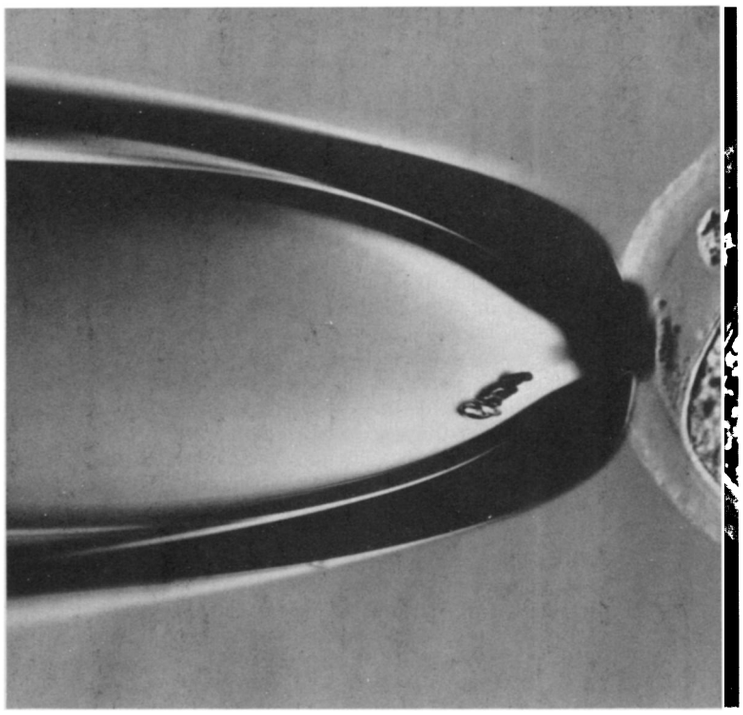
Zona Blasters

There's more than one way to crack an egg

By KATHY A. FACKELMANN



The microscopic human egg floats in its fluid-filled shell. Suddenly, thousands of tiny sperm bombard it. Lashing their tails to power their entry, they bore into the shell, a tough glycoprotein coating known as the zona pellucida. One particularly vigorous sperm pierces the zona barrier, setting off a chemical reaction that shuts the others out. Then, if all goes well, the winning sperm fertilizes the egg and the miracle of human life ensues.

This dramatic micro-scenario can occur in the womb or in a laboratory dish. But what happens when only a few sperm cluster at the zona surface? And what if the sperm can't whip their tails hard enough to bore through the tough outer shell?

For centuries, such setbacks have thwarted conception. But scientists are now developing imaginative methods of cracking, blasting or drilling tiny passageways through the egg's zona envelope, and although their techniques remain experimental and show only limited success so far, they may one day give sperm a better shot at fertilization.

The new work builds on a landmark achievement of 1978: the birth of Louise Brown, the world's first "test tube" baby. A British team pioneered this technique, now called *in vitro* fertilization (IVF), to help women with blocked fallopian tubes. Today, IVF specialists around the world routinely incubate human eggs and sperm in a laboratory dish and then gingerly transfer a fertilized egg to the mother's uterus.

However, IVF alone fails to help most infertile couples, particularly when the fertility problem rests with the sperm. For even a modest chance of pregnancy with IVF, the male partner must produce a healthy number of sperm. Each IVF attempt typically involves at least 50,000 motile sperm, incubated with several eggs in a petri dish, and even the most successful clinics working with sufficient numbers of sperm offer only a 20 percent prospect of pregnancy per attempt. The fertilization rate plummets as sperm counts drop or when the sperm swim feebly or not at all.

Many couples beset with sperm-related infertility undergo repeated IVF attempts in which the sperm never make it past the zona barrier. Until a few years ago, the problem appeared untreatable. But new research findings, including several announced in October at the American Fertility Society's annual meeting, strengthen the growing belief that a variety of novel zona-opening methods will someday revolutionize the treatment of male infertility.

