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Effect of red light on optically trapped spermatozoa

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Abstract: Successful artificial insemination relies on the use of high quality spermatozoa. One measure of sperm quality is swimming force. Increased swimming force has been correlated with higher sperm swimming speeds and improved reproductive success. It is hypothesized that by increasing sperm swimming speed, one can increase swimming force. Previous studies have shown that red light irradiation causes an increase in sperm swimming speed. In the current study, 633nm red light irradiation is shown to increase mean squared displacement in trapped sperm. The methodology allows for comparison of relative swimming forces between irradiated and non-irradiated samples.

OCIS codes: (000.1430) Biology and medicine; (350.4855) Optical tweezers or optical manipulation.

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1. Introduction

Although large numbers of thorough studies of spermatozoa quality and characteristics have been conducted, there is still much that is unknown about sperm motility. Conventionally, percentages of motile sperm and sperm trajectories have been used as measures of sperm fitness. Sperm swimming force is another metric for sperm quality which is highly dependent on the energy dissipation of individual sperm. It is implied that higher swimming force is indicative of increased ability to out-compete other sperm for successful fertilization [1].

It has been suggested that sperm swimming force is instrumental in fertilization of the egg. Sperm must first penetrate the cumulus oophorus to reach the zona pellucida, where fertilization occurs. Although the sperm acrosome contains enzymes to help digest the cumulus oophorus, it has been shown that sperm are capable of penetrating the egg without them [2], indicating the importance of mechanical force. Another study correlated the swimming force of epididymal sperm with fertilization [3] wherein it was shown that sperm with lower swimming force produced fewer embryos, implying a relationship between swimming force and fertilization.

Studies have indicated a positive correlation between sperm swimming speed and swimming force [1,4]. Recently, it has been shown that red light irradiation of sperm can increase curvilinear velocity (VCL) [5,6]. One proposed mechanism for this increase is stimulation of cytochrome c oxidase (Cox), Complex IV of the electron transport chain. It has been shown to be a photoacceptor for red to near-IR light [7]. The absorption of these wavelengths is believed to cause an increase in ATP production, and thus an increase in VCL. It is possible that an increase in VCL by 633nm laser irradiation may cause an increase in sperm swimming force, which may be of benefit to reproductive medicine particularly in increasing fertilization potential. In the current work we describe the effect of red light illumination on optically trapped spermatozoa. We determine the average force exerted by the sperm in the optical trap by observing the time dependent position of the sperm head in the optical trap under red light.

1.1. Measuring forces generated by sperm cells

The forces exerted by individual sperm are difficult to measure due to their small size and inherent motility. Baltz et al. [8] used the forces generated by a suction micropipette to measure sperm escape force. The force was lowered by decreasing the pressure within the pipette until the sperm was able to escape. The swimming force was calculated from this pressure and the area of the pipette opening. This method requires that only the sperm head is captured laterally on the pipette during force measurement, causing only about 20 in 10000 sperm to be measured.

Sperm swimming forces can also be measured by optically trapping sperm. One method to determine the force exerted by the sperm is to lower the trap power until the sperm is able to escape (P_{esc}) [1,4]. The escape force can then be determined based on a predetermined trapping efficiency, Q, and the equation $F_{esc} = Q\frac{nP_{esc}}{c}$, where n is the refractive index of the media and c is the speed of light. Although this method is a simple, mathematically inexpensive method of determining swimming force, it has some drawbacks. For example, it does not account for directionality of force. It also relies on a single measurement obtained per sperm at a single instance in time without being able to take into account direction, position, or amount of sperm movement prior to escape. However, this method does preserve the integrity of the sperm cells by only exposing them to radiation for a small amount of time, thus reducing the possibility of "opticution" and allowing for high throughput measurements.

Another way of determining force is by its relation with the optical trap stiffness. Because optical traps can be approximated as a parabolic potential well, the trapping force can be calculated from Hooke's law with trap stiffness κ as the proportionality constant, defined for a trapped particle under Brownian motion via equipartition theory as [9].

