

Light-emitting diode exposure enhances sperm motility in men with and without asthenospermia: Preliminary results

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Summary *Objective: To evaluate the effect of light-emitting diode (LED) on sperm motility in men with and without asthenospermia.*

Material and Methods: Semen samples from 27 men were assessed and washed. An aliquot was taken from each sample as a control. The remaining amount was exposed to red LED for 2, 5 and 10 minutes. Sperm motility from the test and control tubes were re-checked at the end of each time interval. In 11 of these 27 samples, the same protocol was repeated without sperm washing. Evaluation of sperm creatine kinase (CK) activity, hypoosmotic swelling (HOS) test and aniline blue staining (ANBS) were undertaken after phototherapy in additional 15 samples.

Results: Progressive sperm motility increased significantly after LED treatment at the different time intervals whether in washed ($p = 0.000$) or non-washed ($p = 0.003$) samples. The amount of the increase in motility in washed aliquots was significantly more ($p = 0.000$) than in naive semen.

Sperm CK activity increased, but was not significant whilst there were no changes regarding HOS and ANBS.

Conclusion: Red LED is a promising safe tool to boost sperm motility in vitro. This may have a great implication on maximizing the possibilities and outcomes of intrauterine insemination trials.

KEY WORDS: Sperm; Motility; Light-emitting diode.

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INTRODUCTION

Recently, there has been increasing public concern over the likelihood of reduction in human fertility and semen quality (1). Extensive epidemiological studies reported a clinically relevant range of current infertility rates between 5-15% (2). Male factor infertility represents about 40-50% of etiologies of human infertility (3). Semen quality is considered as a good marker of success to achieve pregnancy (4).

Sperm motility is one important parameter which can define sperm quality. It is an important prerequisite for fertilization. A good sperm motility ensures effective swimming in the female genital tract until reaching the sperm's final destination which is the ovum where fertil-

ization can occur. Asthenospermia or abnormal motility is, therefore, a common etiology of infertility in the male. It may be presented as an isolated anomaly or in combination with other sperm parameter defects in 19% and 63% of patients, respectively (5).

Treatment of asthenospermia has in the past involved an in-vivo approach subjecting the patient to either medical treatment (6, 7), varicocelelectomy (8) or both. With the recent advent of assisted reproductive techniques (ART), enhancement of sperm motility has progressed by other means. The enhancement is done as an in-vitro process (9) using some enrichment media with or without motility-improving agents like platelet activating factor (10). Improvement in sperm motility is essential for a good outcome of some of these ARTs like intrauterine insemination (IUI) (11).

To have a good ranked motility, the sperm has to have a well-developed and functional tail which is the basic tool for sperm motility. In its proximal part, the tail is marked out by the mitochondrial sheath. Mitochondria have the highest amount of chromophores within the cell, particularly the cytochrome of electron transport chain (12). Mitochondria are able to absorb the photon energy due to this rich chromophores content (13). The absorbed photon energy can be utilized in the adenosine triphosphate (ATP) production necessary for the cellular physiological processes like sperm motility (13).

Photodynamic treatment is the application of light to stimulate photosensitizers which can be present as cellular components or introduced as exogenous matter. Light-emitting diode (LED), which is a complex semiconductor, has recently been introduced as a new option in light therapy. It emits non-coherent light with narrow spectrum using the electric current (14). Several studies indicated its efficiency in treating numerous medical disorders. Examples include wound healing (15), pain soothing (16) and allergic and inflammatory skin disorders (17). This impressive effect of the LED was attributed to its photobiomodulating effect. However, no studies so far have investigated the LED effect on human sperm.

The objective of the current study was, therefore, to study any possible effect of the LED on the human sperm motility in men with and without asthenospermia using several time points of light exposure.

No conflict of interest declared.

