In vitro Fertilization: Is It Safe and Repeatable?

Louise Brown, the first baby ever to be conceived in a petri dish, was born on 25 July. Not unexpectedly, her birth generated enormous publicity. Ironically, however, the scientific details of how she was conceived and implanted in her mother's womb are as yet unavailable.

Patrick Steptoe, a gynecologist in Oldham, England, and Robert Edwards, a physiologist at Cambridge University, are responsible for this first successful in vitro fertilization. Steptoe and Edwards, however, did not follow the usual procedure for announcing their methods and results. A number of scientists in the United States feel that when Steptoe and Edwards sold their story to the *London Daily Mail* without publishing their results in a scientific journal, they were in effect thumbing their noses at the scientific community. What bothers many scientists is that they do not know whether the success of Steptoe and Edwards is repeatable or a lucky accident.

For years, Steptoe and Edwards had tried unsuccessfully to achieve pregnancies following in vitro fertilization in married women otherwise unable to have children. An obvious question now is whether abnormal babies might result from this procedure.

No one can give definitive answers to the questions of whether in vitro fertilization is repeatable or safe, but two scientists in the United States speculated on the subject in interviews with *Science*. Joseph D. Schulman of the National Institute of Child Health and Human Development (NICHD) spent a portion of 1 year, from 1973 to 1974, working as a postdoctoral fellow in Edwards' laboratory. As far as he knows, he is the only scientist who ever spent a substantial amount of time working directly with Edwards on human in vitro fertilization. Schulman now works in other research areas as head of the section on Human Biochemical and Developmental Genetics at NICHD. His comments reflect his personal views and not necessarily those of NICHD.

The second U.S. scientist is Fritz Fuchs of Cornell Medical Center. Fuchs, chairman of obstetrics and gynecology at Cornell, went to Cambridge last October to discuss in vitro fertilization with Edwards. Both Schulman and Fuchs hope to see in vitro fertilization used to help childless couples in this country.

When Schulman worked in Edwards' laboratory, he was impressed by the fact that very few people worked with the human embryos. There were several scientists in Cambridge studying in vitro fertilization in animals, but none of them personally participated in the studies with human embryos, which took place 100 miles away in Royton (near Oldham)—a 3-hour drive from Cambridge.

Since these scientists were well qualified to help with the human studies, Schulman finds it unusual that they did not do so. "If I were trying to achieve what Edwards was trying to achieve, I would have made a real effort to interest other investigators in the human work," he says. "Steptoe and Edwards have not reached out with open arms to a large number of collaborators. Things went on within a very small closed group."

Lately Edwards has refused to talk to the press. However, in a telephone interview with *Science* in which he stressed that he had little time to talk, Edwards said that few scientists worked with the human embryos because the scientists lived in Cambridge and would have had to travel to Oldham to work with the humans. "The problem was basically the space between Oldham and Cambridge," he said. "The scientists would have had to make a commitment to stay away from home for days on end." He

How in vitro Fertilization Is Done

The technique for in vitro fertilization is conceptually straightforward. Needed are ripe eggs ready to be fertilized, sperm, a medium in which to mix the two, and a medium in which to support embryo development.

To obtain the ripe eggs, known as preovulatory oocytes, the woman is given a precisely timed dose of the hormone human chorionic gonadotropin (HCG), which causes her ovaries to prepare eggs for release. Then 33 to 34 hours later, it is time to try to recover the eggs. If a few extra hours are allowed to elapse, the eggs will have been released from the ovary and will be unrecoverable.

The woman is put under general anesthesia for removal of these preovulatory oocytes. A small incision is made in her abdomen and a laparascope inserted. A laparascope is a long metal tube containing a light and an optical system. It allows physicians to directly view their patients' ovaries.

Preovulatory oocytes, which look like bulges on the surface of the ovary, are removed by suction. Most patients have between one and three preovulatory oocytes after they have been treated with HCG. Before or after the oocytes are removed, the woman may be treated with additional hormones to help prepare her uterus for implantation.

Before laparoscopy, the woman's husband donates a sperm sample, which is then washed and diluted. The sperm are diluted in order to simulate conditions in the fallopian tubes where fertilization occurs.

The sperm are put in a salt solution, where, within a few hours, they undergo chemical changes, called capacitation, that prepare them to fertilize the egg. Droplets of the solution containing the sperm are placed in a petri dish that is partly filled with inert oil. The droplets sink to the bottom of the dish. Each preovulatory oocyte is pipetted into one of these droplets. The droplets in the oil are used to keep the sperm and eggs in a small volume.

A few hours after the sperm and egg are combined, fertilization occurs. About 12 hours later, the embryo is transferred to a different solution that supports embryo development. The embryo is also kept in a special atmosphere with low oxygen tension and some carbon dioxide.

After 2 days, the fertilized egg has become an eight-celled embryo. After 4 days, it is an approximately 100-celled embryo (called a blastocyst). Some time between 2 and 4 days after fertilization, the developing embryo is inserted into the woman's uterus. (No one knows what is the ideal time for insertion since no one knows how large a human embryo is when it normally leaves the fallopian tube and enters the uterus. All that is known is that embryos normally implant in the uterus when they reach the blastocyst stage.)

