While researchers in many countries engage in political battles over human embryonic stem cells, Israeli scientists have moved to the vanguard of this scorching field

In the Mideast, Pushing Back The Stem Cell Frontier

JERUSALEM AND HAIFA—The other passengers on the flight from Singapore to Australia never suspected that a small flask of reddish liquid tucked in Benjamin Reubinoff's shirt pocket contained what is now one of the hottest commodities in biomedical research. It was September 1998, and the fertility specialist was carrying one of the first preparations of human embryonic stem (ES) cells.

Reubinoff was a long way from his home in Israel. In January 1998, the gynecologist at Hebrew University's Hadassah Medical Center in Jerusalem had come to Australia to spend a 1-year sabbatical with Alan Trounson and his colleagues at Monash University in Melbourne. Reubinoff had joined a high-risk project: Trounson's team had struggled for years to derive ES cells from human embryos. "We could get them formed but couldn't keep them maintained" for long, Trounson recalls, so the effort had stalled. Reubinoff knew nothing about the top-secret work when he first approached Trounson, a well-known embryologist and in vitro fertilization (IVF) expert, about a place in his lab. When Trounson filled him in, Reubinoff jumped at the opportunity.

At the forefront. In 1998, Benjamin Reubinoff helped energize a pioneering stem cell project in Australia before coming home to the Hadassah Medical Center to establish his own research effort.

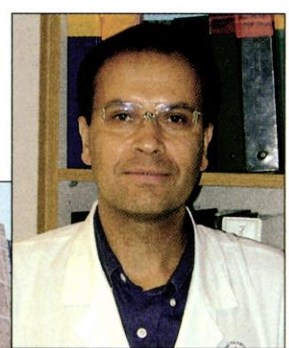
Reubinoff's enthusiasm and grit at the lab bench—along with that of developmental biologist Martin Pera of Oxford University—energized the project. Because the state of Victoria outlaws research using human embryos, Trounson was collaborating with Ariff Bongso at the National University of Singapore, where no such prohibitions existed. So in August 1998, Trounson dispatched Reubinoff to Singapore for another attempt at creating a stable line of human ES cells. Within a few weeks the team succeeded, deriving the cell line that Reubinoff then kept warm in his shirt pocket on the flight to Australia.

Back at Monash, Reubinoff logged 15-hour days for months to concoct a recipe that would keep the vexing cells dividing but not maturing. "His persistence in the face of frustration really made the project work," says Trounson. Reubinoff's sabbatical extended into a 2-year stay and a Ph.D. He also helped put Israel on the stem cell map.

With their magical potential to transform into any cell type in the body, human ES cells have kindled hopes for new treatments for the millions of sufferers of Parkinson's disease, Alzheimer's, and other killers that share a hallmark feature: cell death. But because ES cells are culled from early embryos that are destroyed in the process, they are at the center of heated debates over research ethics and the sanctity of life.

Indeed, in many countries, biologists have spent more time lobbying politicians and courting public opinion than they have in their labs learning about the cells. But that's not the case in Israel. Thanks to liberal regulations governing embryo research and broad public support, scientists here have been at the forefront of ES cell research. Reubinoff and gynecologist Joseph Itskovitz-Eldor of the Rambam Medical Center at the Technion in Haifa were key players in the landmark isolation of stem cells from human embryos in 1998. And of the first 12 publications on human ES cells, 10 included Israeli authors. "There's less of a pall over the work in Israel," says stem cell expert George Daley of the Massachusetts Institute of Technology in Cambridge, who collaborates with Itskovitz-Eldor.

Because of their head start, Israeli scientists have helped set the pace for the rest of the world. Researchers here, with U.S. collaborators, were the first to publish detailed descriptions of the differentiation of human ES cells in culture in October 2000, and they were the first to report the genetic modification of the cells 6 months later. The Israeli teams "are very important players" in doing the fundamental work of figuring out how the human cells work, says Ron McKay of the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, a specialist on mouse ES cells.