MATERIAL AND METHODS

Semen samples

Twenty-seven consecutive patients gave semen samples, between January and March 2014, during evaluation of their fertility status at the *Andrology clinic, Department of Urology, Alexandria Faculty of Medicine*. Exclusion criteria included men with a history of azoospermia, intake of recreational drugs, use of medications which may adversely affect the testis or any systemic disease as diabetes. Each patient collected his semen into a sterile plastic container by masturbation without any lubricants after 3-5 days of sexual abstinence. Semen stayed at the incubator (37° C) for 30 minutes to allow liquefaction. A computer-aided sperm analysis (CASA) automatic system (*Sperm Class Analyser SCA GII, Barcelona, Spain*) checked immediately semen parameters based on WHO criteria, version V (18). The check-up of semen included evaluation of semen volume, sperm count/ml, percentage motility in its different grades (PR: progressive, NPR: non-progressive, IM: immotile) and percentage normal sperm forms. Each patient gave consent to use his semen sample for research purposes. The Departmental Review Board gave approval for the protocol of the study.

Sperm preparation

An aliquot (200 µL) was taken from each semen sample and underwent centrifugation (400 x g, 10 minutes) at room temperature to separate seminal plasma. The supernatant was discarded and the resulting pellet was re-suspended in 1 ml Ham's F-10 culture medium (*Irvine Scientific, Santa Ana, CA*), and the tube contents were thoroughly mixed. The sperm suspension was distributed equally between 2 glass sterile tubes (*BD bioscience, USA*), one tube represented the control (C1) and the other tube was assigned to receive the light treatment (irradiated tube, number 1 or RT1). In the lastly recruited 11 patients, an extra step was taken. An aliquot of the naïve semen (800 µL) was taken from each sample before the separation of seminal plasma. These aliquots did not undergo the previous sperm preparation. Instead, they were distributed equally between 2 tubes, one represented the control (C2) and the other tube received the light treatment (irradiated tubes, number 2 or RT2). This extra step was done to verify the real impact of light exposure on sperm motility as the washing medium, Ham's F-10, in this experiment, is well-known about its antioxidant effect and may indirectly affect sperm motility (19). In addition, we aimed to report the most promising circumstances to get the maximal effect of LED on sperm motility.

Exposure to LED and sperm motility analysis

All tubes settled in a dark room. The RT1 and RT2 were exposed to a red LED source (wavelength 636.6-nm, total output power 1.3 W) which was kept at 5-cm distance. At each time point light exposure (2, 5 and 10 minutes), an aliquot of sperm suspension inside the RT1 and C1 tubes were subjected to the assessment of sperm motility. Accordingly, the irradiation energy imposed on the sperm tubes were calculated to be 496 mJ/cm², 1.241 J/cm² & 2.482 J/cm² for the 2, 5 and 10 minutes of light exposure. The chosen time points of light expo-

sure were previously used by other researchers (20, 21). In the last 11 samples, the naïve semen tubes (RT2 and C2) were treated in the same way. CASA involved checking the tracks of at least 200 motile sperms each tube.

Supplementary studies

A. Determination of sperm creatine kinase (CK) activity

Free energy released from hydrolysis of ATP is required for sperm motility. The CK activity is regarded as an indicator of the rate of ATP synthesis in sperm (22). We hypothesized that, sperm CK activity would be altered under LED treatment. Therefore, it would be useful to check the validity of this hypothesis to explain any possible mechanism behind the changes occurring in the sperm motility after the application of the LED treatment. This step was expedited through studying the sperm CK levels after 5-minute phototherapy. We selected this time point because our early observations identified that LED-enhancing effect on sperm motility was maximal at that time point and any changes in sperm CK would be augmented and detected at ease.

Semen samples from another 7 consecutive patients were collected at the end of the current study. The samples were treated without washing as previously described. Reagents were purchased from Sigma Aldrich. CK concentrations were assayed as previously described (23). In brief, an aliquot of naïve semen (100 µL) was taken from the tubes (RT 2 and C2) at the time point 5 min of the experimental procedure. The aliquots (100 µL) were washed with cold imidazole buffer (0.03 M imidazole and 0.15 M Na Cl, pH 7.0) at a volume/volume of 1: 15. The tubes were centrifuged at 500 x g for 10 minutes. The supernatant per each tube was removed. The remaining pellet was resuspended in a 0.1% Triton X-100 solution with the help of strong vortexing for 20 seconds. The tube was centrifuged again for 10 minutes at 500 x g. The supernatant was retrieved and checked for CK activity by spectrophotometry using a CK kit. The CK activity was expressed in international units/10⁶ sperms.

