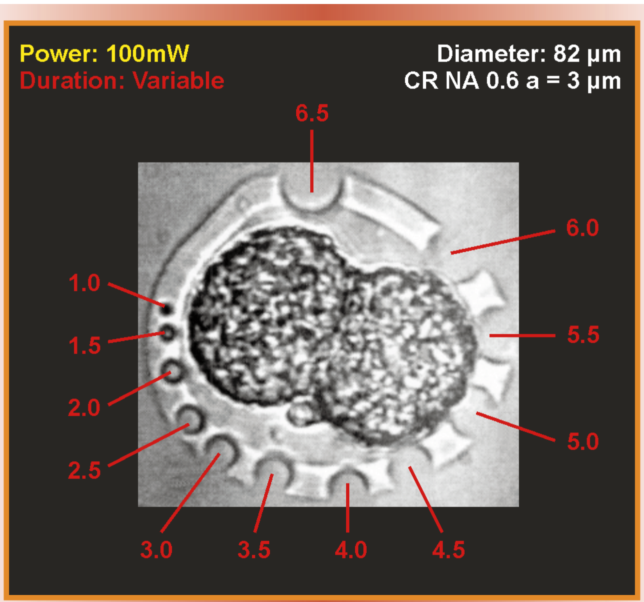
Laser-assisted technique aims to improve infertility measures

In vitro fertilization has been used to successfully treat infertility for a number of years. Even though fertilization may be successful, as evidenced by early division in the pre-embryonic stage, implantation into the uterine wall does not always occur. One reason may be that the covering of the pre-embryo, known as the zona pellucida, may be too thick or tough. Since the early 1990s, lasers have been used to cut a notch or groove in the zona to facilitate hatching and subsequent implantation of the pre-embryo.

The zona pellucida is composed of layered glycoproteins. Researchers have found that lasers in the near-IR range, 1480 nm, are most effective for cutting the zona because it strongly absorbs this wavelength. Studies have reported utilizing pulse durations of 3 to 100 ms, power of 22 to 70 mW and spot size of 2 to 8 µm to cut into the zona. Because heat absorption is the mechanism of tissue ablation by laser, the danger is that the cells of the pre-embryo immediately underlying the zona will be damaged in the process.

Diarmaid Douglas-Hamilton of Hamilton Thorne Biosciences Inc. in Beverly, Mass., and Jérôme Conia of Cell Robotics Inc. in Albuquerque, N.M., published a study in the April issue of the *Journal of Biomedical Optics* calculating the amount of heat generated by a laser beam passing through water and the zona pellucida in various configurations. They also calculated the rate of heat diffusion and the tissue temperatures at various distances from the beam, in the hope of determining a protocol of pulse duration, power and spot size that will effectively lyse the zona with the least collateral damage to adjacent pre-embryonic cells.



A series of 100-mW 1480-nm laser beams measuring 6 µm in diameter drilled the zona pellucida of the mouse embryo in this photomicrograph. Pulse duration varied from 1.0 to 6.5 ms.

For the study, Douglas-Hamilton and Conia used a Nikon TE-300 inverted microscope with an IR laser module mounted beneath the stage. The module, the ZLTS made by Hamilton Thorne Research, consisted of a diode laser, control board, adjustable collimating lens and dichroic mirror. It had a maximum power output of 200 mW at 1480 nm, and the pulse duration was adjustable from 0.5 to 25 ms. The beam traveled up through the objective and was tightly focused on the target. The beam diameter at the focal point was approximately 6 µm, and the estimated delivered energy at the focal point through the objective, container and medium was 103 mW.

In the preliminary experiment, the researchers drilled multiple channels into bovine eggs, using tangentially aimed beams with pulse durations of 25 ms and power of 50 mW. Electron micrographs showed sharply drilled channels, about 25 µm long and 12 µm in diameter, virtually cylindrical, and without evidence of beam convergence or divergence. The beams were aimed tangentially so the zone would be notched without harming the cytoplasm underlying the surface. In a pre-embryo, cells would be directly under the zona.

Using finite element analysis with the standard thermal diffusion formula and assuming the conductivity of the zona to be comparable to water, the scientists calculated the temperature diffusion for the focal point and the immediate surrounding areas for the time frame of concern, figuring in the attenuation for the converging, waist and diverging areas of the beam. They used the formula for water because, as Douglas-Hamilton explained, "In terms of absorbing the energy, and in terms of conducting it, I would expect [the zona's] behavior to be similar to water."

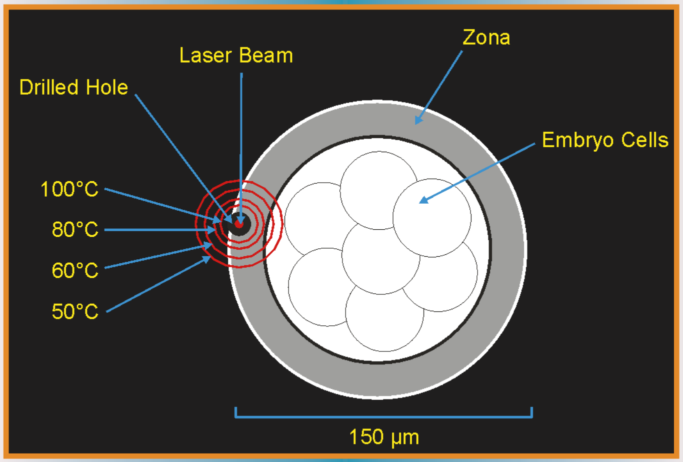
The finite element analysis showed that calculated isotherms were virtually parallel in the area of concern: the area of tangential contact of the beam with the zona and the areas immediately above and below the beam's contact throughout the time span. From this the team concluded that the beam provided even heating along its length. "That calculation shows that, in fact, it is very similar to having the entire beam in a very narrow column all the way," Douglas-Hamilton said. Of the temperature isotherms he said, "The variation is a variation only with radius, no matter what angle you are at. … It's cylindrically symmetric, not spherically symmetric."