Far-flung connections

When James Thomson of the University of Wisconsin, Madison, and his colleagues first announced the isolation of stem cells from human embryos in November 1998, the news took the scientific community by storm. Although several groups had been racing toward this goal, they had mostly kept their progress under wraps—to prevent tipping off competitors and to avoid the tumultuous public attention that has buffeted the young field ever since.

Although the initial successes happened about as far from Israel as one can get—in Wisconsin and in Melbourne—both teams had Israeli collaborators. Thomson was working with Itskovitz-Eldor, who in 1997 had sent him more than a dozen frozen embryos donated by Israeli couples in IVF clinics. One of Itskovitz-Eldor's graduate students, Michal Amit, carried the frozen embryos to Thomson's lab and assisted in the project. Four of the five cell lines the team first described (*Science*, 6 November 1998, p. 1145) came from Israeli embryos. Just before publication, Itskovitz-Eldor carried cells from all five lines back to Israel.

CREDIT: (LEFT TO RIGHT) AVI HAYUN/HADASSAH MEDICAL CENTER; COURTESY OF B. REUBINOFF

Stem cell central. Researchers from six countries (red arrows) have shuttled to Israel to study the fine art of stem cell science. So far Israel has shared its human ES cell lines with scientists in one country, the United States (blue arrow).

Itskovitz-Eldor and Reubinoff credit advances in IVF techniques for making the derivations possible. Scientists could not simply follow the recipe used to create most mouse ES cell lines. These are derived from blastocysts, which develop about a week after fertilization when the embryo forms a shell around a cluster of cells called the inner cell mass. The blastocysts were flushed from pregnant mice to get the inner cell mass, which gives rise to ES cells. As that method would never be morally acceptable in pregnant women, researchers knew they would have to find a way to derive ES cells from test tube embryos. In the mid-1990s, says Reubinoff, "there was a big question whether it could be done."

The problem was that researchers did not know how long human embryos could last outside the body. Until the mid-1990s, the normal routine at IVF clinics was to transplant embryos into a patient about 3 days after fertilization, before the inner cell mass develops. However, hypothesizing that embryos that survive to develop healthy blastocysts would be more likely to establish a successful pregnancy, IVF researchers in the United States and Australia developed methods to keep embryos alive longer in culture. Given the importance of IVF expertise and connections to the research, it isn't surprising that Israeli researchers were involved, says Reubinoff. "According to Jewish tradition, to procreate is very important," he says. "There is a lot of support for infertility treatments and a very large number of IVF clinics in Israel."

Fundamental firsts

When Thomson's team introduced the world to human ES cells, developmental geneticist Nissim Benvenisty of Hebrew University in Jerusalem leapt to take advantage of the breakthrough. As one of a handful of researchers who had studied how mouse ES cells transform into mature cells, Benvenisty had long been captivated by the potential of using human ES cells to probe early development and perhaps to treat diseases. "I had been waiting for years for someone to isolate human ES cells," he says. The day he read about the Wisconsin findings, he phoned co-author Itskovitz-Eldor, just 2 hours away in Haifa, to discuss the work. The conversation went so well that Itskovitz-Eldor drove to Jerusalem later that week to hash out a collaboration.

Their first project was to test whether human ES cells, like those from mice, can form clusters of differentiating cells called embryoid bodies. Initial studies by Thomson's group suggested they didn't. But by learning how to grow the cells suspended in liquid rather than flat on a dish, the labs found that they could. "[Benvenisty] is excellent at taking what has been done in the mouse cells and translating it to human cells," says developmental geneticist Austin Smith of the University of Edinburgh, U.K.

Benvenisty and company scored several other firsts. In October 2000 the team, along with cell biologist Doug Melton of Harvard University, published the first paper on how growth factors, such as bone morphogenic protein 4 and fibroblast growth factor, prompt human ES cells to mature into different cell types. They struck again last spring with the first report on the stable genetic modification of human ES cells. In that work, they inserted into stem cells a gene for green fluorescent protein, which glows in immature cells and shuts off as they begin to differentiate. The cell line should prove a boon to research, as it enables researchers to easily sort immature ES cells from those that have begun to transform.

