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Guest Editorial

Lasers in Infertility Treatment: Irradiation of Oocytes and Spermatozoa

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Laser beams were introduced into the medical field of assisted human reproduction in the late 1970s and early 1980s for reconstructive pelvic surgery through operative microscopes and laparoscopes.[1] In the 1980s and 1990s, lasers began to be used in the treatment of infertility. The terms IVF (*in vitro* fertilization), ICSI (intracytoplasmatic sperm injection), and ART (assisted reproductive technologies) appeared. These terms mean that spermatozoa and/or oocytes (egg cells) are handled outside of the human body and the fertilized egg is planted in the uterus. Lasers have been in use to treat oocytes as well as spermatozoa by IVF, ICSI, and ART technologies. Oocytes were treated by Nd:YAG laser at 1064 or 534 nm or by tunable Ti:sapphire lasers at 650–1080 nm for ablating zona pellucida (a glycoprotein layer surrounding the plasma membrane of the oocyte) thinner or even to drill thin holes through it.[2,3] Nowadays, the most successful equipment for laser-assisted hatching is considered to be a semiconductor laser emitting at 1.48 μm, which is the highest standard for laser ART fulfilling all safety requirements for zona pellucida ablation as well as for spermatozoa immobilizing prior to use.[3,4] By using ICSI, pregnancy was achieved in a couple with male primary cilia dyskinesis, with viable sperm that was detected using 1.48 μm wavelength diode laser.[5] Semen samples showed no motile spermatozoa and a high percentage of spermatozoa had curled flagella. Injection of laser-selected spermatozoa to the oocytes resulted in four fertilized oocytes out of seven. The transfer of two frozen/thawed oocytes of the laser group led to a singleton pregnancy. The authors conclude that the use of noncontact diode laser for sperm viability assessment may be a useful test method.

Also, low-power He-Ne laser has been used in IVF to treat immature oocytes. The use of laser radiation at clinical doses of 0.4 and 2 J/cm^2 produced a negative effect in the maturation process, with significant damage at the nuclear level. At the same time, He-Ne laser radiation at doses of 2, 4, 8, and 16 J/cm^2 stimulated acrosome reaction depending upon the dose; the degree of stimulation was even higher than that with chemical capacitate agents (heparin, calcium, caffeine).[6]

Laser microbeams, most frequently continuous wave (CW) near-infrared (IR) ones in wavelengths ranging from 700 to 1200 nm, are employed as optical traps (laser tweezers) in sperm micromanipulation.[7–9] It appeared that spermatozoa can be manipulated by laser tweezers in two or even three dimensions.[8] However, the beams used in optical traps are of rather high intensity and could be damaging to DNA. Even He-Ne laser radiation (632.8 nm) at a dose of 24 J/cm^2 induced sister chromatid exchange in sheep peripheral mononuclear cells.[10] (It is important to recall that He-Ne laser radiation is not absorbed by DNA directly.)

The common cause of male infertility is a low sperm count; however, some men are infertile because of poor sperm motility. It is known that the amount and the quality of spermatozoa has decreased over the past 50 years, and oligospermia or aspermia (which refer low concentration or full absence of sperm cells in the ejaculate, respectively) are nowadays rather widespread conditions. Also, the total motility of spermatozoa, which refers to the fraction of sperm that displays any type of movement, has decreased in the last decades.[3,9]

Every sperm cell consists of a head (acrosome), which contains tightly packed condensed DNA, followed by a short neck containing mitochondria (midpiece), and a thin tail (flagellum), which is responsible for the motility of the cells. The moving speed of a spermatozoon depends upon energy supply. Spermatozoa maintain low energy consumption during storage in canda epididymis. These cells are motile but unable to fertilize an egg. Enhanced adenosine-5′-triphosphate (ATP) production becomes critical at the time of fertilization. Motility is activated only upon ejaculation, and so-called "hyperactivation" takes place in the oviduct.

