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## Giving In Vitro Fertilization A Helping Hand

Technologies have the potential to move selection methods in assisted reproduction beyond morphology assessment

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### FUTURE BABY

An embryologist transfers an egg to a petri dish for in vitro fertilization.

Credit: Shutterstock

For many infertile couples, assisted reproductive technology such as in vitro fertilization (IVF) offers hope. But success rates are abysmal. In the U.S. in 2011, the latest year for which data have been released, 163,039 such cycles resulted in 47,818 live births and—thanks to twins and other multiples—61,610 infants, according to the Centers for Disease Control & Prevention, **which collects data from U.S. fertility clinics**. That's a take-home-baby rate of less than 30%.

In IVF, doctors give the mother hormone treatments to stimulate the maturation of extra eggs. Back in the lab, they use sperm collected from the father to fertilize the eggs harvested from the mother. The resulting embryo is allowed to develop for three to five days before doctors transfer it to the mother in the hope that it will implant itself in her uterus and result in a pregnancy.

Conventional methods of selecting eggs, sperm, and embryos are based on morphology. Fertility specialists look at each one under a microscope and judge them on the basis of appearance.

Researchers hope to improve assisted-reproduction success rates with technologies that move beyond simple morphology. They want to find methods and criteria that allow them to select the best eggs, sperm, and embryos without destroying these precious resources. These techniques include DNA analysis for counting chromosomes, microscopy and spectroscopy for gauging gamete and embryo health, and microfluidic methods for manipulating and incubating eggs. Although some of these technologies have been in development for more than a decade, most of them are still far from clinical practice. And questions remain about to what extent selection really improves outcome.

So morphological analysis remains the most common selection method. For sperm, that means making sure the cell is moving—because that means it's alive—and checking that the head is properly proportioned. "If a sperm has a small head or a pinhead, it likely has abnormal DNA or lacks DNA," says Gary D. Smith, codirector of the reproductive sciences program at the University of Michigan Medical School. "If it has a big round head, twice as big as a normal sperm head would be, you don't want to select it, because those normally give rise to abnormal embryos."

**With eggs**, embryologists check that the egg is symmetrical and at the proper stage of meiosis, the type of cell division that leads to sex cells. If the egg is at the correct stage, it will have extruded a polar body, which is a small, nonfunctioning cell that forms as a result of uneven cell division during meiosis. They're also checking that the cytoplasm is smooth, without many holes, which can indicate cellular damage.

Embryos are also graded on morphological attributes. Embryologists look at the embryos under a microscope and judge them on the basis of number of cells, symmetry, and amount of fragmentation.

Of course, "people don't measure morphology because they think morphology is the most important thing in the world," says [Daniel J. Needleman](#), an engineering professor at Harvard University whose group is starting to apply fluorescence microscopy methods to assisted reproduction. Instead, he says, they think morphology might indicate whether a given sperm, egg, or embryo is likely to lead to a viable pregnancy.

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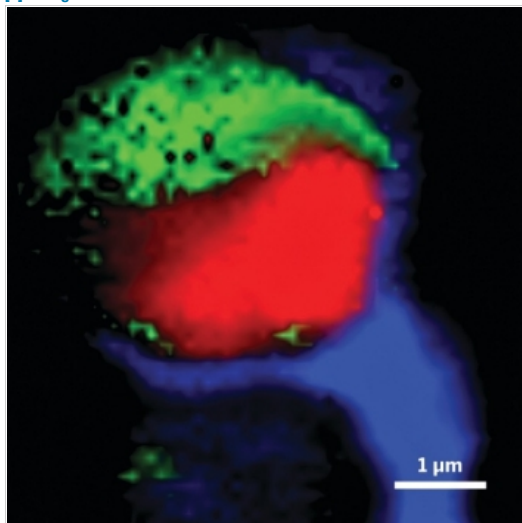
But the problem with just looking at shape is that “often you can have embryos that look really bad under the microscope but actually are quite capable of making a healthy baby,” says **Nathan R. Treff**, an associate professor of obstetrics, gynecology, and reproductive sciences at the Robert Wood Johnson Medical School and director of molecular biology research at Reproductive Medicine Associates of New Jersey. And the inverse can be true as well, Treff says. “The ones that look beautiful under the microscope may not be capable of making a baby.”

