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Laser

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LASER

Laser in Assisted Reproduction and Genetics[1]

Laser beams were introduced to the field of assisted reproduction in the late seventies and early eighties, when the carbon dioxide laser (operating at a 10,600 nm wavelength) was coupled to operative microscopes (1) and laparoscopes (2,3) for reconstructive pelvic surgery. Later, specially designed rigid (4) and flexible (5) delivery systems for this laser, as well as other lasers [argon 540 nm (6); Nd:YAG 1064 nm (7); and frequency doubled YAG 532 nm (8)] were developed and clinically evaluated. Numerous publications described conflicting data on the potential superiority of each of these laser wavelengths for specific applications and compared them to conventional operating accessories (9,10). Different criteria for patient selection as well as personal skill and bias could affect the analysis of these data. There is no doubt that for certain applications, laser light may selectively interact with tissue (i.e., pigmented endometriotic implants), cause minimal effect to surrounding tissue, and thus offer significant advantages over other techniques. Another important characteristic of the lasers in endoscopic surgery (mainly the CO_2 laser) is the "what you see is what you get" effect (WYSIWYG). However, most indications can be handled without the use of the laser (10). Some surgeons claim that the laser is cumbersome for endoscopic application.

The experienced laser endoscopist can take advantage of the laser's special effects if properly handled, i.e., alternate quickly between a small-spot size cutting beam and a superficial vaporization using a large spot, combining maneuvers of cutting and blood vessel sealing at the same time, and using contact fibers with different tip profiles to obtain different effects (11). Controversies on the place of the laser in operative laparoscopy are expected, and one has to view its existence as an additional tool to other accessories. In experienced hands it can offer significant advantages in properly selected indications. Future developments in delivery systems, imaging technologies, and photodynamic therapy might offer significant advantages for the use of lasers in minimally invasive surgery.

Progress in the treatment of infertility, in which oocytes were fertilized outside the human body, has contributed significantly to the understanding of reproductive mechanisms. The first decade of this new era was dedicated mainly to "trial-and-error" studies, followed by controlled trials and the definition of guidelines for proper patient selection. The term in vitro fertilization (IVF) was broadened to assisted reproductive technologies (ART), and the need for more accurate equipment became self-evident. The main goals of ART today are (a) improving the implantation rate following embryo transfer, (b) improving the fertilization rate in the presence of poor sperm quality, and (c) serving as a "platform" for molecular genetic studies at the preembryo stage. Extensive basic research and clinical trials with micromanipulation techniques are being developed to achieve these goals. Procedures such as subzonal sperm insemination (12), various methods of zona opening (13,14), assisted hatching (15), and preembryo biopsy are being perfected.

The high precision of laser beams and the possibility of reducing the beam spot size from 500–1000 μm [used for tissue microsurgery (16)] to 0.5–3 μm (needed for cell and subcellular organelle microsurgery) stimulated its adaptation for ART. Using a laser-generated "optical trap," sperm could be manipulated in two (17,18) and three (19) dimensions. Initially, a Nd:YAG laser was used, at powers as low as 10–40 mW. Recent experiments in our laboratories revealed that similar effects are

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[1] With this article, we are welcoming Yona Tadir, M.D., to the Editorial Board of this journal. Dr. Tadir, who is internationally known as a pioneer of laser applications in reproductive medicine, has agreed to assure the constant update of our readers in this very rapidly developing field. *N.G.*

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achieved with a continuous-wave (CW) titanium sapphire laser (700- to 800 nm wavelength; unpublished data). Some authors discuss the potential photoactivation of sperm following exposure to a helium neon laser at low power levels (20). Drilling holes in the zona pellucida with a tunable dye laser at various wavelengths (266–532 nm) was first described in 1989 (21). Mouse, hamster, and human oocytes were exposed to laser beams in a nontouch technique. The beam was delivered through the microscope objective and the depth of the incision was monitored with a television monitor and activated by a joy stick-controlled motorized stage. This method is fast, simple, and accurate. The xenon chloride excimer laser (operating at 308 nm) in a similar nontouch configuration can be used to perform even more accurate incisions. It can be applied for laser zona drilling (LZD) and for assisted hatching (22). The accuracy of this method enables the drilling of several neighboring apertures without causing damage to the vitelline membrane and to the ooplasm. A krypton fluoride laser (operating at 248 nm) was recently applied to two-cell mouse embryos to create a 2–4 μm opening in the zona pellucida (23). The authors conclude that by selecting the right parameters, the clean cuts did not interrupt blastocyst formation and suggest this method whenever zona pellucida surgery is indicated.

A different approach using laser fibers in a contact mode was recently suggested (24). In this study, an argon fluoride laser (ArFl, at 193 nm) was delivered to the mouse oocyte zona pellucida through a series of mirrors and a long focal-length lens connected to an alumina silicate pipette. The glass pipette was pulled from capillaries with a 1-mm outer diameter to a tip of about 3–5 μm and filled with a positive air pressure. Insemination at low sperm densities led to fertilization and further development to the blastocyst stage. An early human pregnancy following erbium:YAG LZD was recently published (25). The authors discuss the potential advantage of a infrared laser (2940 nm) with regard to the potential DNA damage, as compared to the shorter-wavelength laser systems.

According to our experience, the noncontact mode may prove to be superior by taking advantage of the unique characteristics of the laser beam as a "light scalpel." Using the noncontact mode, one can eliminate the need for additional control and manipulation with holding and cutting tools (micropipette or an optical fiber). The control of the laser effect in the zona pellucida (shape and orientation) can be instantly achieved by simple adjustment of laser parameters [e.g., energy per pulse, pulse repetition rate (22)] or by moving the precision microscope stage with respect to the cutting beam. This can also be performed through culture medium or oil. Such control with the contact fiber/pipette-based system is more cumbersome and might be difficult to apply through culture medium or oil. Inactivation of a human extra pronucleus following polyspermic fertilization (26) and welding single cells with a nontouch laser beam have also been described (27). Further evaluation of laser efficacy, safety, and the preferred delivery mode is needed in these studies.

As previously mentioned, the high precision of a laser beam's small spot diameter may be applied on tiny subcellular organelles and may play a role in genetic studies. Methods describing trapping, dissecting, and manipulations of chromosomes have also been published (28).

There are more questions than answers and more concerns than conclusions with respect to the use of lasers in reproduction and genetics. The biblical quotation, "And *there was light,*" was connected to the process of creation and might also be relevant for applications of assisted procreation.

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