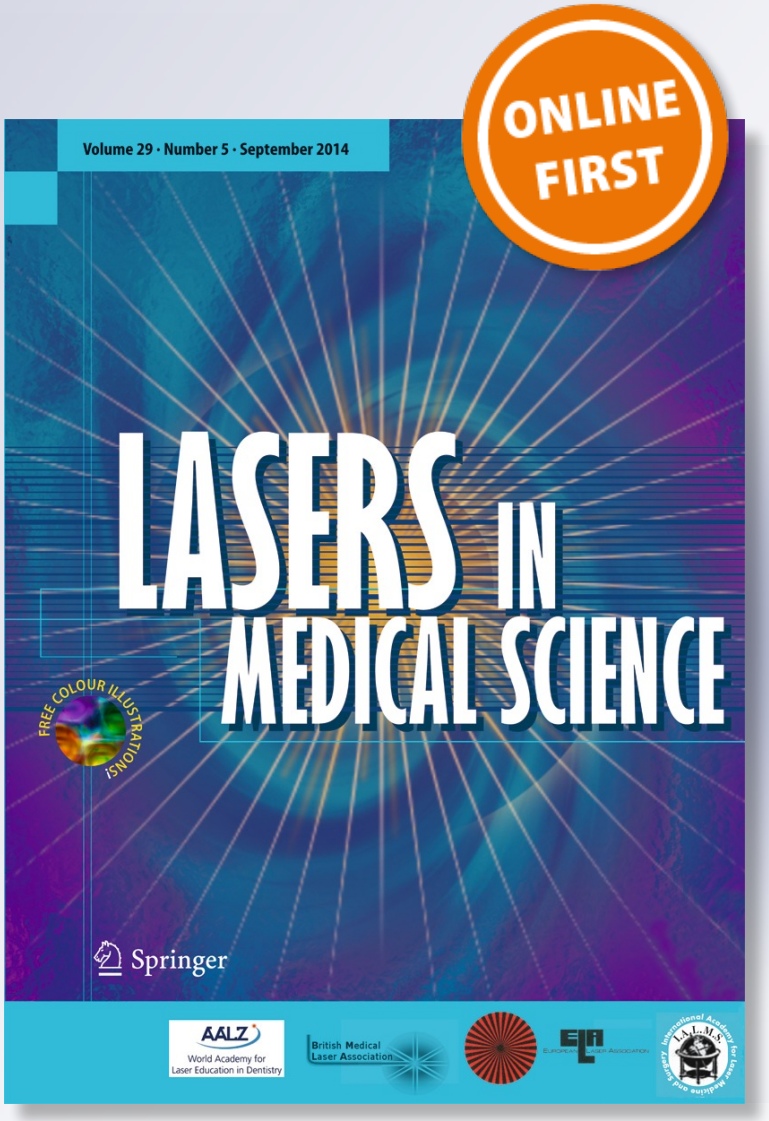
Lasers in Medical Science

ISSN 0268-8921

Lasers Med Sci
DOI 10.1007/s10103-014-1653-x



Springer

Lasers Med Sci
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ORIGINAL ARTICLE

Photobiomodulation with light-emitting diodes improves sperm motility in men with asthenozoospermia

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Received: 19 February 2014 / Accepted: 29 August 2014

Abstract Sperm motility is an important parameter of male fertility and depends on energy consumption. Photobiomodulation with light-emitting diode (LED) is known to stimulate respiratory chain in mitochondria of different mammalian cells. The aim of this research was to evaluate the effect of photobiomodulation with LED on sperm motility in infertile men with impaired sperm motility—asthenozoospermia. Thirty consecutive men with asthenozoospermia and normal sperm count who visited the infertility clinic of University Medial Centre Ljubljana between September 2011 and February 2012 were included in the study. Semen sample of each man was divided into five parts: one served as a non-treated (native) control and four parts were irradiated with LED of different wavelengths: (1) 850 nm, (2) 625, 660 and 850 nm, (3) 470 nm and (4) 625, 660 and 470 nm. The percentage of motile sperm and kinematic parameters were measured using a Sperm Class Analyser system following the WHO recommendations. In the non-treated semen samples, the average ratio of rapidly progressive sperms was 12 % and of immotile sperm 73 %. Treating with LED significantly increased the proportion of rapidly progressive sperm (mean differences were as follows: 2.83 (1.39–4.28), 3.33 (1.61–5.05), 4.50 (3.00–5.99) and 3.83 (2.31–5.36) for groups 1–4, respectively) and significantly decreased the ratio of immotile sperm (the mean differences and 95 % CI were as follows: 3.50 (1.30–5.70), 4.33 (2.15–6.51), 5.83 (3.81–7.86) and 5.50 (2.98–8.02) for groups 1–4, respectively). All differences were highly statistically significant. This finding confirmed that photobiomodulation using LED improved the sperm motility in asthenozoospermia regardless of the wavelength.