Embryologists already have one way to go beyond morphology: They have the option of removing a few cells from a developing embryo to perform more detailed genetic analyses.

For example, they can use those cells to count the chromosomes in an embryo before that embryo is transferred to the mother. This is important because a common problem in IVF is aneuploidy, having the wrong number of chromosomes. In such cases, an embryo contains an extra copy or is missing a copy of one or more chromosomes. This problem increases with the age of the mother. Aneuploidy can lead to miscarriage or birth defects.

This sort of preimplantation genetic screening (PGS) has been available for more than two decades. (PGS differs from preimplantation genetic diagnosis, or PGD, in that the latter is used to detect particular genetic disorders when one or both parents are known to be carriers.)

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#### DAD'S CELL

Raman imaging can delineate the parts of a sperm cell—protein (blue), DNA (red), and protein overlaid on DNA (green).

Credit: Hum. Reprod. Update

**Early versions** of PGS used fluorescence in situ hybridization (FISH). FISH involves use of fluorescently labeled DNA probes to identify the presence or absence of specific sequences. In PGS, it has been used as a way of counting chromosomes, but it can look at only a subset of the genome at a time.

PGS with FISH has been disappointing, Treff says. “All of the randomized, controlled trials using FISH failed to demonstrate that it improved outcomes,” Treff says. “In some of the more famous studies, it actually caused harm and reduced pregnancy rates.”

**Sjoerd Repping**, head of the Center for Reproductive Medicine at the Academic Medical Center at the University of Amsterdam, led one of those studies. In a randomized trial, he and his colleagues found that with PGS implantation rates increased but the overall pregnancy rate actually declined (*N. Engl. J. Med.* 2007, DOI: [10.1056/NEJMoa067744](#)). FISH was used in that study, and they were able to look at only nine chromosomes.

To count all chromosomes at once, newer versions of PGS swap FISH for DNA microarrays, quantitative real-time PCR (polymerase chain reaction), or next-generation DNA sequencing methods. In each case, researchers use the

sequencing technique only to count chromosomes—not to examine the sequence itself.

One such method combines a whole-genome amplification method called MALBAC (multiple annealing and looping-based amplification cycle) with DNA sequencing. The method was developed by the group of **X. Sunney Xie**, a chemistry professor at Harvard with a part-time appointment at Peking University, and coworkers.

Xie and his clinical collaborators **Jie Qiao** and **Fuchou Tang** at Peking University recently showed that they could use MALBAC-based sequencing to determine aneuploidy in eggs (*Cell* 2013, DOI: [10.1016/j.cell.2013.11.040](#)). In their strategy, Xie's team counts chromosomes in polar bodies to deduce what's in the fertilized egg. Analyzing polar bodies is less invasive than analyzing the egg itself.

The advantage of MALBAC over other methods used for PGS and PGD, Xie says, is that the amplification is more uniform across the genome, which results in a clearer readout. In addition, with MALBAC, Xie says, identification of aneuploidy and undesirable mutations related to genetic disease can be done simultaneously by actually sequencing the DNA taken from developing embryos, rather than just counting chromosomes.

Other technologies are under development to improve selection of the various players in IVF.

For example, Con Mallidis and coworkers at the University of Münster, in Germany, are developing Raman imaging as a way to select sperm cells. A significant fraction of fertility problems can be traced to the male, but it's often hard to identify the actual problem, Mallidis says. Although there are tests for gauging the health of sperm, those tests are destructive and provide information about a population of cells, not individual cells.

In contrast, Raman microscopy is noninvasive and nondestructive. Mallidis's team is using Raman microscopy to look at nuclear DNA in individual sperm cells. In a baseline study, they mapped each sperm cell by taking 2,000 Raman spectra at 50-nm intervals across the sperm head (*Hum. Reprod.* 2011, DOI: [10.1093/humrep/der122](#)). Mathematicians devised algorithms to determine which peaks were indicative of DNA damage.

