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The Effects of Low-Level Laser Light Exposure on Sperm Motion Characteristics and DNA Damage

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ABSTRACT: The objective of this study was to determine the effects of low-level laser light exposure on the motility of spermatozoa and on DNA damage. Thirty-three semen samples were collected for routine analysis and were classified as normospermic, oligospermic, or asthenospermic. After routine semen analysis was performed, residual semen was divided into treated and control aliquots. Treated samples were exposed to a 30-second infrared laser pulse of 50 mW/cm^2 at 905 nm, a wavelength thought to increase light-sensitive cytochrome *c* oxidase in the mitochondrial electron transport chain. Samples were then incubated at 37°C, and aliquots were analyzed at 30 minutes and 2 hours using computer-assisted semen analysis. After incubation, 250 μL of each sample was frozen at −80°C until DNA fragmentation analysis by flow cytometry. A significant increase in motility, most prominent in oligospermic and asthenospermic samples (85% increase), was observed 30 minutes after the treatment ($P < .0001$). No significant increase in DNA damage compared with control samples was observed. Significant changes in sperm motion kinetics were observed. Low-level laser light exposure appears to have a positive short-term effect on the motility of treated spermatozoa and did not cause any increase in DNA damage measured at 2 hours. We conclude that some cases of asthenospermia may be related to mitochondrial dysfunction. The implications of this study in terms of future clinical applications needs further investigation.

Key words: Assisted reproduction ICSI, semen analysis.
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Infertility is commonly caused by male factor infertility, which typically accounts for 40%–50% of all cases (Brugh and Lipshultz, 2004; Cooper et al, 2010). Often the cause of male factor infertility is associated with poor-quality sperm—specifically, those that would be classified as teratospermic (less than 30% normal morphology), asthenospermic (less than 50% motility), or oligospermic (less than 20 million spermatozoa/mL) (Isidori et al, 2005). Although males affected by azoospermia, a condition resulting in a complete lack of spermatozoa in semen, have virtually no chance of conceiving spontaneously, those affected by teratospermia, asthenospermia, and oligozoospermia could have reduced chances for conception.

At present, few treatments are potentially effective for male factor infertility, including medications such as clomiphene citrate (Moradi et al, 2010) and aromatase inhibitors (Raman and Schlegel, 2002) or operative therapy such as varicocoelectomy. More recently, coenzyme Q10, a mitochondrial nutrient, has been shown to improve sperm motility. These therapies are variably effective, and all require between 3 and 6 months to assess their success. More commonly, treatments include various procedures associated with advanced reproductive technology (ART), such as selection of motile sperm for intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). All of these treatments typically involve the use of a sperm preparation technique that attempts to improve sperm motility and functional capacity for successful fertilization (Younglai et al, 2001). One of the substances commonly used to achieve such improvement is a nonspecific inhibitor of phosphodiesterase, the methylxanthine derivative–pentoxifylline (Tesarik et al, 1992; Henkel and Schill, 2003). The improvement of motility seen with pentoxyfyline could be attributed to the increased intracellular levels of cyclic adenosine monophosphate (cAMP) and cAMP-dependent kinase (Tash et al, 1986). A beneficial effect of pentoxifylline on sperm motility and kinetics has been described, but the use of pentoxifylline remains controversial because both premature acrosome reaction in the spermatozoa and embryotoxicity have been associated with the use of this chemical (Tesarik et al, 1992; Stanic et al, 2002; Henkel and Schill, 2003).

Procedures like ICSI require a living spermatozoon, which does not need to be motile. Therefore, within a

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sample of spermatozoa classified as immotile, some spermatozoa could still be of use to a couple trying to conceive. At present, the only means of differentiating dead spermatozoa from living, immotile ones, while preserving sperm capability to fertilize an oocyte by means of ICSI, is the hypo-osmotic swelling test (HOST) (Casper et al, 1996). The HOST measures functional sperm membrane integrity, which is one characteristic of sperm vitality (Moskovtsev et al, 2005).