Scientists must resolve many technical glitches before such methods can play a routine role on the road to the nursery, cautions Robert J. Stillman, a reproductive endocrinologist at George Washington University in Washington, D.C. Nonetheless, he says, the recent work points to encouraging avenues for treating previously intractable infertility.

Perhaps the most dramatic means of achieving conception when sperm don't measure up involves drawing a single human sperm into a tiny glass needle and injecting it just beneath the zona. Australian scientists first described this technique in the October 1987 FERTILITY AND STERILITY.

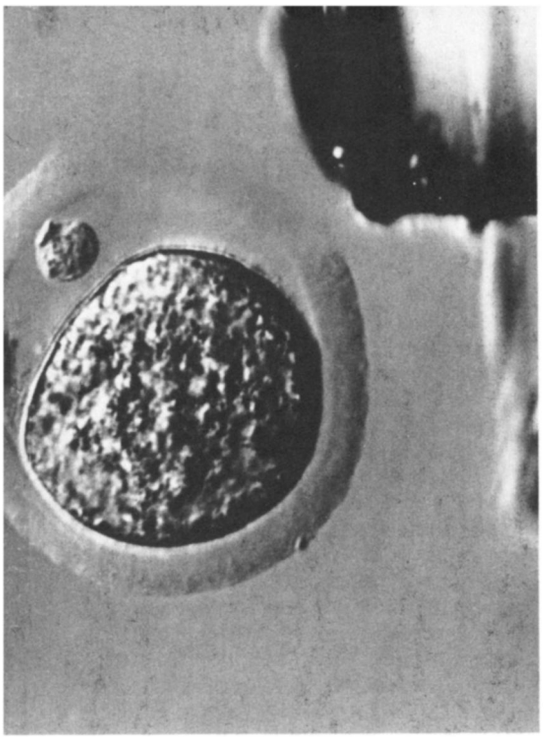
The research team, led by Andrea Laws-King and Alan Trounson of Monash University in Melbourne, squirts one sperm into the perivitelline space, a fluid-filled area between the zona and the membrane enclosing the egg itself. In their 1987 report, they described successful fertilization of five of seven human eggs.

The Melbourne project has not produced any babies. However, other researchers using similar techniques are reporting some success.

For example, another Australian team used a similar microinjection method with 40 infertile couples. In all cases, the male partner had a low sperm count or abnormal sperm. Working under a microscope, Janine Lippi and her colleagues at Sydney IVF Propriety Limited used a microneedle to suction off about six sperm, and then inserted the captured sperm beneath the zona. Because this method bypasses the normal zona-closing mechanism, it sometimes allows more than one sperm to penetrate the egg membrane, creating a "polyspermic" egg that can't develop properly and must be discarded. After repeated attempts, the researchers obtained at least one usable embryo (fertilized egg) for 32 of the 40 women in the study. They then carefully transferred each embryo from its laboratory dish to the mother's uterus.

Three pregnancies resulted, and two of those women delivered healthy babies, Lippi reported at the October meeting.

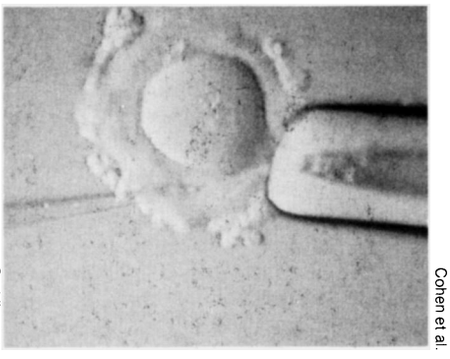
While two births among 32 couples isn't much of a success rate, Lippi points out that many of the couples in her study had already failed conventional IVF attempts and seemed to have no other hope of producing a child. Even under natural conditions, the average embryo has only a 25 to 35 percent chance of completing its incredibly complex, nine-month journey to delivery; the extra complications of laboratory tinkering can further lower those odds. For instance, scientists might unwittingly damage an egg during micro-



