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ASSESSMENT OF SPERM ROTATIONAL DYNAMICS AND MOTILITY BEHAVIOR USING LASER-BASED OPTICAL TRAPPING: IMPLICATIONS FOR MALE FERTILITY POTENTIAL AND ASSISTED REPRODUCTIVE TECHNOLOGY

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Abstract
Laser-based optical trapping provides non-invasive access to cellular physiological and mechanical properties. The aim of this technique is to study the rotational dynamics and motility behavior of sperm cells in order to assess the male fertility potential. An integrated optical system with near-infrared laser beam was used to measure rotational dynamics of live sperm cells from oligozoospermic and asthenozoospermic cases. As the sperm are optically trapped, their translational motions become rotational motions without adversely affecting them. As compared to controls, infertile men's sperm cells rotate at a significantly slower speed. As part of modern reproductive biology, it may be crucial to distinguish between normal and abnormal sperm cells based on beat frequency. It has been proposed that the application of laser-assisted techniques to biology may be a useful tool for improving assisted reproductive technology by assessing sperm fertilization capacity.

Keywords: Laser-Based Optical Trapping, Sperm Rotational Dynamics, Male Fertility, Assisted Reproductive Technology, Cellular Physiology

Introduction

Biophotonic optical tweezers (optical traps) trap particles whose size is less than one micron in size by using a sharp gradient of laser intensity. Several decades ago, Ashkin described this physical phenomenon, and it was used for the first time to trap live cells twenty-five years later [1, 2]. An object that is trapped and its optical geometry determine the strength of the trapping. A microscope objective with a high numerical aperture (normally 100X) produces sharp gradients of spatial intensity [3]. Cells are increasingly being studied mechanically, behaviorally, and physiologically, and individual cells are being spatially manipulated using optical tweezers [4–6]. Recent studies of Chlamydomonas reinhardtii flagellar mutants under physiological conditions demonstrated the utility of this technique for determining the dynamics of flagellar rotation [7]. Contemporary interest has focused on the dynamics of spermatozoa, specifically their motility forces [8–11]. Sperm cells with abnormal forms of motility have been studied through the use of optical traps [12–14]. Laser tweezers and tracking software combined with robotic technology have enabled high-throughput sorting and measurements of sperm characteristics [15–19]. Scientists interested in determining fertilization rates

are measuring sperm motility and swimming force. The force generated by swimming sperm is correlated with the speed of swimming sperm [20]. It has also been possible to deduce evolutionary patterns of sperm based on swimming forces and swimming speeds of various primate species, including chimpanzees, rhesus macaques, humans, and gorillas [21]. In traditional optical trapping, near-infrared lasers with spectral ranges of 700–1200 nm have been used since live cells weakly absorb these wavelengths [22, 23]. In this spectral range, laser light is assumed to be non-invasive and safe for cells, but certain types of living specimens may be damaged by laser light, including DNA damage, membrane damage, diminished viability, reduced cloning efficiency, and reduced cloning efficiency [24–27]. It has been studied to a certain extent how spermatozoa behave prior to and after optical trapping in different mammalian species, but there is no report on how a single live sperm cell behaves within a trap when it rotates. The study compares oligozoospermic and asthenozoospermic human spermatozoa that have been optically trapped to normal sperm cells in real time. Also, before and after the trapping process, we study the individual's motility.

Materials and Methods

Sperm collection and subject selection

Randomly collected semen samples were collected from 27–39 years old males with asthenozoospermia (n = 48) and oligozoospermia (n = 44). We compared the results of 40 proven fertile men whose partners had delivered healthy babies without assisted reproductive technologies (ART) in the last six months with those of 40 men without ART. During sexual abstinence, sterile containers were used to collect sera ejaculates, and within an hour, they were tested in the laboratory. Each sample was optically trapped to analyze 50 sperm cells. Informed consent was obtained from all couples in this study through the Research Ethics Committee. A normal sperm concentration, motility, and morphology are all above 4% according to WHO guidelines (2010).

Setup of optical tweezers. A diode-pumped Nd:YVO4 laser produces a continuous wave beam coupled to an inverted microscope equipped with fluorescence. Lasers were focused through microscope objectives with numerical apertures of 1.3 and 100X to achieve trapping. Laser power could not exceed 20 mW when it was incident on trapped sperm cells in order to prevent possible damage. Live sperm cells under the trap were monitored and captured in real time with CCD cameras connected to computers.

Trapping Efficiency and Rotational Motion. The optical tweezers used for sperm cells are set up to measure the movement of sperm cells based on the trapping efficiency.

An ineffective trap has 100% trapping efficiency if its effect is insignificant and its velocity is unchanged before and after trapping. Pretrap and posttrap velocities must, however, be different if the process is to be efficient. Optically trapping sperm also results in rotational movement, as previously mentioned. Real-time video footage has been analyzed frame by frame to determine how many cells are rotating, how fast they are rotating, and when the sperm tail beats.