Low-level laser therapy is believed to increase the adenosine-5′-triphosphate (ATP) production in mitochondria (Koppers et al, 2008). Laser treatment is typically used to treat muscle and ligament damage, primarily in athletes who incur traumatic injury, and joint damage in patients with rheumatoid arthritis (Brosseau et al, 2005). The treatment uses light between 600 and 1000 nm in wavelength (Tafur and Mills, 2008) to stimulate the light-sensitive cytochrome *c* oxidase complex in the mitochondrial electron transport chain to increase ATP production (Tafur and Mills, 2008). An increase in ATP is thought ultimately to promote healing by powering faster cell division.

The midpiece of the spermatozoon contains a tightly coiled mitochondrial sheath, which powers the movement of the tail (Huang et al, 2009). Thus, if one were to increase the mitochondrial activity of the midpiece, one would expect to see an increase in the motility of the spermatozoa. We hypothesized that low-level laser light exposure could increase energy for sperm motility in poor-quality semen samples.

Materials and Methods

Semen samples from patients attending the Toronto Centre for Advanced Reproductive Technology and the CReATe Fertility Center were allowed to liquefy for 30 minutes after collection before routine semen analysis. Remaining semen, after the routine analysis, was included for the study if the residual volume was 1 mL or more. Patients provided written consent for use of their residual semen samples in this research project approved by the Sunnybrook and Women's Hospital REB, University of Toronto (project ID 346-2008). Of the 33 semen samples collected, 10 were considered normospermic, 12 asthenospermic, and 11 both asthenospermic and oligospermic by World Health Organization (WHO) criteria.

Residual semen samples after routine analysis were divided into 2 equal volumes in 10-mL conical tubes: 1 designated for control and 1 for treatment. Samples marked for treatment were irradiated with a laser system using 50 mW/cm^2 (1.5 J/cm^2) for 30 seconds at a 905-nm light wavelength (Theralase® TLC-1000, Toronto, Canada), keeping the distance between the conical tube and the light emitter as small as possible. After irradiation, both the treated sample and untreated samples were incubated at 37°C for 30 minutes and then subjected to an initial integrated visual optical system (IVOS) computer-assisted semen analysis (CASA; HTM-CEROS Sperm Analyzer, Hamilton Thorne Inc, Beverly, Massachusetts). The samples were then returned to 37°C for an additional 90 minutes, followed by a second IVOS CASA.

Assessment of Semen Characteristics

Routine semen analysis included assessment of semen volume, sperm concentration, sperm vitality, sperm morphology, sperm motility, path velocity (VAP), curvilinear velocity (VCL), straight-line velocity (VSL), linearity (LIN), amplitude of lateral head displacement (ALH) (Agarwal et al, 2003), and mucus penetration ability, calculated as previously described (WHO, 1999; Mortimer 2000). The manufacturer's established parameters and the definitions of measured sperm kinetic parameters were used for the study. Aliquots of semen samples (5 μL) were placed in MicroCell 20-μm chambers (Conception Technologies, San Diego, California). A minimum of 200 spermatozoa from at least 10 different fields was analyzed from each specimen.