B. Examination of sperm membrane integrity

To check for the safety of the sperm-LED treatment, the sperm membrane integrity was checked by the hypoosmotic swelling (HOS) test (24). The same 7 semen samples described above were used. After the maximum duration of LED treatment used in the present study (10 minutes), 100 µL from RT2 was mixed with 1.0 ml of the hypoosmotic buffer (1.35 g of fructose and 0.73 g of sodium citrate.2 H₂O in 100 ml of distilled water). The mixture was incubated in a 5% CO₂ incubator, at 37° C for 2 hours. The control samples (C2) were also included. The sperms were observed under 400 x magnification. Two hundred spermatozoa were analyzed in each sample. Percentage of sperms having curled tails was calculated.

C. Evaluation of sperm head chromatin condensation

This was done for further confirmation of the safety of our treatment after the maximal duration of light treatment (10 minutes). It was accomplished using aniline blue staining (ANBS) method (25). Eight new and consecutive semen samples were pooled during August 2014. They were treated without washing with LED for

10 min, as previously described. Smears were taken from these samples, air-dried and fixed in 3% glutaraldehyde in phosphate-buffered saline for 30 min. Then, they were stained in 5% aqueous aniline blue solution (pH 3.5) for 5 min. The controls were included. If the sperm head does not hold condensed chromatin, it stains blue, but if not it does not pick up the stain. The percentage of stained sperms were calculated by assessing 200 sperms, and 25% was taken as the cutoff point (25).

2.5. Statistical analysis

The study data were expressed as mean ± s.d. The data were examined using SPSS statistical software for Windows Release 16 (SPSS Inc., Chicago, USA). The impact of LED phototherapy on sperm motility, CK activity, ANBS and membrane integrity was studied using the Wilcoxon-singed ranked test. The effect of the duration of the LED exposure on the sperm motility was studied using one-way ANOVA with Tukey post-hoc test. We also arranged the semen samples into 2 groups. The first group included samples with isolated asthenospermia while the second group consisted of those with normal semen parameters. The size of the increase in percentage sperm PR motility was calculated as the difference between initial motility once the samples were received and that percentage at the end of each time point of the study. A comparison between the size of the increase in this motility at the different time points for asthenospermia and normal semen groups was made by the Mann-Whitney test. The same test was also used to compare between percentage normal sperm forms of the 2 groups. P-value < 0.05 was considered significant.

RESULTS

Semen parameters

All the initial 27 patients had normal sperm count (42.4 x 10⁶/mL ± 13.98). Sperm motility was normal in 9 (33%) but low in 18 (67%) patients. The normal sperm forms in either group had normal percentage (≥ 4%) but significant difference could be seen between both (16.6% ± 2.2 for the normal motility group vs 12.5% ± 2.8 for the asthenospermic group, p = 0.002).

Effect of LED exposure on sperm motility in washed samples

The LED irradiation induced significant increase in sperm motility at the different (2, 5 and 10 minutes) time points of the study, as demonstrated by the increase in PR sperm category (Figure 1). This increase went parallel with a significant decrease in IM sperm category (Table 1). The PR motility showed a rising trend at the 5-minute time point compared with that of the 2-minute point. However, the difference between both time points was not significant (p = 0.39). Then, this rising trend started to decline to be significantly lower at the 10-minute point compared with that of the 5-minute point (p = 0.000). This behavior of the LED-associated

Table 1.

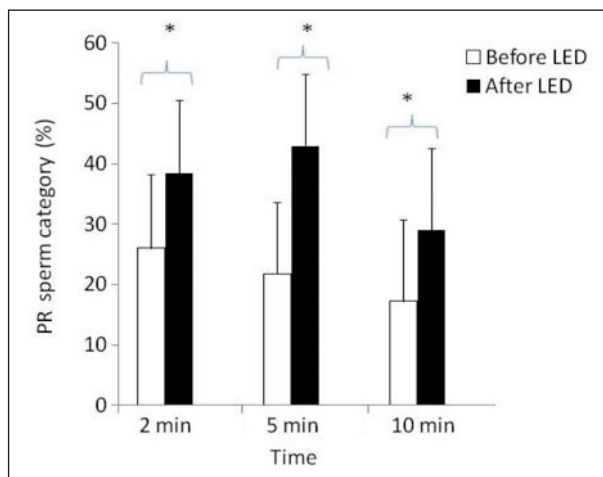
The changes in percentage (mean ± SD) NPR and IM washed sperms at the different time points (n = 27).*