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$$\kappa = \frac{k_b T}{< x^2 >} \tag{1}$$

In the current article we take advantage of sperm dynamics in the optical trap to generate a qualitative force metric which can serve as a measure of the overall forces present in differing populations of sperm.

2. Methods

2.1. Sample preparation

Cryogenically frozen human sperm samples obtained from Infertility, Gynecology, and Obstetrics Medical Group (San Diego, CA) were collected from healthy men and frozen according to standard freezing protocol [10, 11]. Samples were thawed in a water bath at 37°C before centrifugation at 208g for 10 minutes. The pellet was then resuspended in 1 mL modified human tubal fluid (HTF) (Irvine Scientific) with 5% serum substitute supplement (SSS) (Irvine Scientific) and centrifuged again. This wash technique was performed twice for every sample used for a final concentration of about 30000 sperm/mL. $10\mu L$ of the suspension was plated in a 35mm glass bottom dish with 2mL of modified HTF. To minimize the effect of diminishing motility over time, samples were discarded within 3 hours of preparation.

2.2. Optical setup

Motile spermatozoa were optically trapped in a single beam gradient trap before and after red light irradiation. A Nd:YVO_4 continuous wave 1064nm wavelength laser (Spectra Physics, BL-106C) was used as the trapping beam. After power attenuation, the beam was sent through a 4f imaging system and into an Axiovert S100 2TV microscope (Zeiss) and a 63x NA 1.4 phase III oil immersion objective (Zeiss) (transmission ≈25% at 1064nm). Samples were trapped at a power of 150 mW at the focal spot. For red light irradiation, samples were irradiated from above using a 633nm He-Ne laser light source (Intense 7404) coupled to a multimode, homogenizing fiber (Medlight FD) at a power density of 31 mW/cm^2 for 30 minutes. Experiments were conducted in the absence of excess lighting to prevent the effect of other wavelengths of light. Images were taken using a CMOS camera (Mako G-030) at 500 frames per second (Fig. 1(a)).

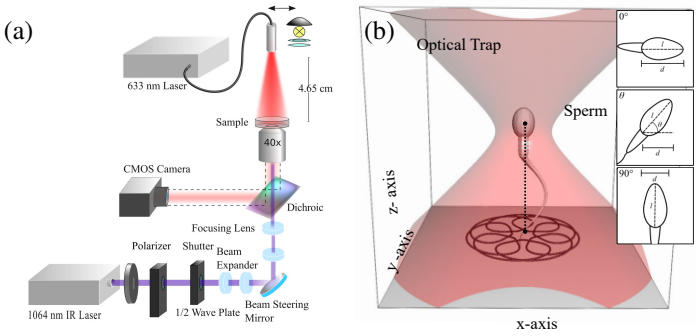

Fig. 1. (a) The experimental setup showing 1064nm laser (Spectra Physics) optical shutter (Unibliz), beam attenuation and 4f imaging system for optical tweezers and dichroic mirror and camera (Mako G-030) for high speed imaging of sperm position. (b) Representation of sperm in the optical trap. over short time scales the sperm traces out a "rose curve" like trajectory in the xy-plane. A schematic is also shown depicting how sperm orientation was determined based on length measurements, d.

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2.3. Sperm position analysis

Sperm within the optical trap were tracked over a maximum duration of 20 seconds to avoid photo damage using center of mass (COM) tracking, with an error of 0.023 μm, implemented in LabVIEW. The image is thresholded to eliminate background pixels and COM is computed. Though it is possible to use this algorithm to track in three dimensions, here we track only in the x and y dimensions [12].

