Test-Tube Diagnosis

Analyzing embryos for genetic flaws

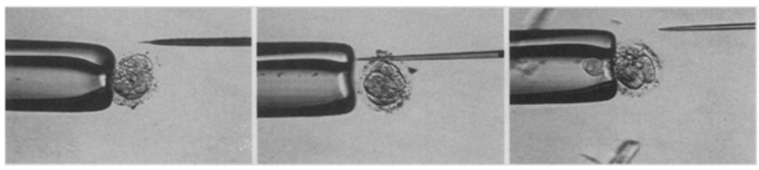
By KATHY A. FACKELMANN

Renee and David Abshire's only child, Maigon Nicole, died at age 3 of Tay-Sachs disease, an incurable inherited disorder. Infants born with Tay-Sachs appear healthy at birth but within 6 months begin to show signs of mental retardation, blindness, and paralysis. They usually die by age 4.

The Abshires wanted children, but they couldn't face the risk of having another child die from this disease.

So the Louisiana couple made their way to the Jones Institute for Reproductive Medicine in Norfolk, Va. Researchers Gary D. Hodgen and William E. Gibbons told them that an experimental procedure called preimplantation diagnosis might help them deliver a healthy child.

Renee and David agreed to participate in a clinical trial of this method, which combines in vitro fertilization (IVF) with genetic testing of the embryo.



Genetics & IVF Institute

In preimplantation diagnosis, a researcher first holds the embryo in place (left) and makes a tiny incision in the outer shell (center). He or she uses a pipette to remove a single cell (right) and later tests it for genetic defects.

Standard IVF involves uniting egg and sperm outside the body in a petri dish. In such cases, technicians look at the embryos under a microscope and transfer those that appear normal to a woman's uterus. With preimplantation diagnosis, researchers harness a powerful molecular technique to peer into the embryo's genetic code.

Rather than have to agonize over whether to abort a fetus with a devastating disease like Tay-Sachs, a couple can choose to transfer to the uterus those embryos free of a particular genetic defect. At this stage of development, the embryos are tiny — only a fraction of the size of the period at the end of this sentence.

Doctors at the Jones Institute removed and fertilized seven eggs from Renee Abshire's ovaries. For technical reasons, the team could test only four of the seven embryos. They found that only one had inherited the genetic flaw causing Tay-Sachs. Doctors transferred to Renee Abshire's womb the remaining three healthy embryos, one of which implanted.

For Renee and David, the new technology brought about a joyful conclusion to their struggle to have another child. Their healthy baby girl was born in January 1994 — "the first successful birth of a child screened for Tay-Sachs," notes Gibbons.

Worldwide, an estimated 29 children have been born with the help of this technology, according to a report by the Human Embryo Research Panel. Last month, that advisory group of 19 scientists, lawyers, ethicists, and others recommended that the National Institutes of Health begin paying for research involving human embryos (SN: 10/1/94, p.212). The panel specifically okayed the use of federal funds to study preimplantation diagnosis.

Many U.S. clinics have not waited for the federal okay. Using private funds, they have forged ahead with clinical trials of this technology. In addition to the scientists at the Jones Institute, researchers at another Virginia facility are analyzing human embryos for a genetic flaw that causes a form of mental retardation. And a New York team has devised a method that may help older women, who are at risk of having a child with chromosomal abnormalities such as the one causing Down's syndrome. Still others are working on a method to select a baby's sex based on the type of sperm that fertilizes the egg (see sidebar).

Couples who opt for preimplantation testing aren't necessarily infertile. In many cases, they know they're at risk of having a child with an inherited disease. They turn to this conception-in-a-dish technique because they wish to avoid an abortion later.

In the past, at-risk couples often went ahead with Mother Nature's roulette, then opted for amniocentesis, usually in the 16th week after a pregnancy was established. Using this prenatal testing method, doctors can detect a genetic flaw in the fetus. Still, a pregnant woman must face the prospect of a second-trimester abortion if the news is bad. Chorionic villus sampling can be done in the 10th week of pregnancy, but a bad result often means a first-trimester abortion.

Preimplantation genetic diagnosis allows some couples to reject affected embryos before a pregnancy is ever established.