Study time point	Before LED therapy	After LED therapy	p-value*
2 minutes	74.1 ± 9.4	62.2 ± 12.8	0.000
5 minutes	78.1 ± 9.5	56.8 ± 12.01	0.000
10 minutes	81.1 ± 10.99	71.5 ± 12.4	0.000

* Wilcoxon-singed ranked test.

Figure 1.

*PR motile sperms (washed samples) before and after the LED phototherapy at the different time points of the study. *p-value = 0.000 (Wilcoxon-singed ranked test)*



PR motility was in contrast to the corresponding pattern in absence of LED treatment. In the latter case, the PR motility progressively declined with time (Figure 1).

Response to LED exposure in relation to the presence/absence of asthenospermia

Initially, the 2 sample groups (asthenospermic and normal) in the present study had significant differences in between in relation to the PR and IM sperm motility (Table 2). After light application, enhancement of sperm motility was found in both types. However, the size of this enhancement for PR motility was significantly higher in the normal semen samples than that in the asthenospermic samples at the 2-minute point only (Figure 2).

Non-washed sperm samples

These samples showed the same behavior as the washed ones on the LED irradiation. There were significant increases in PR motility at the different time points (Table 3). The increase in PR motility reached also its peak after the 5-

Table 2.

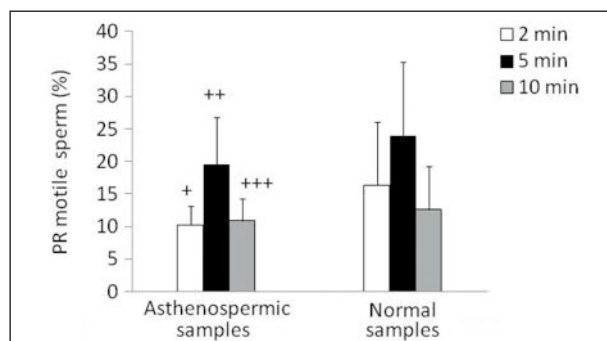
Initial motility (%) in the 2 groups of the study semen samples (n = 27).

Motility grade	Asthenospermia group	Normal semen group	p-value*
PR	23.4 ± 5.03	40.9 ± 5.7	0.000
NPR	21 ± 6.5	22.8 ± 9.4	0.68
IM	55.6 ± 9.1	36.3 ± 9.5	0.000

* Mann-Whitney test.

Figure 2.

The size of the increase in PR sperm motility (washed samples) after the LED application for the different time points in the 2 study groups (asthenospermia & normal). *p*-value: +0.035, ++0.135, +++0.179 (Mann-Whitney test was done between each bar in the asthenospermia type & its correspondent bar in the normal type).



minute light exposure, although this rise was not significantly higher than the rise happening after 2 minutes ($p = 0.85$). Then, the PR motility dropped significantly ($p = 0.01$) after that on arriving at the 10-minute point. These enhancements in PR motility were also associated with a significant drop in IM sperms (Table 3). The size of the increase in PR motility in non-washed samples were significantly lower than those of the washed samples (Table 4).

Supplementary studies

A slight increase in sperm CK activity after 5-minute LED treatment was detected as compared to that of the control

Table 3.

The changes in the motility % (mean \pm SD) of the different grades during the study at the different time points for non-washed samples ($n = 11$) *.