Keywords Low level light therapy · Sperm motility · Human sperm

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Introduction

Visible light in form of low level laser therapy or photobiomodulation with light-emitting diodes (LED), known also as low level light therapy (LLLT) is currently being used in different fields of medical treatment [1–3]. Previous studies have shown that the beneficial effect of LLLT is achieved by the acceleration of mitochondrial respiration and ATP synthesis [4–6]. The favourable effect of photobiomodulation is mostly visible in stressed or hypoxic cells but not in healthy cells [5–7]. The proposed mechanism was that in stressed cells nitric oxide (NO), which is produced in the mitochondria is bound to cytochrome c oxidase and thus competitively displaces oxygen and inhibits respiratory chain. LLLT probably works by photodissociating NO from its binding sites on the heme, iron and copper centres thus allowing an immediate influx of oxygen, resumption of respiration and generation of reactive oxygen species (ROS) [4–7]. Researches performed on somatic (fibroblasts, skin cells, skeletal muscle cells…) and sperm cells support the described mechanism as it has been established, that there are increased NO and ROS concentrations after LLLT [7–13]. Also, NO concentrations are

Published online: 10 September 2014

increased due to the photo-relaxation from other intracellular stores such as nitrosylated haemoglobin and nitrosylated myoglobin [4, 5, 11]. Furthermore, changes in respiratory chain alter the flow of Ca^{2+} ions between mitochondria and cytoplasm [4, 12]. Increase in NO, ROS and intracellular Ca^{2+} initiates the signalling cascade that promotes cellular proliferation and cytoprotection [4–6].

In spermatozoa, mitochondria have the key role in normal sperm function. Adenosine triphosphate (ATP) as well as intracellular Ca^{2+} and NO formation has a pivotal role in control of sperm motility, capacitation and acrosome reaction [13].

Considering the described effects of LLLT on cell function, our hypothesis was that LLLT improves the sperm motility. According to the described mechanism, the beneficial effect could be expected in cases of asthenozoospermia rather than in sperm with normal motility. Improvement in sperm motility, capacitation and acrosome reaction in asthenozoospermia could perhaps improve the pregnancy rate in intrauterine insemination or even classical in vitro fertilization. Therefore, the aim of our study was to evaluate the effect of LLLT on sperm motility in cases of asthenozoospermia.

We have searched the Pubmed database and found 18 publications reporting the effects of LLLT on sperm cells with approximately half of them being done on human sperm [12, 14–23]. The sources of light (He–Ne laser, diode laser or LED) as well as wavelength, power and exposure time used were different between the publications, and all of the parameters were seldom fully described. Since the effect of photobiomodulation on cells is known to be dose-dependent, all parameters of LLLT exposure should be in an optimal range in order to achieve the desired effect [7]. Therefore, we decided to compare four different wavelength regimes of LLLT on sperm motility in asthenozoospermia.

Materials and methods

This research was performed at Infertility clinic of the University Medical Centre Ljubljana. Thirty (30) consecutive men with asthenozoospermia and normal sperm count and volume were included between September 2011 and February 2012. Asthenozoospermia was defined as ≤25 % of rapidly progressive sperms, less than 32 % of progressive motile sperm and >40 % of immotile sperms according to the WHO criteria [24]. The research was approved by National Medical Ethics Committee, and all patients have signed informed consent prior to participation. Semen was obtained for research purposes only and was disposed after the analysis.