Coddington

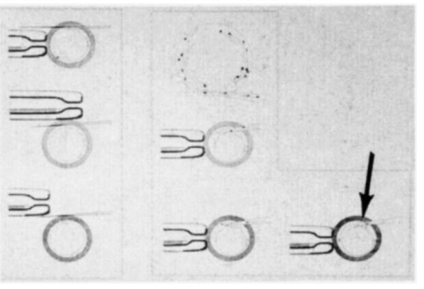
Left: A suction pipette holds a hamster egg in place while scientists blast an opening in its shell with a laser.

Below: Microinjection in progress.



Cohen et al.

DISSECTION MICROINJECTION



Cohen et al

Above: Two techniques for penetrating the egg's outermost shell. In zona dissection, scientists steady the egg with a holding pipette, spear the zona shell with a tiny needle and mechanically grind an opening in the shell. In the microinjection method, researchers draw sperm into a needle, pierce the zona (arrow) and release the sperm into the underlying perivitelline space. Sperm still must burrow past a thin membrane enclosing the egg.

injection or when transferring it to the uterus after fertilization, preventing successful implantation.

Moreover, researchers still need to overcome technical problems that severely limit their fertilization rates. At present, the Sydney team and others must gather hundreds of eggs to obtain a few usable embryos. Lippi says her group injected a total of 585 eggs with sperm but fertilized only 95 of them (16 percent). Of those, 35 proved polyspermic and had to be discarded.

Lippi thinks part of the problem traces to the injection of some sperm that retain the "acrosome cap," a glycoprotein coating normally shed from a sperm's head as it approaches the zona. Sperm injected into the perivitelline space with these caps still in place cannot fertilize the egg, she says. Because researchers cannot distinguish between sperm with and without acrosome caps, they must inject more than one sperm into an egg in order to get a decent shot at fertilization – a practice that sometimes produces useless, polyspermic eggs, says Lippi.

She and her co-workers hope to develop a reliable method of stripping the caps from sperm. At this point, they are working on a technique for dissolving the structure by zapping sperm with an electric shock. Lippi says it's too soon to tell whether the shocks can produce enough acrosome-free sperm – without damaging them – to boost fertilization rates.

A number of research teams in the United States are racing to develop or perfect their own zona-penetrating techniques. At the IVF lab at Cornell University Medical Center in New York City, Jacques Cohen and Henry E. Malter are testing a microinjection method similar to that of the Australian groups, using a microneedle to suction up several sperm and inject them into the perivitelline space. They have also developed a new method called zona dissection, using tiny instruments to grind an opening through the zona. Eggs with a breach in the zona are then incubated with sperm, which must find the artificial channel and wriggle their way through.

So far, the Cornell team has used either microinjection or zona dissection with 229 women. The results include 48 pregnancies and 19 healthy babies, Cohen told SCIENCE NEWS.

Trying to spear a microscopic egg with an even tinier needle isn't easy. And Cohen says the grinding technique often creates a gaping slash in the zona, letting more than one sperm inside. Such problems have prompted several other investigators to seek alternative strategies for getting sperm past the zona barrier. One of the most promising of these involves punching a discrete hole in the zona with a tiny laser beam.

At the American Fertility Society meeting, Charles C. Coddington of the Jones Institute for Reproductive Medicine in Norfolk, Va., described his group's work with a yttrium-aluminum-garnet laser. The researchers blasted a microscopic hole in the zona of 12 hamster eggs, then incubated the eggs with human sperm from a fertile male volunteer. The sperm clustered around the laser-created opening in 11 of the eggs, reports Coddington, who says this boosts hopes that the team can refine its technique for use with infertile human couples.

His team used human sperm because it withstands laboratory handling better than hamster sperm. "You can throw [human sperm] on the floor, pick it up, wash it off, and you're ready to go," Coddington says. Human sperm can enter a hamster egg but cannot fertilize it, he adds. This means the researchers must find a way to use eggs *and* sperm from hamsters, or perhaps switch to another animal model, before attempting human trials.