"The best indicator for working out whether a stretch of DNA is intact or fragmented is the phosphate backbone, which forms a particularly prominent peak in the Raman spectrum," Mallidis says. If DNA is damaged, that phosphate peak shifts, and a shoulder peak becomes visible. Researchers can't gauge how much damage has been done, but that's not actionable information right now anyway.

"Is 50% of its DNA damaged? Is 40%? Is 10%? Is 90%? This is not an academic question because the egg has the ability to repair a certain amount of sperm DNA damage; how much we don't know. Our test picks up everything above 5%," Mallidis says. "We could be throwing away perfectly good sperm that could perform the job without any problem."

Mallidis's goal is to develop a system that could quickly analyze individual sperm and segregate them on the basis of whether their DNA is intact. Then, embryologists wouldn't have to worry about selecting a particular sperm because they would know that any of the sperm cells in a group was usable. The method is still in preclinical development. If progress continues at its current pace, "it would not be unreasonable for something to be available for clinical trials in five years," Mallidis says.

Other microscopy methods look at eggs and embryos. For instance, Harvard's Needleman has long focused on learning the details of how cells divide. Much of that work is done with frog and mouse eggs. Now he wants to apply those techniques to humans.

**Eggs and embryos** can suffer from metabolic problems, he says. Needleman's group is working to use a type of microscopy called fluorescence lifetime imaging to measure metabolic states in eggs and embryos. "A lot of small molecules that are part of metabolism are autofluorescent," he says. That means that measurements can be made using unlabeled molecules that are naturally in the embryo. "Everything we want to do is totally noninvasive."

The challenge is to figure out whether or to what extent such measurements can predict the outcome of IVF and related fertility treatments. Needleman is in discussions about spinning off a start-up venture that can take on some of these questions.

An existing early-stage company, **Auxogyn** of Menlo Park, Calif., uses microscopy to record time-lapse videos of embryos prior to implantation rather than just looking at the embryos at discrete time points. Time-lapse imaging has been available for several years, but Auxogyn's assay uses automated software to measure specific events—the timing of each of the first three cell divisions. This information is used to compute a score that predicts the development potential of embryos.

"All of our measurements are done in the first two days of embryo development," says Lissa Goldenstein, president and chief executive officer of Auxogyn. "By day three, when the embryologist is ready to really look at the embryos and decide which one, they have the data they need."

In a prospective, multicenter clinical trial with 160 patients and approximately 1,800 embryos, the assay correctly predicted at day two 85% of the embryos that arrested development by day five (*Fertil. Steril.* 2013, DOI: [10.1016/j.fertnstert.2013.04.021](https://doi.org/10.1016/j.fertnstert.2013.04.021)). Predictions made at day three with standard morphology alone predicted only 57%.

The assay has received the CE marking, which is needed to use it in Europe, but it has not yet received clearance from the U.S. Food & Drug Administration.

