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Red Light Irradiation of Human Spermatozoa Increases Motility without Significant DNA Damage

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Abstract: Red light has been shown to increase sperm swimming speeds, but there is little characterization of its effect on DNA or swimming force. In this study 633nm laser light irradiation did not induce significant levels of oxidative DNA damage.
OCIS codes: (000.1430) Biology and medicine, (350.4855) Optical tweezers or optical manipulation

1. Introduction

The conservation of endangered species is important for preserving biodiversity. One major obstacle in animal conservation is low reproductive rates. Due to the complex reproductive systems of many wild animal species, many fertility treatments developed for human use are not very successful [1]. One potential method for increasing reproductive rates is to improve the swimming speed of sperm *in vitro*. Irradiation of sperm with red light has been shown to increase sperm swimming speeds.

The use of red light to aid in wound healing has been well-studied [2–5]. The proposed is the absorption of photons by cytochrome c in the mitochondria to increase ATP production [6]. The increased supply of ATP is believed to increase curvilinear velocity (VCL). Other studies have measured VCL using computer-assisted sperm analysis (CASA) systems. Although the CASA system is the standard of sperm motility analysis, it is unable to measure VCL directly under red light. Additionally, it relies on the use of a high-powered flash lamp to measure VCL, which may bias measurements [7]. In this study, a method of sperm tracking under any light source is used to address these issues.

Although there have been studies on the benefits of red light irradiation, few have addressed the possibility of DNA damage. Laser light irradiation has been shown to increase production of reactive oxygen species (ROS) in cells, which can cause oxidative DNA damage. The presence of oxidative DNA damage can be measured with antibody staining. Sperm have been shown to form γH2AX, a sensitive double-strand DNA break marker, in response to ROS [8]. This marker can be detected to determine the locations of DNA damage within the cell. The nitrogenous base guanine has been known to form 8-oxo-dG in response to ROS [9]. Detection of 8-oxo-dG with a quantitative ELISA can quantify the amount of DNA damage produced as a result of red light irradiation.

2. Methods

Sperm samples were cryogenically frozen following collection according to a standard freezing protocol [10,11]. Samples were thawed in a water bath at 37° C and washed twice. Samples were kept at 37°C until use.

For motility analysis, samples were irradiated from above using a 633 nm monochromatic, coherent laser light source coupled to a multimode, homogenizing fiber at a power density of 5.66 mW/cm^2 for 30 minutes. For oxidative DNA damage assessment, samples were irradiated from the same source at a power density of 31 mW/cm^2 for 30 minutes. Experiments were conducted in the absence of external lighting to minimize the effect of other wavelengths.

Sperm VCL was measured using a wavelet-based tracking algorithm for use directly under red light. Multiple measurements were taken from a single sperm sample and averaged. The algorithm analyzes videos of swimming sperm. Individual sperm are detected using wavelet analysis and tracked using a Kalman filter. The CASA measurements were consistently 1.59 ± 0.18 times the algorithm measurements.

To assess the effect of red light on DNA integrity, three samples were prepared: one exposed to red light, one exposed to H_2O_2, and one untreated sample. Sperm were exposed to 1 mM H_2O_2 to induce double strand DNA breaks. All samples were fixed and stained with anti-γH2AX antibody and DAPI. The mean pixel intensity of the sperm head as well as the number of γH2AX foci were measured to determine the localization of double strand breaks.

The effect of red light irradiation on sperm swimming forces was characterized. Individual sperm were trapped with a 1064nm continuous-wave IR laser at a power of 500 mW. The trapped sperm were irradiated with phase contrast and 633nm red light in 30 second intervals. Their x and y positions were recorded and motion characteristics analyzed in MATLAB to determine their power spectral densities.

3. Results

633nm red light irradiation of sperm produced a 17-47% increase in speed compared with their untreated counterparts.

Sperm were dyed and imaged (Figure 1). In the H_2O_2 treated sample, double strand breaks were concentrated around the sperm head. The untreated and irradiated samples did not show this overlap. An ANOVA test showed that there were significantly higher levels of γH2AX in treated than in untreated sperm (Figure 2A). A Student's t-test showed no significant difference in γH2AX levels between the untreated and irradiated sperm (Figure 2B).

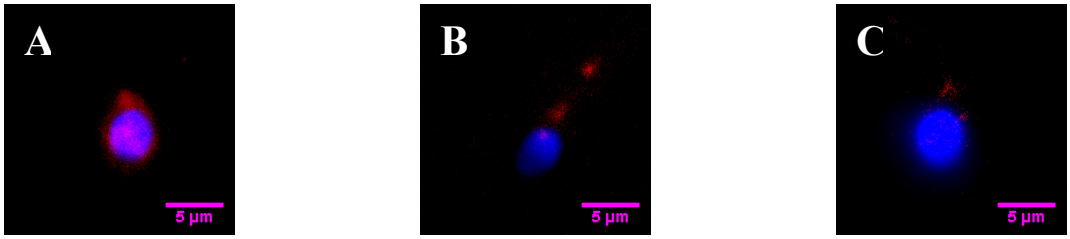

Fig. 1. Visualization of γH2AX Localization. DAPI staining is shown in blue. Anti-γH2AX staining is shown in red. **(A) H_2O_2 Treated Sperm. (B) Untreated Sperm. (C) Irradiated Sperm.**