The procedure begins, like standard IVF, with a woman taking powerful drugs to induce the ovaries to release a slew of eggs. Technicians then combine the eggs with sperm in a laboratory dish, where fertilization takes place. After about 2 or 3 days, the resulting embryo has become essentially a collection of eight genetically identical cells called blastomeres.

The eight-celled embryo then undergoes the biopsy procedure.

Technicians put a culture dish containing the embryo under a microscope. Using an extremely fine pipette, they apply suction to the embryo to hold it in place. Next, they use a sharp instrument to make an incision in the zona pellucida, the tough outer membrane that protects the embryo. With a second pipette, they gently suck one or two of the eight blastomeres out of the embryo. Such an embryo can go on to develop normally.

What happens next varies. In many cases, researchers simply analyze the one or two sample cells to determine the sex of the embryo. Thus couples at risk of delivering a child with an X-linked disease, such as hemophilia or Duchenne muscular dystrophy, can choose to have only female embryos transferred to the uterus. In X-linked disorders, female children may carry the mutant gene on one of their X chromosomes, but generally only male children will actually develop the disease.

In another type of preimplantation diagnosis, researchers home in on the genetic flaw itself. For example, Alan H. Handyside of Hammersmith Hospital in London and his colleagues reported the first successful use of the technology to test for the mutant gene that causes cystic fibrosis (SN: 10/10/92, p.237).

In the latest twist on this technique, a group at Cornell University Medical Center in New York City has developed a method for diagnosing chromosomal defects in human embryos. This technique is likely to benefit older women, who face a higher than average risk of having a child with too many or too few chromosomes.

Researchers know that fertility starts to decline after a woman reaches her 30th birthday. Cornell geneticist Santiago Munné and his colleagues believe that

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one reason for the sharp drop-off is that embryos from older women are more likely to contain genetic abnormalities. Indeed, the Cornell team will present evidence to that effect at the 50th annual meeting of the American Fertility Society, to be held in San Antonio, Texas, in November.

Munné points out that older eggs, once fertilized, may divide abnormally, leading to a high proportion of embryos with chromosomal problems. Thus women age 35 and older run the risk of having a child with Down's syndrome, which is caused by the presence of an extra chromosome 21.

The Cornell laboratory has targeted women age 39 and older undergoing IVF. Munné's team decided that rather than transfer all healthy-looking embryos, they would first test each one. The researchers looked for embryos with the correct number of chromosomes, then placed only those embryos in a patient's uterus.

The team hopes both to increase fertility for women who have delayed motherhood and to reduce older women's risk of having a genetically defective child. So far, the researchers have tried the experimental procedure with just 12 women, 1 of whom is now pregnant. Munné says the team will have to conduct a larger study in order to draw any conclusions about the efficacy of the procedure.

Still, if it works, the method could offer thousands of older women who are having trouble conceiving the hope of having a healthy child.

Preimplantation diagnosis can't provide guarantees, at least when it comes to testing for fragile X syndrome, an inherited form of mental retardation.

Previous research has shown that an abnormally long string of repeated elements, called nucleotides, in a specific gene on the X chromosome leads to this disorder (SN: 6/8/91, p.359). This "stuttering" gene may be unable to give the proper instructions, thus causing the cell to produce no protein at all.

Gene Levinson and his colleagues at the Genetics & IVF Institute in Fairfax, Va., have developed an improved method of searching for those repeated nucleotides. They were ready to test the method, when Janice and George Hill consulted them. (The names of this couple have been changed to protect their privacy.)

The Hills already had a young son who was mentally retarded. Without medical intervention, they knew they had a 50 percent risk of having another mentally retarded child. They decided to try to improve their odds.

With the Hills' consent, the researchers performed a standard IVF procedure and obtained three embryos. Then the team suctioned off two blastomeres from each for genetic analysis. They used a technique known as polymerase chain reaction to amplify key genetic material more than a millionfold. The repeated nucleotide sequences would show up as a characteristic band on a gel.

Fragile X inheritance patterns are extremely complex. For some couples, the researchers can detect this disorder in both male and female embryos. But because of the Hills' genetic makeup and the limitations of the test, the researchers told the couple they could rule out fragile X only in male embryos. For female embryos, the geneticists were back to offering the couple risk estimates.