Results

The optical tweezers setup was used to analyze the rotational dynamics of various sperm cell categories. Upon trapping, all motile spermatozoa ceased their normal linear translational motion. If the cell had a functional tail, the rotational direction would have been stochastic, with cells rotating clockwise or anticlockwise. Sperm tail forces push the sperm head down tangentially to the optical trap's force, causing the sperm to spin. Oligozoosperm and asthenozoosperm sperm cells rotate at a significantly slower speed than controls. For differentiating normal and abnormal sperm cells, the cut-off value for receiver operating characteristic (ROC) curves was 5.6 Hz. In order to differentiate abnormal from normal spermatozoa in oligoospermic and asthenozoospermic cases, sensitivity and specificity are discussed. Asthenozoospermic cases have 73% sensitivity and 69% specificity, whereas oligozoospermic cases have 85% sensitivity and 69% specificity. In addition, sperm cells with different rotational speeds or beat frequencies exhibited positive correlations with sperm swimming speeds. Sperm cells from oligozoospermic and asthenozoospermic men exhibited

significantly slower swimming speeds compared to those from controls. Sperm cells from oligozoospermic, asthenozoospermic, and normal individuals showed no significant differences in swimming speed. By using formula (1), it was determined that oligozoospermic, asthenozoospermic, and normal sperm cells can be trapped by the tweezers at 96%, 92%, and 95%, respectively.

Discussion

CASA systems, which have been commercially available since the mid-1980s, are typically used in research and infertility clinics to evaluate sperm motility. Optically tweezers may provide an alternative method to measure sperm swimming force, speed, and energetics. Using this technology, one is able to better understand the characteristics of sperms, such as motility and energetics, and create assays to diagnose abnormal sperm behavior in infertility and fertility issues.

When sperm cells were trapped, translational motion became rotational motion. The transition from translational to rotational motion may be attributed to reversible changes in molecular components of the mitochondrial apparatus following trapping. Due to this change, the laser light becomes more polarizable, subsequently interacting with the electric field associated with the laser light to develop a torque. An increase in laser intensity results in a decrease in torque generated by the optical field. Rotational motion of red blood cells is influenced by the intensity of laser light incident in our laboratory earlier this year.

A certain frequency is assigned to the sperm as it rotates upon being optically trapped. As a result, this frequency correlates well with the speed at which the sperm swims and depends upon the force generated by the beating of the cell. Reduced rotational or beat frequency indicates alteration of flagellar apparatus or proteins in trapped abnormal sperm cells. Oligozoospermic and asthenozoospermic sperm cells were differentiated from control sperm cells using beat frequency with a cutoff value of 5.6 Hz.

Monitoring sperm physiology before, during, and after optical trapping requires documenting sperm swimming speed. As a result, the optical tweezers set-up with nIR laser beam do not damage spermatozoa, as demonstrated in our current study. According to a study published by who concluded that long wavelength nIR laser beams, when used to manipulate spermatozoa, cause significantly less damage than ultraviolet light. Laser induced temperature increase may slow down sperm swimming speed and negatively affect mitochondrial membrane potential after long exposure to the laser trap. Domestic dog spermatozoa exposed to optical tweezers were also found to have depolarized mitochondria.

It is considered the gold standard for determining the quality of semen to use the WHO manual on the examination of human semen and sperm cervical mucus interaction. As per WHO guidelines, oligozoospermia is defined as a lack of spermatozoa in the semen, but the motility of the sperm cells remains normal. In contrast, beat frequency, another measure of sperm motility, was significantly lower in the oligozoospermic group than in the control group. Oligozoospermic sperm samples are found to have low motility for the first time. Further investigation is definitely warranted in light of this observation. As sperm quality is described by WHO parameters, this valuable information can be incorporated into the definition of sperm parameters after it has been validated in a larger sample size.

Conclusion

Considering the rotational dynamics and motility of sperm cells, we suggest that optical tweezers are becoming an important tool for optically selecting and sorting sperm cells. In spite of the need for further research to gain a deeper understanding of a trapped sperm's physics, this study suggests that this technique can be utilized to select sperm cells with beat frequencies above 5.6 Hz when optically trapped in order to begin selecting good quality sperm cells. As a result of observing the rotational dynamics of one live sperm cell trapped by an optical tweezers, we have further demonstrated that its normal physiological characteristics are not damaged by the nIR laser beam. A variety of parameters can be measured with this system, giving us a better understanding of the speed at which sperm swim.

Infertile men can be selected using this technique for good quality sperm cells that can be used to support ART procedures involving intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF), where sperm quality plays a key role in determining embryo quality.

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