At the Mount Sinai School of Medicine in New York City, molecular biologist Jon W. Gordon and his colleagues have pioneered another method, known as zona drilling. In 1986, they reported using acid to dissolve a hole in the zona of eggs removed from mice. Many scientists had speculated that sperm would immediately penetrate the zona when incubated with such acid-drilled eggs. At the October conference, however, Gordon presented new findings suggesting that sperm need plenty of time to find the artificial opening and wriggle inside.

The researchers began their recent experiment by washing 139 mouse eggs in acidic chemicals to strip away the entire zona, leaving the eggs completely exposed. Then they used a microneedle to squirt tiny droplets of the same chemicals on another 49 mouse eggs, "drilling" an opening into each zona. Finally, they doused the 139 zona-stripped eggs and the 49 drilled eggs with sperm. The sperm penetrated the stripped eggs immediately, but with drilled eggs they took 30 to 60 minutes to locate the hole, penetrate the egg membrane and reach the egg interior, Gordon told SCIENCE NEWS.

The finding, he says, may help clinicians fine-tune the incubation of human sperm and drilled eggs and thus increase the odds of fertilization.

Still another strategy for slipping sperm past the zona barrier comes from

Continued on p.379

Nearly three-quarters of its total R&D budget will support Space Station Freedom (at $1.9 billion) and space-science research. However, Congress warned NASA to keep tight reins, particularly on the development of the space station, now consuming almost one in every three NASA R&D dollars. The lawmakers have required the agency to submit a less costly design for the space station by February, and has restricted increases for this program to no more than 10 percent a year (regardless of inflation) and a total of $2.6 billion annually.

Though Congress killed plans to launch new programs aimed at sending human missions to the moon and Mars – a much-publicized goal of President Bush – the lawmakers did provide $263 million for one new project. Known as Mission to Planet Earth, this program aims to study Earth's climate from orbiting platforms and small spacecraft.

EPA: Congress increased the Environmental Protection Agency's overall R&D funding to an estimated $433.6 million, a figure that did not keep pace with inflation. The 25 percent increase (to about $18.6 million) in the agency's global climate change programs increased at five times the inflation rate. And for the first time in EPA's history, research spending in its Superfund (toxic-waste cleanup) program suffered a decline – of $5.6 million, or roughly 12.5 percent after inflation. Congress did appropriate $400,000 for one new program: an independent evaluation of the nation's environmental research – a possible first step toward creating a national environmental institute akin to NIH.

DOD: The $35.9 billion that Congress appropriated for defense research, development, test and evaluation programs represents about $2 billion less than the President had requested – and a decrease of roughly 7 percent in spending power, after accounting for inflation. Lawmakers saved their single largest R&D cut for DOD's Strategic Defense Initiative. Though the President had sought $4.5 billion, Congress appropriated only $2.89 billion – 19 percent less than last year – and directed DOD to reevaluate this program with an eye toward cutting costs and boosting efficiency. Congress also approved the appointment of a joint DOD/civilian commission to analyze DOD's options for cutting costs and reorganizing the Pentagon's 72 R&D labs.

Although the President had sought to cut funding for DOD's Advanced Research Projects Agency by 12 percent, Congress boosted that agency's budget by 14 percent, to $1.4 billion. This figure targets increases for advanced submarine technology and X-ray lithography, and money to create a $50 million program aimed at developing new technologies. The new budget package also directs DOD to spend $20 million more on graduate training, $50 million to establish an engineering training institute and $150 million to launch a Strategic Environmental Research Program that will share DOD's environmental and global-change data with other U.S. agencies.

DOE: The 1 percent increase ($74.7 million) Congress approved for the Department of Energy's total R&D budget – $6.9 billion – does not keep pace with inflation. Moreover, most of the increase is earmarked for projects "that amount to pork-barrelling," says DOE spokesman Jeff Sherwood. That leaves only about $191 million – the amount being cut from the agency's defense programs – for reallocation elsewhere in DOE's civilian programs.