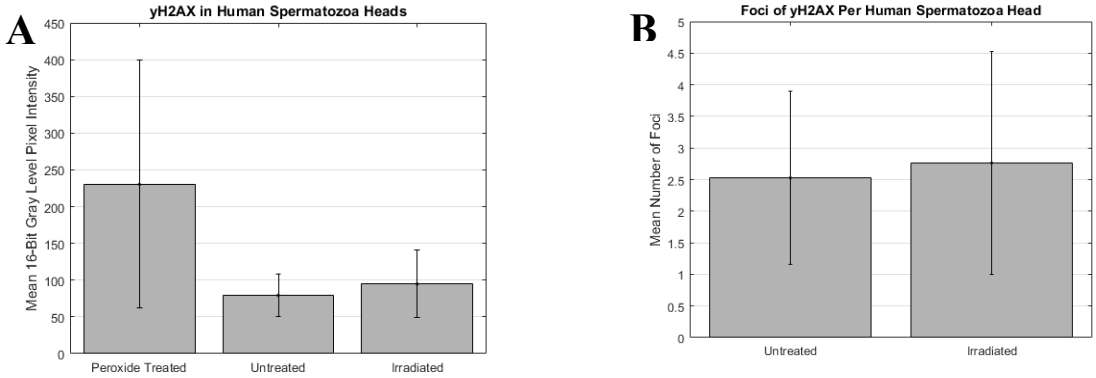

Fig. 2. γH2AX Formation in Spermatozoa. (A) γH2AX in Human Spermatozoa Heads (B) γH2AX Foci in Human Spermatozoa Heads. Error bars indicate standard deviation.

A Student's t-test showed no significant difference in oxidative DNA concentration between the irradiated and untreated groups (Figure 3). The untreated sample contained 425.4 ± 34.51 pg/mL of 8-oxo-dG and the irradiated sample contained 470.9 ± 21.44 pg/mL of 8-oxo-dG.

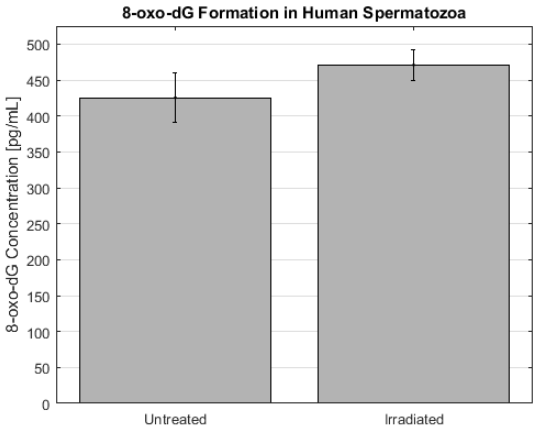

Fig. 3. Concentrations of 8-oxo-dG in Untreated and Irradiated Samples. Error bars indicate standard deviation.

Power spectra were acquired from x and y position data. Peaks in the power spectra represent the frequency of sperm tail movement, shown in Figure 4 to be on the order of 10 Hz.

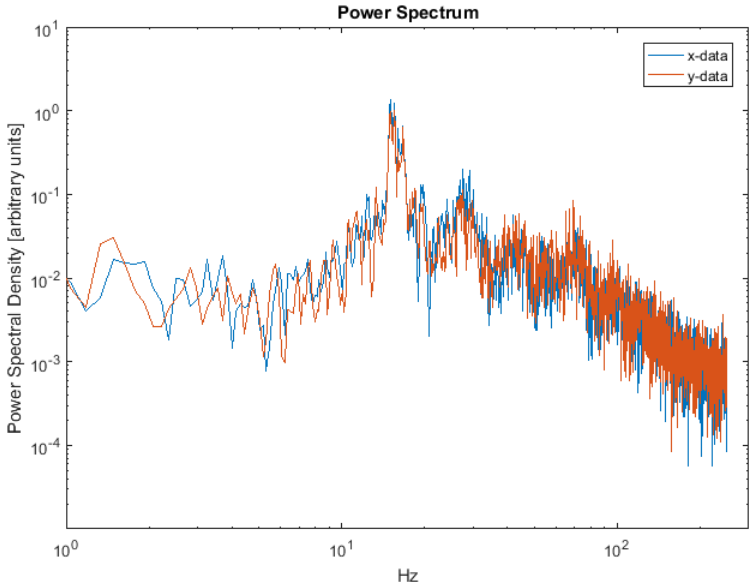

Fig. 4. Power Spectra for Trapped Sperm.

4. Conclusion

Red light irradiation of sperm increases VCL without significant oxidative damage to DNA. The small amounts of DNA damage present in the untreated and irradiated samples are likely an artefact of cryopreservation, thawing, and centrifugation. This study also validated the use of a wavelet-tracking algorithm to measure VCL as opposed to a CASA system. The results of this study offer a potential alternative or complement to current fertility methods used to treat animals as well as humans.

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