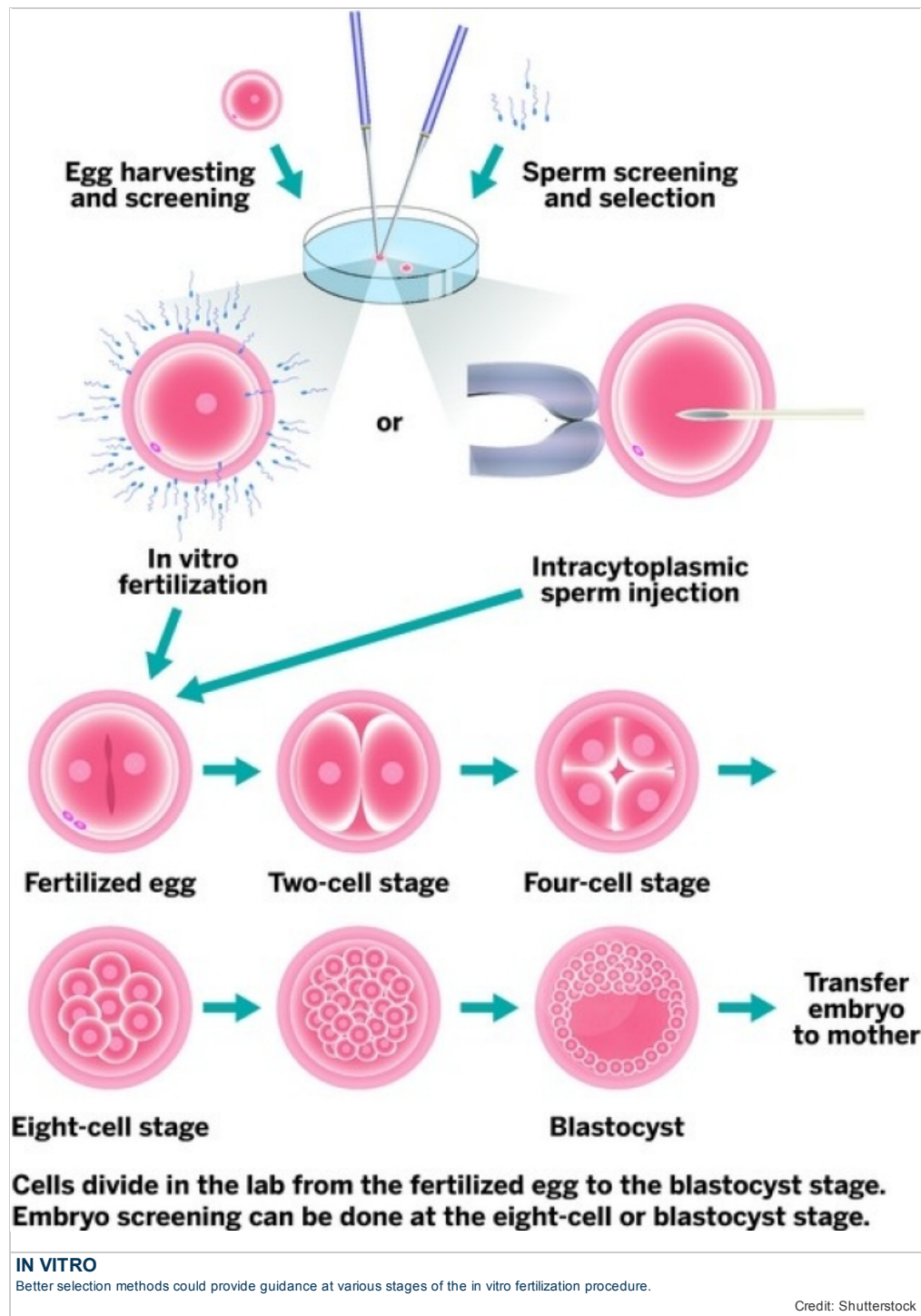
Other groups are developing ways to assess embryonic metabolism. For example, Smith and his collaborators at the University of Michigan are using microfluidic devices to culture embryos and to analyze their metabolism. In proof-of-concept experiments, they monitored glucose consumption by mouse embryos at the blastocyst stage, a structure formed at five days in which the cells differentiate into those that will become the baby and those that will form the placenta (*Lab Chip* 2012, DOI: [10.1039/c2lc21050a](https://doi.org/10.1039/c2lc21050a)). Measurements of an embryo's efficiency of glucose consumption appear to be a good way of selecting embryos with the best development potential, Smith says.

But those lab-on-a-chip studies were done in animals, not humans. Smith and his collaborators are preparing a manuscript on the first clinical trial using microfluidic devices for human embryo culture. "But it's taken us over 10 years to get here," he says, because research on human reproduction is such a sensitive topic. "Nobody's going to do the study for humans without data first from animal models. These aren't leftover pieces of tissue. These are embryos from people who are trying to make children."

Many of these technologies are far away from clinical practice, but others, such as PGS, are already available. Meanwhile, fertility clinics continue to wrestle with how commonly PGS—or any of these technologies—should be offered.

"Some programs are a little more conservative and rarely offer PGS to patients because they're not convinced it helps; other places will offer it to every patient who comes through the door," Treff says. He says that most people working in IVF agree PGS should be offered to women over 35. "Whether or not they want to use it should be their choice. It may not be a cost-effective solution for everyone." But every patient needs to have the option of making that decision, he says.

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