All three embryos survived the biopsy procedure. The one male embryo would almost certainly develop the disorder. The other two were female. The researchers told the Hills they had a 1 in 3 chance of having another child with fragile X syndrome if one of these female embryos implanted and established a pregnancy.

The Hills decided to proceed with the transfer, knowing full well the uncertainties that haunt the road ahead. □

Vive la Différence

How do you tell a Y-bearing sperm from an X-bearing sperm?

Some researchers believe that Y sperm move faster than X sperm; these scientists use a swimming competition to sort the tadpole-shaped critters. But Lawrence A. Johnson, a reproductive physiologist at the U.S. Department of Agriculture in Beltsville, Md., and his colleagues believe they've devised a better way. Their experimental technique, based on DNA content, has been used successfully to help farmers boost their stock of dairy cows.

Johnson and his colleagues believe that if their method can be used to sort human sperm, it will eliminate some of the ethical dilemmas that crop up when researchers sort embryos to avoid an X-linked disorder. With preimplantation diagnosis, researchers generally discard all male embryos — even healthy ones.

The Johnson group's technique is based on the fact that the X chromosome is physically larger than the Y chromosome. For example, a human sperm with an X chromosome has 2.8 percent more DNA than a sperm with a Y chromosome, Johnson says. He and his colleagues used that variation to fashion a mechanical sperm sorter.

The group obtains sperm from human donors and treats the cells with a fluorescent dye. Then it forces the sperm in solution to pass single file through a laser beam, which excites the dye molecules.

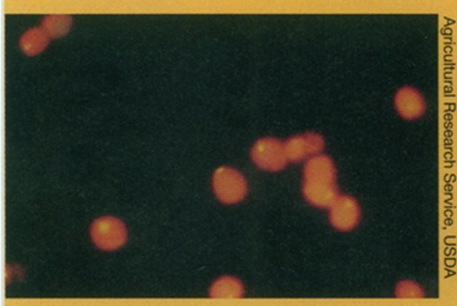
"An X-carrying sperm glows brighter than a Y," Johnson says, adding that the machine evaluates each sperm's glow-in-the-dark capacity and mechanically sorts them. Brighter, presumably X-bearing sperm, go down one collection tube, while Y-bearing sperm swim down another.

Ordinarily, the ratio of X- to Y-bearing sperm is 50:50. But using the mechanical sorter, Johnson and his group ended up with samples that contained about 85 percent Y-bearing sperm or X-bearing sperm. They reported this research in the October 1993 HUMAN REPRODUCTION.

The method isn't perfect. Some sperm of the opposite sex will invariably swim down the wrong collection tube, Johnson notes. However, it's a more efficient way to get sperm of a particular sex than some of the folk methods people have relied on for centuries.

The technology should benefit couples at risk of bearing children with X-linked genetic diseases, Johnson says. Indeed, his collaborators at the Genetics & IVF Institute are beginning to use the method in such cases. In a meeting held in Washington, D.C., last month, the institute's Gene Levinson reported using the technique to try to prevent hydrocephalus, an X-linked condition in which infants are born with an abnormal amount of fluid trapped in the cranium.

The researchers have yet to establish a pregnancy using this method, but that's probably only a matter of time, Levinson says. "This is a revolutionary advance," he adds. — *K.A. Fackelmann*



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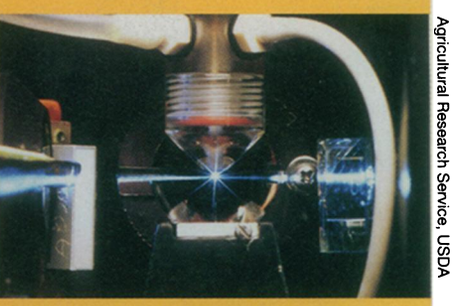
A sample of human sperm containing mostly female-producing cells. About 85 percent of the sperm show a brightly glowing spot, which represents the X chromosome.



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Sperm flow single file past this laser beam. By evaluating the brightness of the signal, scientists can separate the X-bearing sperm from the Y-bearing sperm.