Study time point/Grade	Before LED therapy	After LED therapy	p-value*
2 minutes/ PR	23.6 \pm 8.7	27.7 \pm 8.9	0.003
NPR	21.2 \pm 7.2	21.2 \pm 5.8	0.52
IM	56.1 \pm 9.5	51.1 \pm 9.95	0.014
5 minutes/ PR	22.7 \pm 10.3	31.3 \pm 7.8	0.003
NPR	17.4 \pm 7	15.7 \pm 5.4	0.128
IM	59.9 \pm 10.4	52.3 \pm 7.6	0.008
10 minutes/ PR	19.5 \pm 11.3	22.9 \pm 10	0.003
NPR	19.2 \pm 8.7	17 \pm 5.9	0.065
IM	60.4 \pm 10.5	60.1 \pm 9.7	0.86

* Wilcoxon-singed ranked test.

Table 4.

The size of the increase in PR motility (%) with and without washing of the sperm samples *.

Time point	Non-washed samples	Washed samples	p-value
2 min.	4.1 \pm 1.97	12.3 \pm 6.6	0.000
5 min.	9.3 \pm 2.8	21 \pm 8.8	0.000
10 min.	4.4 \pm 1.9	11.8 \pm 4.8	0.000

* Mann-Whitney test.

samples (7.9 ± 4.7 vs 7.4 ± 4.7 unit/ 10^6 sperms, $p = 0.61$). Both the sperm membrane integrity and head chromatin condensation remained stable after the treatment for 10 minutes as the HOS test did not show notable changes ($58.4\% \pm 13.2$ (before) vs $59.2\% \pm 18.8$ (after), $p = 0.61$), and also the ANBS ($86.5\% \pm 6.3$ (before) vs $85.8\% \pm 6.7$ (after), $p = 0.5$).

DISCUSSION

The present work is an in-vitro study which investigated the effect of red LED exposure on the sperm motility in semen samples pooled from men with and without asthenospermia. The retrieved data indicated that exposure to red LED induced a significant improvement in sperm motility. To the best of our knowledge, this is the first report presenting an investigational evidence for the enhancing impact of red LED on human sperm motility. The current data come in line with the previous workers who also showed enhanced sperm motility after treatment of semen samples with low level laser (9, 20, 21). Good motility with the ability of forward progression is an essential element to ensure the sperm successful trip inside the female genital tract and achieve its final objective for fertilization. Poor sperm motility or asthenospermia is a common cause of male factor infertility. It is implicated as a possible causative factor in up to 82% of these cases (5). It is diagnosed when there is PR < 32% or total motility < 40% (18).

A cell holds several components which contain chromophores. Mitochondria may be the most important of these components containing the highest concentration of chromophores inside the cell, particularly the cytochrome of electron transport chain. Mitochondria are, therefore, able to absorb the photon energy due to its rich chromophores content (13). Photons with wavelength 655-nm are mainly absorbed by cytochrome a/a3 inside the cell (26) with production of ATP, which can be used in the physiological processes within the cell. In sperm, the mitochondria activity is closely linked to the sperm motility. This occurs through the mitochondria-generated energy (27, 28).

The red LED-enhancing effects on sperm motility, in the present study, are suggested to be related to an increase in the level of net energy available for sperm motility. This energy was agitated after absorption of the LED-associated photon energy and its interaction with the chromophores contained within sperm mitochondria. In support of this speculation, the level of the sperm CK activity, in the enrolled semen samples, showed an increase although insignificant after LED treatment for only 5 minutes. Recent studies considered the activity of sperm CK as an indicator of the rate of ATP synthesis in sperm (22). Extension of the current work to include more semen samples and treat them with more powerful LED for longer duration may change this insignificant CK level; giving a clear clue about the real

role of the enzyme during LED treatment. The LED-augmenting effect on motility can also be attributed to the change in the level of calcium inside the sperm cell after exposure to LED (29). Calcium is an essential element to trigger sperm motility (30). Future research is highly recommended to address the exact mechanism explaining the motility-enhancing red LED effect on sperm motility. Intrauterine insemination (IUI) is considered as the most cost-effective and first-line treatment of moderate cases of male infertility (31). One important prognostic marker to achieve a good result with IUI is the availability of processed total motile sperm count of > 10 million (11). The success of IUI may be questioned if this motile sperm count is < 10 million. Men who does not fulfill such sperm count will not be suitable candidates for IUI. The motile sperm count must be considered before deciding to proceed with IUI. In the present study, the significant enhancement in sperm motility after the LED phototherapy can help improving the IUI prerequisites, and unsuitable candidates for the insemination procedure may change to suitable ones. Sperm treatment with LED can be, therefore, an alternative to medical/surgical treatment of asthenospermia which still have non-encouraging results in several patients after waiting for many months (32, 33).