Sperm DNA Fragmentation Analysis

After semen analysis and within 4 hours from the time of ejaculation, aliquots of raw semen from control and laser-treated samples containing 1–2 million spermatozoa were frozen at −80°C for later DNA assessment performed as previously described (Evenson et al, 2002). Chemicals were obtained from Sigma-Aldrich Ltd (Oakville, Canada) unless otherwise stated. Briefly, at the time of analysis, the samples were thawed on ice and diluted with TNE buffer (0.01 M Tris-HCl, 0.15 M NaCl, and 1 mM EDTA, pH 7.4) to 1–2 $\times$ 10^6 cells/mL; 200-μL aliquots of diluted sample were mixed with 400 μL of a low-pH (pH 1.2) detergent solution containing 0.1% Triton X-100, 0.15 M NaCl, and 0.08 N HCl for 30 seconds followed by staining with 1.2 mL of 6 μg/mL chromatographically purified acridine orange (AO; Polysciences Inc, Warrington, Pennsylvania) in a phosphate citrate buffer (pH 6.0). At 3 minutes after the staining procedure started, the cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, California) equipped with an air-cooled argon laser. Measurements were collected at sperm flow rate less than 300 cells/s in duplicate on 5000 cells/sample, and 2 aliquots were analyzed for each semen specimen. The reference donor semen samples with known low level of sperm DNA damage were used to set the red and green photomultiplier tube voltage gains to give the same means for red and green fluorescence levels (130/1000 and 500/1000 channels ± 5) and were run every 5 samples to avoid drift. Under these conditions, AO intercalated with double-stranded DNA emits green fluorescence and AO associated with single-stranded DNA emits red fluorescence. DNA damage was expressed as the DNA fragmentation index (DFI), which is the ratio of red to total amount of red plus green fluorescing sperm in an individual semen sample. FCS Express version 2 (De Novo Software, Thornhill, Canada) was used for off-line analysis of the flow cytometric data. On the basis of the previously published categorical system, sperm DNA damage has been classified into 3 categories—low, ≤15%; moderate,

Table 1. *A statistically significant increase in the average motility of 33 semen samples, measured by computer-assisted semen analysis, at 30 minutes after treatment was observed in response to treatment. No significant change in motility was observed at 120 minutess after treatment*

	Untreated Control (n = 33)	Treated (n = 33)
Percent motility ± SEM (30 min)	29.8 ± 3.51%	34.9 ± 3.55% ($P < .002$)
Percent motility ± SEM (120 min)	24.9 ± 3.26%	25.8 ± 3.04% (P = NS)

Abbreviation: NS, not significant.

>15 to <30%; and high, ≥30%—and has been reported to be associated with excellent, good, and fair to poor fertility potential, respectively (Evenson et al, 2002).

Statistical Analysis

Because all of the data were normally distributed, a 2-tailed paired Student's *t* test was performed with the Statistical Package for the Social Sciences (SPSS 18). A value of $P < .05$ was considered statistically significant.

Results

Thirty-three semen samples of varying baseline motility and concentration were collected and treated with low-level laser light exposure. Motility was assessed after 30 minutes, and both motility and DNA damage were assessed 2 hours after treatment.

The CASA assessments showed a statistically significant increase in motility in the treated samples at 30 minutes after exposure (17.1%) when compared with the untreated samples, as shown in Table 1. This difference was not observed 120 minutes after exposure.

The magnitude of the treatment effect was also significantly different for the different WHO classifications of the 33 samples studied (Table 2). Those samples deemed normospermic (motility ≥ 50%, concentration ≥ 20 M/mL) exhibited a slight but significant relative increase of 5.5% in motility after the treatment. Those classified as asthenospermic (motility < 50%) showed a moderate relative increase of 11.8% in motility after the treatment. Most significantly, those samples that were classified as both oligospermic and asthenospermic (motility < 50%, concentration < 20 M/mL) exhibited the greatest increase in motility (83.5%) as a result of the treatment (Table 2).

DFI assessment by flow cytometry in 20 semen samples resulted in a mean (±SEM) value of 23.2% ± 0.034% in the control samples and 22.8 ± 0.033% in the treated samples at 2 hours. The levels of DNA damage in the treated samples compared with the control samples were not significantly different at 2 hours after laser exposure (see the Figure).

The results of the semen kinetic analysis showed a significant increase in VSL and LIN of the oligospermic and asthenospermic samples 30 minutes after treatment, as shown in the Figure.

Discussion

In the present study, 33 semen samples were each irradiated with 50 mW/cm^2 (1.5 J/cm^2) of low-level laser light for 30 seconds at a wavelength of 905 nm. Motility of the treated samples increased an average of 17.1% compared with the untreated samples 30 minutes after the initial exposure, but no significant increase was seen in the motility of treated samples 120 minutes after the initial exposure. The laser is thought to function by stimulating the light-sensitive cytochrome *c* oxidase complex in the mitochondria (Koppers et al, 2008), and a 30-second exposure would only affect the cells for a finite amount of time before the protein complex's excitation ceased. Additionally, motility in samples of poor quality had greater improvement compared with normospermic samples, suggesting that decreased mitochondrial activity could be a possible underlying mechanism of asthenospermia.