Benvenisty's group is also collaborating with Melton to tackle a mountain on the stem cell landscape: targeting genetic changes to a specific gene or spot in the genome. This would allow researchers to knock out or modify specific genes. Success would bring them closer to the Benvenisty lab's ultimate goal: creating human ES cells that are not attacked by the immune system. Cells in the body display proteins called HLA antigens that help the immune system tell friend from foe. Like organ transplants, ES cells infused in a patient would trigger a potentially fatal reaction unless the immune system were suppressed, but suppression triggers a host of side effects that could doom a potential treatment. One way to avoid the problem, says Benvenisty, might be to knock out or shut off the HLA genes. Such modified cells could be a universal donor, like type-O blood, accepted by all patients' immune systems.

A northern leading light. Joseph Itskovitz-Eldor's lab in Haifa has managed to coax ES cell lines to grow on human "feeder" cells.

Mouse-free ES cells

A few hours north of Jerusalem, in a 13th-floor lab with a sweeping view of the sun lovers on Haifa beach and the Mediterranean's turquoise waters, Itskovitz-Eldor's team has notched its own set of firsts. The group has recently managed to overcome one practical hurdle standing in the way of using human ES cells as therapy. To keep cells undifferentiated, scientists grow them on a "feeder layer" of embryonic mouse cells, which generates an as-yet-unknown cocktail of proteins that signal cells to remain immature. That means all existing human ES cell lines have been exposed to mouse cells—and possibly to unknown pathogens. Itskovitz-Eldor's group has figured out how to remove mice from the picture by growing ES cells on feeder cells derived from human fetal tissue.

Itskovitz-Eldor and Amit also continue to create new cell lines. They are working on a new method, in which embryos are allowed to develop several days beyond the blastocyst

CREDIT: (BOTTOM) COURTESY OF J. ITSKOVITZ-ELDOR

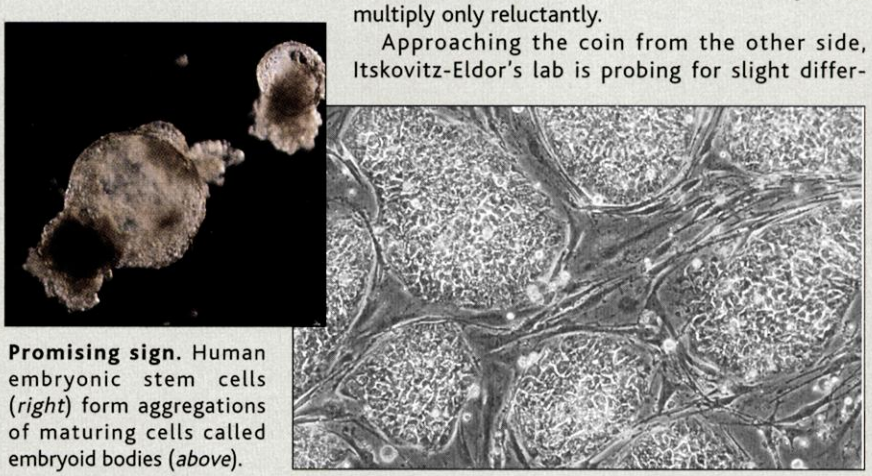
Are Any Two Cell Lines the Same?

HAIFA AND JERUSALEM—Although the U.S. National Institutes of Health lists 72 human embryonic stem (ES) cell lines as "approved" for use by NIH-funded researchers, fewer than half a dozen so far have been distributed to scientists outside the labs where they were derived. That has hampered efforts to explore one of the field's burning questions: whether the cell lines, with their different pedigrees, act differently in culture.