In this study, sperm motility decreased significantly after 10 minutes of the phototherapy. This may attract attention to the power and energy densities of the currently used LED. Both may not be enough to generate more ATP production to sustain the improved motility noticed at 5-minute exposure. The LED phototherapy has a definite role in enhancing sperm motility as is shown by the improvement noticed while applying LED into naïve semen samples. However, there was a significant difference in the size of the increase in motility grades between samples with processed sperms versus naïve semen. Two lines of evidence were present, which may contribute to this difference. First, centrifugation of semen helped discarding seminal plasma. This means: A) No semen viscosity which may hinder sperm motility. B) A significant decline in the level of sperm-associated reactive oxygen species (ROS) which have a detrimental effect on sperm motility (34). Second, the use of cell culture medium, the Ham's F10 which is a well-known potent antioxidant (19) opposing the sperm-associated ROS.

In the present study, most sperm samples (67%) had poor motility, but they showed good response to LED therapy. This may go in line with the suggestion that the effects of photodynamic therapy like that delivered by low-level laser therapy will be striking if applied to stressed cells (35). The current data, at the same time, revealed that sperm samples with good motility had significantly more motility enhancement than those with low motility. This may be attributed to the presence of significantly more normal sperm forms in semen samples with good motility as compared to those samples with asthenospermia. Previous reports indicated that anomalies in mid-piece may be associated with variable degree of mitochondrial dysfunction and low membrane potential which affect sperm motility (36).

Thanks to the novel work of NASA and outstanding effort of Nichia Chemical in Japan, LED has been intro-

duced into our daily life with tremendous applications. LED treatment in the current study has several advantages compared to laser. In addition to its cheaper costs, it has a large planar array which can help application to a large area containing many cells at the same time and in a hand-free manner. This is in contrast to laser which is applied in a pointed manner which would limit the treatment to a certain small area with limited number of targeted cells (37). In the present study, we have been using a red LED with a wavelength 636.6-nm and total power 1.3 W. The selection of this LED with such criteria came by the merest chance as we do not have any access to consultation facilities about LED especially from physics and engineering points of view. However, we think that this random selection of this kind of LED was suitable as it was well absorbed by the sperm mitochondrial chromophores giving a good photobiological response. It was also a safe procedure as the HOS test and ANBS did not show any remarkable changes until 10 minute application; indicating a steady sperm membrane integrity and chromatin condensation. As the biological response to phototherapy is wavelength-dependant at equal doses (35), we do not know the potential response of sperm to LED therapy with different wavelengths, power and energy densities. Therefore, an extension of the current work to include these variables will be the only avenue to provide suitable answers.

The current study is a preliminary report. Some limitations are, of course, present in it. The sperm motility parameters were not checked. One of these parameters is straightness, which may show how much the sperm motility is effective which in turn can save energy (20). However, both WHO manual (38) and ESHRE guidelines devised by the Andrology Special Interest Group (39) did not give any recommendation on which of the sperm motility kinematic parameters should be calculated because of its biological importance. In addition, there is no standardized mathematical algorithm to compute these sperm kinematics, which can be used by the different CASA machines (38, 39). Another limitation was the lack of follow-up checks on motility beyond 10-minute irradiation to establish how long the LED-enhancing effect would continue. This may be an important point during the IUI procedure. A last limitation was that we checked the sperm ATP, HOS and head chromatin condensation in a non-integrated way using a relatively small number of patients. This was due to our financial shortage. However, our chromatin condensation and HOS results come in accordance with other investigators (9, 20).

CONCLUSION

The results of this *in vitro* study may have practical implications for those involved in assisted reproductive techniques. We could reveal for the first time that treating sperm with red LED could safely enhance its motility; especially if preceded with sperm processing.

Results from this pilot study should be corroborated in larger studies with a detailed investigation on the effect of red LED treatment on sperm quality and functional characteristics.

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