The researchers were then able to calculate the temperature changes for beams of varying power and duration, and to plot the temperature rise for various distances from the beam. For instance, a 100-mW beam with a 3-ms duration and a diameter of 6 µm, starting at 37 °C, will rise to 170 °C at beam center at 3 ms. The temperature at 20 µm from the beam will peak at about 65 °C at 4 ms. The temperature at both locations will be about 42 °C at 15 ms and normal at 30 ms.

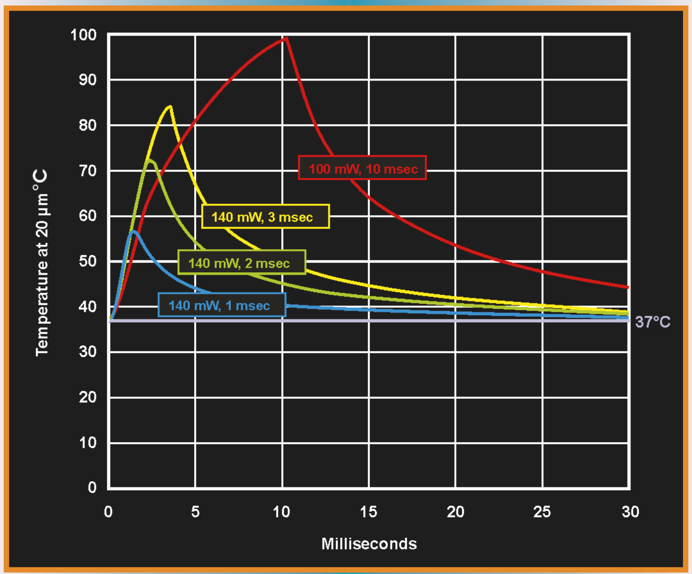
Douglas-Hamilton and Conia then set up another experiment to check the temperature excursion. Using the same laser module/inverted microscope setup, they placed a rectangular 1 × 1-mm tube full of distilled water in the area where the vertical beam would pass. The beam was focused 200 µm above the bottom of the tube. Its diameter remained about 6 µm, and the power and pulse duration could be varied. A continuous-wave HeNe probe beam approximately 45 µm in diameter was then directed at a right angle to the diode beam, and the refraction of the HeNe beam was measured at various pulse durations and power. Because the refractive index of the medium (water) varies a predictable amount with the temperature, it is possible to calculate the limit to which the temperature has been raised by measuring the refraction. The data show that the predicted high thermal gradient was present.

Douglas-Hamilton and Conia applied the formulas to their work with bovine eggs and mouse pre-embryos and estimated the central beam temperature at which the respective zonas lyse, based on the various pulse duration/beam strength paradigms. For the bovine zona, they calculated 140 to 150 °C, and 5 to 10 °C lower for the mouse zona. They estimate that approximately 140 °C will be appropriate for ablation of human zona.

The problem, however, is not in lysing the zona, but in calculating the temperature rise in the cells underlying it. They estimated that the nearest cell is 20 µm from the zona. Using their thermal diffusion formula, they plotted the maximum temperature attained by cells 20 µm from the beam center for the pulse duration/beam strength paradigms that would produce temperatures in the beam adequate to lyse the zona. For the various combinations of pulse time/beam strength that would produce lysis, the longer pulse durations produced the most increase in temperature at 20 µm. Therefore, using a higher strength beam for shorter pulse durations is clearly the more appropriate direction for this protocol.



The maximum temperature is at the center of a focused beam and decreases as it diffuses outward. The red lines depict the highest temperatures reached at various distances from the center of a 140-mW 1480-nm beam with a 1-ms pulse duration and beam diameter of 4 µm.



The temperature changes at 20 µm from the axis of 100- and 140-mW beams at different pulse lengths. The distance from the center of a zona-notching beam to the nearest cell of the pre-embryo is estimated to be 20 µm.

Douglas-Hamilton is interested in refining the refractive-index measurements that were made with the HeNe probe: "I think we will be interested in getting more exact measurements, using a finer probe than the one we used, and [making] sure that we have got the temperature measurement correct." In the experiment, the scientists used the changes in refractive index to put a limit on the temperature. He said it is difficult to measure temperature in the small areas and time spans involved. Still, "I'd like to measure it precisely," he added.

Douglas-Hamilton sees all aspects of this research going forward. "What we're interested in doing is making these lasers safe and effective for this type of zona ablation to work." Since the study was published, he has continued the research in this area and has updated the data to include beams of 140 mW. He believes that future research will include using zona ablation to assist in pre-embryonic cell biopsy and stem-cell extraction.

Laser-assisted zona-pellucida notching is another step forward in the treatment of infertility. Douglas-Hamilton and Conia's work appears to be making this technique safer and more effective. □

Dale Ott