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. The observation that the specific wavelength light exposure increases sperm motility does suggest that the abnormal sperm motility in the asthenospermic group is related to low ATP production and, by inference, decreased mitochondrial function. It is not possible to determine whether the mitochondria are damaged, have increased mitochondrial DNA

Table 2. *Separation of samples into World Health Organization (WHO) catagories showed the most dramatic increase associated with oligospermic and asthenospemic samples, with a minimal increase observed in normospermic samples*

Sample WHO Classification	Normospermic (n = 10)	Asthenospermic (n = 12)	Oligospermic and Asthenospermic (n = 11)
Percent change in motility	5.5% ($P = .05$)	11.8% ($P < .001$)	83.5% ($P < .01$)

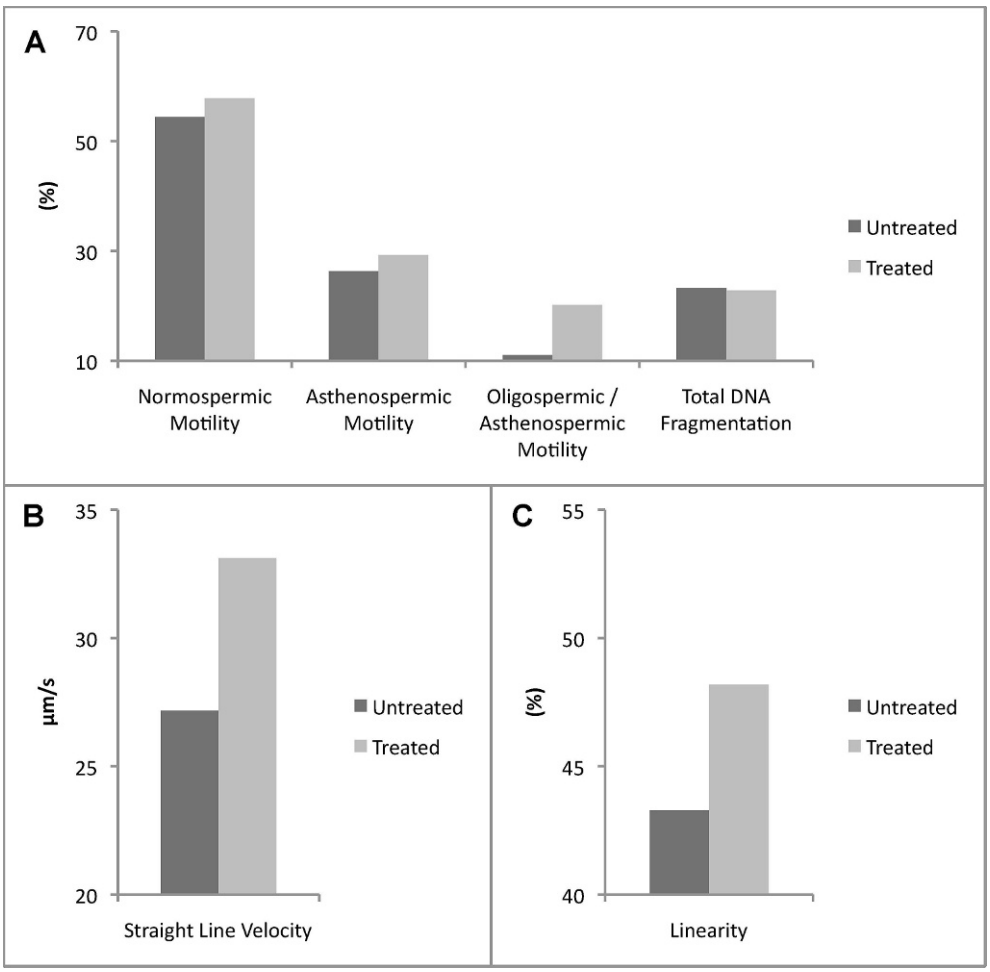

Figure. **(A)** Spermatozoal motility and DNA fragmentation in oligospermic and asthenospermic samples before and 30 minutes after low-level laser light exposure at 905 nm for 30 seconds. **(B, C)** Significant differences in straight-line velocity ($P = .02$) and and linearity ($P = .03$), respectively, measured by computer-assisted semen analysis in oligospermic and asthenospermic samples before and 30 minutes after low-level laser light exposure.

mutations affecting ATP production, or have reduced tricarboxylic acid substrates for ATP.

It was important to determine whether the laser treatment had any negative effect on the viability of the sperm. We have also checked the effects of the treatment on DFI as a marker of potential sperm damage. A DFI assessment was done, on both the treated and control samples 120 minutes after treatment to determine the total amount of DNA breaks that might have been caused by laser light exposure. As shown in Table 1, an increase in DNA damage was not detectable as a result of the laser treatment.

Analysis of the changes in the semen kinetic parameters 30 minutes after the treatment showed a significant increase in VSL and LIN (the Figure), but not in ALH, VAP, or VCL in the samples that were both oligospermic and asthenospermic, as shown in Table 3. An increase in motility in a given semen sample might increase the probably of conception in assisted reproductive technologies such as IUI or in IVF (Küçük et al, 2008). Low-level laser therapy could potentially be an effective treatment for those individuals with asthenospermic spermatozoa, especially if a more prolonged effect could be achieved.