The semen sample of each man was liquefied (37 °C, 30 min). After liquefaction, semen sample was gently mixed using glass stick and five drops of semen sample were taken and divided onto five objective glasses and each was included in one of five different groups in terms of LLLT treatment. Group N was a control group and received no LLLT treatment (native semen). The remaining four groups received different LLLT regimes using LED (VOTAN, Ljubljana, Slovenia). To exclude the influence of visible light, each semen sample was put in one of five separated dark plastic boxes that were 10 cm high. Four boxes for LLLT had on top a hole for LED with a surface approximately 88 cm^2 which enabled illumination of the whole bottom of the box including the objective glass with semen drop with a surface approximately 4 cm^2 (Fig. 1). Sperm motility was evaluated 30 min after LED treatment.

The irradiation power density figures shown were measured at 10 cm distance from the source for all systems. In group 2 and 4, a mix of three wavelengths was used and the contributing ratio of power density of each wavelength is shown in percentages in Table 1. All sources were square wave modulated at a frequency in the kHz range, with a 50 % duty cycle. Treatment time in all groups was 3 min.

After LLLT treatment, the percentage of motile sperm and kinematic parameters were measured using a Sperm Class Analyser system following the WHO recommendations [24]: grade A sperm (rapidly progressive), grade B (slow/sluggish progressive), grade C (nonprogressive motility) and grade D (immobile). All semen samples were evaluated by two experienced technicians with more than 20 years of experience. The semen samples (treated and non-treated parts) of each man were evaluated by the same technician who did not know to which group the investigated semen samples belonged.

For statistical analysis, the paired t test and ranks were performed to evaluate the difference in sperm motility after LLLT treatment using LED in comparison with non-treated semen. Mean differences and 95 % confidence intervals (95 % CI) were calculated with two-sided probability (p) values, and p value of <0.05 was considered to be significant. Statistical analysis was performed using IBM SPSS Statistics, version 19 (IBM Corp, Armonk, NY).

Results

In non-treated (native) samples, the average ratio of rapidly progressive sperm was 11.8 % and of immobile sperm 72.5 %.

Fig. 1 Box for LLLT treatment of semen with LED. After treatment, semen samples stayed in dark until sperm motility was evaluated

Table 1 LED treatment regimes of four investigated groups

	LED wavelengths (nm)	Total power density (mW/cm^2)
Group 1	850	2.16
Group 2	625[a], 660[b], 850[c]	3.92 (23 %[a], 67 %[b], 10 %[c])
Group 3	470	5.06
Group 4	470[a], 625[b], 660[c]	8.23 (65 %[a], 10 %[b], 25 %[c])

[a, b] and [c] represent the contributing ratio of power density of corresponding wavelength

The LLLT-treatment using LED shifted sperm cells toward faster motility—therefore, increase in the average ratio of rapidly progressive sperm (grade A) and slow/sluggish progressive sperm (grade B) on the account of decreased ratio of immotile sperm (grade D) has been proved in all investigated groups of semen samples. The ratio of non-progressively motile sperm (grade C) remained the same in all groups. Clinically, most important results (ratio of rapidly progressive and immotile sperms) are presented in Table 2.

After LLLT treatment, the increase in ratio of rapidly progressive sperm was statistically significant in all investigated groups of semen samples in comparison with non-treated (native) groups of samples (Fig. 2). The improvement of sperm motility was largest in group 3 semen samples, although the differences in sperm motility improvement between the LLLT-treated groups were not statistically significant.

On the other hand, the decrease in ratio of immotile sperm was significant in all investigated groups of semen samples in

Table 2 Sperm motility of non-treated (native) semen samples and semen samples after LLLT-treatment using LED