Activation of sperm flagella motility involves both energy metabolism in mitochondria and the motile apparatus of the cells. Mammalian spermatozoa can produce ATP both by anaerobic glycolysis and aerobic breathing.[11,12] It is well documented that low- power laser irradiation of spermatozoa can increase their motility as well as the ATP amount in cells. To the best of our knowledge, the first publication on this topic appeared in 1984.[13] First, publications[13–17] clearly evidenced that human sperm motility as well as velocity can be improved by He-Ne laser irradiation. Second, it was found in publications[13–17] that the irradiation stimulated nonmotile and badly moving but live spermatozoa to move. Later, an important study in this particular field was done by H. Breitbart and R. Lubart with coworkers. Stimulation of motility of bull, ram, mouse, and human spermatozoa as well as mouse oocytes by irradiation with visible light of laser and non-laser origin at 632.8, 660, and 780 nm as well as

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with broad band visible light 400–800 nm were studied.[18-23] It was found that irradiation of human sperm with broad band visible light (400–800 nm) caused a significant increase in hyperactivated motility, but not in total motility, of human sperm. A rapid increase in intracellular Ca^{2+} concentration and hyperactivated motility caused by irradiation were significantly reduced when voltage-dependent Ca^{2+} channel was blocked or when Ca^{2+}-deficient medium was used.[23] Biochemical and topological analysis evidenced that fertilizing increased in irradiated spermatozoa.[24]

The quality of stored turkey semen was found to be improved significantly following He-Ne laser irradiation[25] and irradiation with He-Ne laser prevented their *in vitro* liquid storage-dependent damage.[26] It was found in one study[26] that irradiation increased the sperm motility index, viability, and cell energy charge. The authors concluded that laser irradiation might be a useful technique for enhancing the quality of semen in long-term storage.

Irradiation at 655 nm was found to increase the motility and velocity of dog sperm 15 and 45 min following irradiation, average velocity, linear coefficient, and beat cross frequency were statistically improved.[27] Fresh human sperm of asthenospermic patients was irradiated by 830 nm diode laser in doses of 4, 6, and 10 J/cm^2. At 30, 45, and 60 min following irradiation, sperm motility was assessed. It was found that sperm motility of the control group decreased significantly depending upon the time passed, whereas in all irradiated groups, it remained constant or even increased slightly. Significant increase in sperm motility was observed with irradiation of cells at doses of 4 and 6 J/cm^2 at 60 and 45 min after the irradiation.[28]

Both the eggs and sperm of the echiuroid *Urechis unicinctus* exhibited CO-insensitive, CN-sensitive respiration whose rate was enhanced by irradiation with white light or with visible light of various wavelengths.[16] The authors believe that these are the mitochondria that are responsible for the photoactivated CO-insensitive respiration. It is remarkable that the respiratory rate of spermatozoa in the presence of CO was enhanced in proportion to the light fluence rate. A sharp and large peak was obtained at the wavelength of 430 nm in the action spectrum of photoactivated respiration of sperm. Broad and small peaks were also found at $\sim$530 and 570 nm. Respiration in the presence of CO was inhibited by antimycin A. The authors concluded, first, that the described action spectrum could mirror the absorption spectrum of reduced cytochrome b. Second, they believed that the light absorption by reduced b type cytochrome activated the redox reaction of this cytochrome to enhance the respiratory rate.

To finish the data about the use of laser and nonlaser light sources in infertility treatment, one has to emphasize that the data are not yet abundant. There are more questions than answers. Without any doubt, this field seems to be promising. Exact mechanisms of spermatozoa movement stimulation can be explained. However, the data gathered so far suggest that primary photoacceptors are connected with oxygen metabolism and, in particular, with respiratory chains. It is important to recall that respiratory chain molecules in eukaryotic as well as in prokaryotic cells are considered as photoacceptors and photosignal transducers in these cells.[29,30] This sentence was about possible primary mechanisms of light activation of spermatozoids.

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