"Everything which is different is better," explains Joseph Itskovitz-Eldor of the Rambam Medical Center at the Technion in Haifa, as subtle variations in some cell lines might point to novel properties that could be exploited to transform the cells into specific tissues at will. Guiding this maturation process has so far proved quite difficult—but it will be essential if scientists are to realize their dreams of using the cells to treat disease.

First, however, researchers have had to determine whether human ES cells behave similarly to the well-characterized mouse ES cells. And that's not so straightforward. Although in 1999 researchers in Nissim Benvenisty's lab at Hebrew University in Jerusalem were able to grow human ES cells derived in Wisconsin into embryoid bodies—a hallmark of the maturation process in mouse ES cells—a team in Australia said its cells wouldn't form the aggregations. Was there something inherently different about the cell lines?

Benvenisty's lab is now the only one in the world using cells from Wisconsin (three lines) and Australia (one line) side by side. The team's results are still preliminary, but they appear to suggest that cells under the same conditions do respond similarly.



Promising sign. Human embryonic stem cells (*right*) form aggregations of maturing cells called embryoid bodies (*above*).

At the same time, each line has its own "personality." So far, says Maya Schuldiner, a graduate student in Benvenisty's lab, the most finicky cells in their hands are from the H1 line from Wisconsin. This happens to be the only cell line, out of five derived there, that WiCell—the company that the University of Wisconsin, Madison, set up to distribute the cells—is sending to scientists. The cells are difficult to sustain, Schuldiner says, and multiply only reluctantly.

Approaching the coin from the other side, Itskovitz-Eldor's lab is probing for slight differences among various lines. In his lab, graduate student Michal Amit is screening dozens of subclones—lines propagated from a single ES cell—for those that might form cardiac muscle cells more readily. According to Amit, some lines spawn cardiac muscle cells only rarely, whereas others consistently form beating clusters. The team hopes to use gene chips to discover whether different patterns of gene expression among the cell lines help determine their fate. **–G.V.**

stage. The researchers hope their latest cell lines might grow in culture more easily or develop into target tissues more readily. Although Itskovitz-Eldor is reluctant to discuss the issue, the new technique might also fall outside the broad patents owned by the University of Wisconsin that cover cell-line derivation using Thomson's method.

A few floors below Itskovitz-Eldor and his colleagues in Haifa, Karl Skorecki's lab in the Rappaport Institute at the Technion is studying lines of ES cells that have been tweaked genetically to churn out loads of telomerase, a protein that adds "caps" to the ends of chromosomes to protect them from degradation after multiple divisions. The team has shown that the enzyme, which is active in undifferentiated ES cells, normally shuts off as cells begin to differentiate. The hope is that differentiated cells in which telomerase stays active undergo more divisions, enabling researchers to grow larger batches of a tissue—a boon for the development of potential therapies.

Masters of translation. Getting human ES cells to perform like the well-studied mouse cell lines is what Nissim Benvenisty (far right) and his team at Hebrew University do best.

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Four musketeers

In a field known for its secrecy and competition, the Israeli teams stand out in another way: The four groups have linked up on a grant proposal to the Israeli Ministry of Science for up to 2 million shekels ($430,000). Their goal is to study how human ES cells develop into four key tissues: blood, pancreas, neurons, and liver. They hope that banding together on a broad proposal will convince the ministry to give them a relatively large chunk of Israel's limited medical research funding. "There is a lot of technology developed within the groups here," says Reubinoff. "If we can join forces, we can move the field more quickly forward."

And as more researchers worldwide gain access to the cells, the Israeli labs are becoming ever more popular, with scientists from half a dozen countries (see map, p. 1819) making pilgrimages to Jerusalem or Haifa to learn from the masters. "For scientists to make this technology applicable one day to patients requires collaboration with the whole world," Benvenisty says. And with stem cell advances pouring out of Israel, the steady flow of visitors seeking knowledge is unlikely to abate anytime soon.

–GRETCHEN VOGEL