Patient no	Native sample		Group 1		Group 2		Group 3		Group 4	
	Grade A	Grade D	Grade A	Grade D	Grade A	Grade D	Grade A	Grade D	Grade A	Grade D
1	25	50	25	50	30	50	35	40	30	45
2	5	80	5	80	10	75	10	75	15	70
3	5	85	0	95	5	85	5	85	5	85
4	20	65	30	45	25	50	30	45	25	50
5	5	85	5	85	5	85	5	80	5	80
6	10	70	15	70	10	70	10	70	10	70
7	10	75	10	75	10	75	15	70	15	70
8	20	65	25	60	25	60	20	65	20	65
9	5	80	5	80	10	75	15	75	10	75
10	5	85	5	85	5	85	5	80	5	80
11	10	75	15	70	15	70	20	65	10	75
12	10	75	10	75	10	75	15	70	15	70
13	10	75	10	75	5	80	10	75	10	75
14	5	80	10	75	10	75	10	75	5	80
15	20	65	20	65	10	75	20	65	20	65
16	5	80	10	75	10	75	10	75	10	75
17	5	80	5	80	10	75	5	85	10	75
18	5	80	5	80	10	75	10	75	5	80
19	20	55	25	50	25	50	25	50	30	50
20	20	65	30	55	30	55	25	50	25	50
21	20	65	25	55	25	50	25	50	25	55
22	20	65	25	50	25	50	25	55	25	50
23	15	70	15	70	20	65	20	65	25	40
24	20	65	25	55	25	55	25	55	20	60
25	10	75	20	65	20	65	20	65	15	70
26	5	70	10	70	15	70	20	65	20	65
27	20	60	20	55	25	50	25	50	20	65
28	5	85	5	85	5	85	5	85	5	85
29	10	75	10	75	5	75	10	75	15	70
30	10	75	20	65	20	65	15	65	20	65
Mean±SD	11.8±6.8	72.5±9.1	14.7±8.8	69.0±12.8	15.2±8.5	68.2±12.0	16.3±8.3	66.7±12.4	15.7±8.0	67.0±12.1

Grade A—ratio of rapidly progressive sperm (%), grade D—ratio of immotile sperm (%)

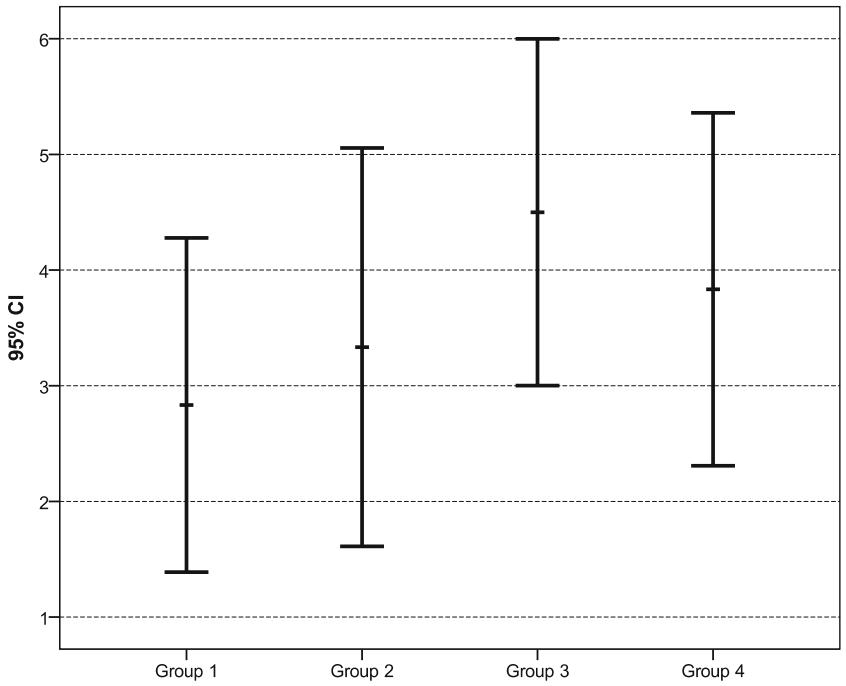

Fig. 2 Increase in proportion of rapidly progressing sperm: mean differences and 95 % CI were as follows: 2.83 (1.39–4.28), 3.33 (1.61–5.05), 4.50 (3.00–5.99), 3.83 (2.31–5.36) for groups 1–4, respectively. *P* value for all four groups compared to the native control was <0.001

comparison with non-treated (native) groups of samples (Fig. 3). The decrease in immotile sperm ratio was the largest in group 3 semen samples but the differences in immotile sperm decrease between the LLLT-treated groups of samples were not statistically significant.