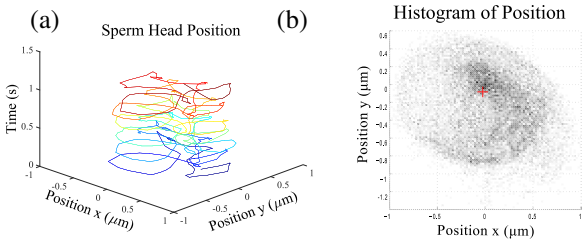

Fig. 2. (a) Trajectory of the sperm head in the xy-plane over a 1 second interval. (b) 2D histogram of sperm head position. Dark areas represent a greater number of counts in that location. The red crosshair denotes the trap center.

Empirically we found the trapped sperm traces a "rose curve" like trajectory in the xy-plane i.e. one which resembles a sine wave in polar coordinates, shown in Fig. 1(b). This is due to the elliptical motion of the sperm head caused by flagellar motion as the sperm tries to escape the optical potential. Brownian noise and stochastic reorientations serve to perturb this behavior causing the ensemble position data to be largely Gaussian in distribution despite the periodic oscillations of the sperm head. Fig. 2 illustrates this by showing a time sequence of sperm motion in the xy-plane and the position histogram of the sperm head taken over 10 seconds. Without a rigorous mathematical model of this behavior it is difficult to predict the dynamics of the sperm head in the optical trap nor is it within the scope of the current article. Instead we adopt the notion of an optical pseudo-potential [13]. Because the trap stiffness is a property of the geometry of the optical trap and of the trapped particle, there may be variation in trap stiffness due to particle orientation. This can be accounted for by measuring the trap stiffness for immotile sperm over a range of orientations and averaging over the occupation probability of each orientation state. The pseudo-potential then gives a measure to quantify the effect of the trap on the sperm. The extra energy imparted into the system by the motion of the flagella can therefore be quantified by observation of the variations of the mean squared displacement (MSD) between different sperm.

The force imparted by the optical trap can be estimated using Hooke's Law and Eq. (1). The trap stiffness can be evaluated using Eq. (1) applied to immotile sperm. The trapping force is then approximately proportional to the square root of MSD, which represents an average net displacement over the duration of measurement. Since the instantaneous velocity of the sperm is found empirically to average to zero at all points in the xy-plane, the MSD due to the motion of the sperm cell can be approximated as proportional to the force exerted by the sperm (Fig. 3).

3. Results

Assuming a symmetric ellipsoid distribution, the trap stiffnesses for different rotational orientations of trapped sperm can be determined. The occupation probability of each orientation was determined by mapping the length of an imaged sperm to an orientation based on measured lengths in axially and laterally oriented sperm. A schematic of this method is shown in Fig. 1(b). 2314 images of a single motile trapped sperm were analyzed and a Gaussian fit was applied to the histogram of orientations to produce a probability density function. Trap stiffnesses were

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determined from the MSDs of 5 immotile sperm trapped axially and 2 trapped at about 65° determined using the method described above. The trap stiffness when sperm were in the axial position was estimated to be 10.32 ± 4.04 pN/μm and 10.83 ± 5.57 pN/μm at 65°. Weighted averaging yielded a trap stiffness of 10.68 pN/μm.

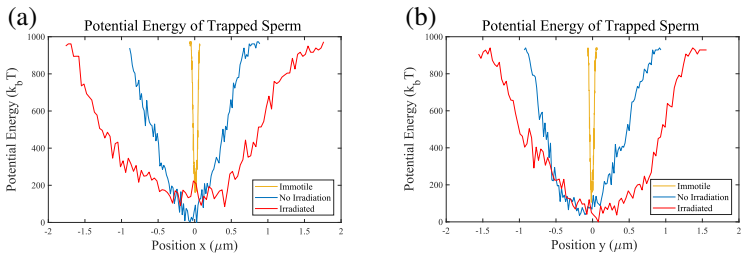

Fig. 3. Comparison of potential energy for irradiated, non-irradiated and immotile sperm calculated via optical potential analysis (a) in the x-direction for trapped sperm (b) in the y-direction for trapped sperm.