The Superconducting Super Collider received just $242.9 million (including $25 million held over from the 1990 budget), a "paper" increase over 1990's allocation. Solar energy programs, by contrast, reaped a real bonanza – an increase of 46 percent above 1990, to $131 million. Fossil energy programs, primarily coal research, also increased notably – to $459 million, an increase of 11.8 percent. Congress slashed "hot" fusion funds to $275 million, however, for a decrease of 15 percent.

NIST: The 31 percent increase Congress slated for R&D programs at the National Institute of Standards and Technology exceeds that for any other agency, and will allow NIST to launch several new programs: a $900,000 "intelligent processing of materials" initiative, a $1.48 million "intelligent machines" venture and a $1.5 million advanced semiconductor program. Funding for its chemical measurements and standards program more than tripled (to $1.4 million); its Manufacturing Technologies Centers received almost a tripling in funds ($11.9 million); its lightwave technology program more than doubled (to $4.23 million); and a computer-security program focusing on "viruses" increased by 40 percent (to $3.5 million).

As the White House prepares to release its 1992 budget request in January, a Nov. 13 update of a Congressional Research Service report warns that things look less rosy for science over the next two fiscal years. The 1991 Budget Reconciliation Act limits the annual growth for all "nondefense discretionary funding" to 4.7 percent in 1992 and 3.7 percent in 1993. "This will place severe limits on future increases for civilian R&D funding and could force the restructuring of a number of big science projects," the report concludes. But Presidential Science Adviser D. Allan Bromley says that for now at least, "Congress had done very well by science and technology." □

Continued from p.377

Johns Hopkins University in Baltimore, where John Hesla and his colleagues use a variation on Cohen's grinding method.

Hesla's group first placed rabbit eggs in a sugar solution to enlarge the perivitelline space. Taking care not to damage the eggs themselves, the researchers speared the zona with a microneedle and mechanically ground away a portion of the zona shell. Then they mixed the altered eggs with rabbit semen containing a high concentration of sperm. At the infertility conference, Hesla reported an 80 percent fertilization rate – comparable to that achieved when the team incubated unaltered rabbit eggs with high concentrations of sperm.

With sperm concentrations, the fertilization rate for unaltered eggs dropped to 7.5 percent, whereas the zona-opened eggs showed a much better rate of 36 percent, he says.

"The technique, if perfected, could be a new dimension for *in vitro* fertilization," Hesla suggests.

Stillman and others speculate that if researchers can improve on their fertilization rates and adapt the still-experimental techniques for widespread human use, zona opening might offer hope not only to couples with sperm insufficiencies but also to those whose infertility results from other factors, such as zona abnormalities that block the entry of sperm.

The new micromanipulation techniques do raise some safety concerns, however. Richard D. Amelar of New York University, a specialist in male infertility, suggests that such methods might give an edge to genetically defective sperm. He wonders whether immobile or feeble sperm – which normally don't stand much chance of penetrating the egg's inner sanctum – might be more likely to harbor serious DNA mutations than their more vigorous counterparts. "Are we creating embryos that are genetically abnormal?" Amelar asks.

So far, researchers have reported no evidence of such problems. Cohen says he finds no correlation between poorly swimming sperm and genetic abnormalities, and Lippi says her group did not detect an increase in genetic problems when comparing embryos fertilized by needle with those produced in the standard IVF procedure, in which the sperm do the work of cracking the zona.

In any case, clinicians remain cautious about recommending the experimental procedures to infertile couples, noting that investigators have yet to achieve significant success rates in humans.

"Unfortunately, progress to date has been disappointing. We would have liked, over the last three or four years, a bit more of a breakthrough," says Stillman. "It may come, but we haven't seen it yet."

Cohen adds, "I think there's hope." □