These results indicate that LLLT-treatment using LED significantly improves the sperm motility regardless of the wavelength.

Discussion

The results of this research have shown that LLLT-treatment of semen samples using LED significantly improves the sperm motility (higher ratio of rapidly progressive sperm and lower ratio of immobile sperm) in infertile men with asthenozoospermia regardless of the investigated regimes of LED application.

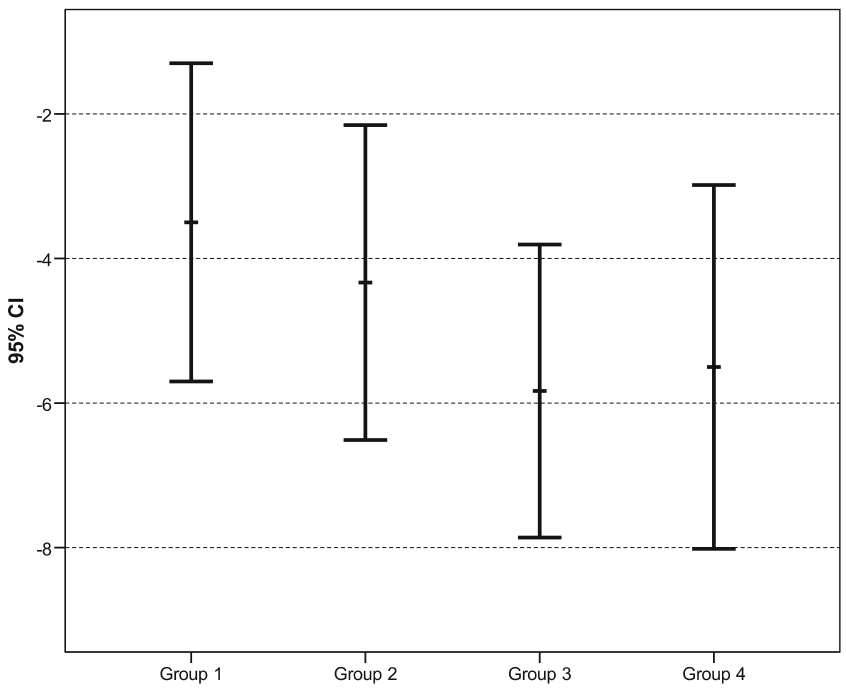

Fig. 3 Decrease in proportion of immotile sperm: the mean differences and 95 % CI were as follows: 3.50 (1.30–5.70), 4.33 (2.15–6.51), 5.83 (3.81–7.86), and 5.50 (2.98–8.02) for groups 1–4, respectively. *P* value for group 1 is 0.003 for groups 2–4 is <0.001 compared to native control group

Our results are in accordance with previous publications that also confirmed improved sperm motility after LLLT treatment [12, 14–23]. According to our knowledge, after the year 2000, there are less than ten publications considering the influence of LLLT on sperm cells and only half of them have been performed on human sperm. The authors of these publications reported that laser light stimulated the sperm motility [15, 19], as confirmed in our research. They all agree that total sperm motility is enhanced by LLLT, but the reports about the improving of sperm velocity are conflicting [15, 16].

The mature human sperm cell is composed of head, which contains the nucleus (genetic material), neck (centriole), midpiece with several mitochondria and tail. Mitochondria are the cell organelles that produce the energy needed for sperm tail motility. In asthenozoospermia, the sperm motility is impaired, due to mitochondrial malfunction or structural defects of the tail. The low-level laser therapy acts on different cells and tissues by improving of mitochondrial function; therefore, the improved sperm motility may be expected in asthenozoospermia.

Sperm motility evaluation is one of the best and easiest tests to evaluate the influence of LLLT on sperm cells. Some previous reports showed that beneficial influence goes beyond the enhanced motility [22]. Good sperm motility is necessary, but not enough for natural conception that is achieved after sperm capacitation and acrosome reaction. The process of sperm capacitation starts after ejaculation. During this process, sperm intracellular Ca^{2+} starts to rise, reactive oxygen species (ROS) generation is initiated, cAMP concentration increases and sperm develops a highly vigorous form of motility known as hyperactivation that enables it to reach the oocyte [25]. After hyperactivation, a functional acrosome reaction is needed for sperm to fertilize the oocyte [25]. The increase in intracellular Ca^{2+} plays a pivotal role during acrosome reaction [26, 27]. The previous findings showed the increased Ca^{2+} and hyperactivation after LLLT in human sperm; therefore, the improved acrosome reaction could not be excluded [12, 22]. The improved acrosome reaction was confirmed in bull sperm treated with low level He-Ne laser irradiation [14].