From the position data, the relative potential energies of sperm with and without irradiation were calculated using Boltzmann statistics. Fig. 3 compares the distributions of potential energy over position for irradiated, non-irradiated, and immotile sperm. The potentials for the trapped sperm appear parabolic, indicating a near-linear relationship between position and force. The broader distribution of potential energy in irradiated sperm compared with non-irradiated sperm implies that there is more energy in the irradiated state.

The MSDs for 31 motile sperm before red light irradiation and 43 motile sperm after red light irradiation were measured. Although more sperm were trapped, data were excluded if (a) a second sperm entered the trap, (b) the sperm swam out of the trap before position data was attained, or (c) a histogram of the sperm position indicated tracking errors. Sperm had an average MSD of 0.026 ± 0.0035 μm^2 and 0.016 ± 0.0027 μm^2 with and without red light irradiation, respectively (Fig. 4). From the measured average trap stiffness, this produces trapping forces of 1.76 pN and 1.38 pN with and without red light irradiation, respectively. A Shapiro-Wilk test indicated that the distributions of MSD data were non-normal. Thus, a Kruskal-Wallis test was used to compare the two groups. The increased MSD in the irradiated group was found to be statistically significant ($p < 0.05$).

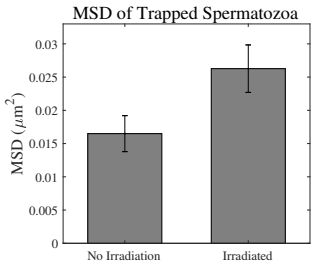

Fig. 4. MSD of trapped spermatozoa with and without red light irradiation. Error bars represent standard error. The data were found to be statistically significant ($p = 0.01269$).

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4. Discussion

MSD is a measure of the average squared distance a particle has traveled and is representative of aggregate displacement over time and is highly correlated with optical trapping force. Thus, a larger MSD is indicative that a higher trapping force is necessary to hold a particle in the trap. The irradiated sperm in this study had a larger MSD than the untreated sperm, implying greater relative swimming force in the irradiated sperm. It should be noted that this model is meant to compare sperm swimming forces within a population, rather than to produce absolute quantitative measurements, which would require more robust mathematical modeling.

Due to their nonuniform shape, sperm head position data may be prone to error through COM tracking, as it relies on the pixel intensities within an image. Although computationally inexpensive, this method is unable to take into account the effects of asymmetrical objects rotating within optical traps. Other tracking techniques such as cross-correlation based algorithms may provide a more accurate measure of cell position [14]. However, because they require more computational time, this method was not utilized in the current work. The study outlined here relies on large data sets, limited by camera frame rate and image processing time. The current methodology was necessitated by the relatively short time window over which useful statistical data could be gathered.

It is possible that the 20 second trap duration could have adversely affected sperm motility. With a 1064nm beam, significant decreases in VCL were observed after 45 seconds at 1 W (in a 2-3μm focal spot) for fresh human sperm [15] and after 15 seconds at 420 mW in the focal spot for fresh dog sperm [16]. It is possible that the observed decreases are velocity or species dependent. Further experiments should be conducted to verify whether frozen-thawed human sperm experience a decrease in VCL at the power and duration used in this study.

5. Conclusion

Optical trapping provides a viable method of estimating sperm swimming forces. 633nm red light irradiation produced a statistically significant increase in mean squared displacement, which correlates with an increase in swimming force. We observed an average MSD of 0.026 μm^2 and 0.016 μm^2 with and without red light irradiation, yielding an estimated trapping force of 1.76 pN and 1.38 pN with and without red light irradiation, respectively. In artificial insemination for humans and other animals, it is important to select high quality sperm for insemination to ensure successful fertilization. Like previous techniques, the method described here produces relative force measurements for comparison between cells, but based on thousands of individual measurements per sperm rather than on a single value.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.