Additionally, low-level laser stimulation might cause immotile, yet viable, spermatozoa to begin movement, thus providing a clinically useful and nondamaging method for determining which immotile spermatozoa in a given sample are alive. This technique could provide a simple and practical alternative to the HOST to select immotile sperm for ICSI. Moreover, treating spermatozoa with low-level laser therapy could potentially result in a significant increase of the likelihood of conception in an infertile couple undergoing ART for male factor infertility. Much more research is needed to gain a better understanding of the effects of low-level laser light on spermatozoa motility. Both dose-response and time course experiments are required. It would also be

Table 3. *Changes in sperm kinetic parameters by computer-assisted semen analysis were observed as a result of the treatment. Similar to overall motility, samples classified as oligospermic and asthenospermic showed the largest changes*

	Untreated	Treated (t = 30 min)	P value
Normospermic			
VAP	53.60	53.38	.79
VSL	43.68	42.83	.11
VCL	83.16	83.36	.93
ALH	4.01	4.00	.93
LIN	52.80	52.00	.43
Mucus penetration	35.00	37.10	.11
Asthenospermic			
VAP	43.30	43.70	.62
VSL	34.70	34.70	.94
VCL	72.00	73.70	.62
ALH	3.70	3.90	.33
LIN	50.30	48.80	.18
Mucus penetration	24.20	34.60	.79
Oligospermic/asthenospermic			
VAP	37.81	42.12	.07
VSL	27.17	33.11	.02[a]
VCL	65.26	68.49	.35
ALH	2.91	3.99	.24
LIN	43.28	48.18	.03[a]
Mucus penetration	23.18	27.45	.31

Abbreviations: ALH, amplitude of lateral head displacement; LIN, linearity; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.

[a] Statistically significant.

valuable to assess the functional effects of the treatment on different sperm parameters, for example, with a sperm penetration assay.

The results of this preliminary study showed a significant increase in the motility of spermatozoa in response to low-level laser light exposure for 30 seconds, especially in those samples classified as both oligospermic and asthenospermic. The effect of the laser to increase spermatozoa motility could involve the mitochondrial electron transport chain activity and was transient in the present study. The treatment did not appear to have any negative effects on spermatozoon DNA fragmentation, suggesting that further studies of the clinical utility of this treatment are warranted.

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