All of the studies mentioned support the finding that LLLT improves the mammalian sperm motility, and to the best of our knowledge, none of them proves the opposite. Despite favourable results, the LLLT treatment of sperm has never been tested in clinical practice. It is questionable, if such LLLT-induced improvement of sperm motility would indeed increase the pregnancy rates achieved by intrauterine insemination. Our results confirm the statistically significant increase in the ratio of rapidly progressive sperm motility from 12 to 16 %, but this is too low increase to predict the improved clinical outcome. Perhaps, more benefits could be expected from LLLT treatment of frozen and thawed semen (e.g., semen cryopreservation in cancer patients before chemo- and radiotherapy for fertility preservation); it is known that the sperm motility (viability) is decreased after freezing and thawing, and reports describing turkey and bull semen suggest that LLLT enables better motility of frozen-thawed sperm [14, 23]. Moreover, the method of LLLT treatment could be beneficial to select the vital sperms from ejaculate or testis from patients with immotile sperm.

Of course there are some ethical concerns, which arise and need to be resolved before the use of LLLT of sperm in processes where new life is created. However, the lasers are not a new item in assisted reproductive technologies [28]. The laser-assisted hatching of human embryos to improve the implantation was first reported in 1991 and was accepted with some concern about possible harmful mutagenic side effects [29]. The reports on children born after this technique were quite encouraging and did not show any harmful effects [30]. Also, the sperm has already been a target of laser application where the spermatozoa were trapped using continuous laser microbeams as laser tweezers [31]. Laser tweezers usually work in the ultraviolet or near infrared range; therefore, they may result in irreversible cell damage so the wavelengths above 800 nm were suggested [32]. Possible applications of 1,480-nm diode laser in assisted reproduction have already been presented and suggest the sperm immobilization before ICSI; the first clinical outcomes confirmed that the use of this methodology is safe [31]. Moreover, the laser system is routinely used for human embryo biopsy in the preimplantation genetic diagnosis (PGD) programme [33].

The energies used to enhance the sperm motility in our study were much lower than those used for assisted hatching and sperm trapping, the procedures mentioned above. In photobiomodulation with LLLT, (LED) the main goal is to stimulate the function of light-sensitive enzyme cytochrome c oxidase. The energies used were below the threshold for ionization. As mentioned, there are some processes in assisted reproductive technology where such stimulation of sperm mitochondria and motility could be beneficial, like treatment of sperm before intrauterine insemination (e.g., with asthenozoospermia), after thawing of frozen sperm (e.g., in sperm cryopreserved before cancer treatment) or to select the vital ejaculated or testicular spermatozoa in patients with immotile sperm before microinjection into the oocyte. Before introducing the method into clinical practice, further studies considering the safety of this procedure, especially at the sperm nuclear, genetic and epigenetic levels, need to be conducted. To the best of our knowledge, the only published study on this subject did not show any negative impact of LLLT on sperm DNA fragmentation [19]. In clinical practice, different chemical substances are used to improve the motility of sperm before use in in vitro fertilization such as pentoxifylline that might be more harmful for sperm cells than LED treatment [34].

Conclusion

Photobiomodulation with low-level light therapy shows to be effective in enhancing the sperm motility in infertile men with asthenozoospermia. The first results are promising. Considering the safety of this new methodology, further research is needed for verification in order to avoid any possible chromatin and DNA damage in human sperm. If safe, this methodology could be used in the assisted reproduction programmes to treat or select the human sperm and to increase the chance to achieve pregnancy in infertile couples.

Acknowledgments The authors thank all patients for their participation, laboratory technicians in the Andrology Laboratory for their additional work that enabled this research, Votan who donated LED for the purpose of this study and Erik Margan for sharing his knowledge of experimental physics with us.

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