Insertion of the embryo into the uterus entails drawing up the embryo in a fine plastic cannula, inserting the cannula into the uterus, and expelling the embryo. Then, if all goes well, the embryo may implant.—G.B.K.

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added that he is now trying to set up a laboratory in Cambridge for human studies and anticipates that he will have no trouble finding collaborators once he gets such a laboratory set up because "Lots of people in Cambridge are dying to get to grips with human embryos."

Martin Johnson of Cambridge University worked in Edwards' laboratory when Schulman was there and says that Edwards assigned scientists in his laboratory to projects but that everyone in Edwards' laboratory worked on things they wanted to work on. Johnson was not interested in the human studies, he says, because he considered the problems with the human in vitro fertilization "clinical problems, not susceptible to scientific analysis."

Whatever the reason, Edwards and Steptoe worked mostly by themselves on the human studies. They also declined to share details of their unpublished findings with other researchers. And their recent work remains unpublished, including the successful pregnancy method. (Edwards has now published one detail of his work—that he implanted an eight-cell embryo. He says that he plans to publish more details soon but that he has been delayed because he is too swamped with letters and phone calls.)

According to press reports, Edwards has implied that his success was not just due to blind luck. And there are rumors that other babies are on the way. However, no one knows how Edwards achieved his breakthrough or even if there was a breakthrough.

When Schulman came to Edwards' laboratory in 1973, Edwards and Steptoe had already tried several dozen times to implant embryos that were produced by in vitro fertilization. All these attempts failed. During 1973 to 1974, 15 to 20 additional unsuccessful attempts were made. The difficulty was not in fertilizing the eggs and supporting the initial stages of embryo development. Rather, it was in inducing the embryo to become implanted in the uterus.

Schulman speculated that trauma to the ovaries or uterus may contribute to the low success rate. The trauma to the uterus when a cannula is inserted to introduce the embryo may impede implantation.

The ovaries are traumatized during the laparoscopy and needle puncture used to remove ripe eggs. Ordinarily, an egg is released spontaneously, and about this time the ovary begins to sharply increase its secretion of progesterone. The ovary also secretes estrogen, which, unlike progesterone, is secreted in substantial amounts both before and after ovulation. These hormones play a role in preparing the uterus for implantation.

When the ovaries are surgically manipulated they may not secrete the proper amounts of estrogen, progesterone, or other hormones precisely on schedule. The woman might require some combination of these or other hormones to help prepare her uterus to accept the embryo. When Schulman was at Edwards' laboratory, various combinations of hormones were being tried.

Schulman speculates that Edwards and Steptoe were successful because they finally hit on a relatively minor variation of their basic technique that worked. Fuchs agrees. "Edwards' success was due to a combination of perseverance and luck," he says. He is nearly certain that there was no conceptual breakthrough.

Fuchs believes that some of Edwards' problems may have been alleviated if he had the facilities to do same-day hormone analyses of women. Edwards had to wait several days before he could know the hormonal concentrations in his patients' blood. For that reason, he could not know precisely when a woman would normally ovulate. He therefore gave the women a hormone, human chorionic gonadotropin (HCG), that induces ovulation. He then knew to within a few hours when ovulation would occur and Steptoe could remove the ripe ova before they ruptured from the ovaries. The problem with this method, however, is that the HCG treatment may have a deleterious effect on the ovaries. Edwards says same-day hormone analysis "would be useful" for in vitro fertilization. This technique is available to a number of American researchers, including Fuchs.

With all this laboratory involvement in what is usually a natural process of conception and implantation, many people have questioned whether the resulting babies will be normal. Schulman says that "There are no data to support the hypothetical fears that in vitro fertilization will lead to abnormal babies. But more research in this area is clearly desirable." A rather substantial amount of work has been done with animals, he points out, and there is no good evidence that in vitro fertilization leads to genetic or morphological abnormalities in the offspring of any species. He reports that species in which in vitro fertilization has been achieved include hamsters, mice, rabbits, rats, cats, guinea pigs, cows, Mongolian gerbils and pigs. Animals whose embryos have developed in culture, although they were not necessarily conceived there, include rabbits, sheep, cows, and mice. Animals whose embryos have been transferred and successfully implanted, although the embryos were not necessarily previously maintained in culture, include mice, rats, rabbits, pigs, sheep, horses, cows, and a baboon.

According to Schulman these preimplantation animal embryos are surprisingly resistant to manipulation. "You can remove cells from embryos, you can take two embryos and fuse them, or you can freeze embryos. Yet the resulting offspring are reported to be normal."

Even supposing there were an increased risk of abnormalities, Schulman says, the decision to have a child should be left to the prospective parents. This is common medical practice. For example if a couple has a child with a genetic disease, there is often 1 chance in 4 that subsequent children will also have the disease. Yet no one tells such couples that they cannot have children. Schulman says that, to his mind, "there is no conceivable way that in vitro fertilization could result in a 25 percent risk of having a seriously abnormal child."

One reason there is little experience in the United States with the methods for implanting embryos in humans and little known about the risks of human in vitro fertilization is that U.S. research involving in vitro fertilization in humans has been halted since 1975. There has been a moratorium on the use of federal funds for this work, and private foundations have hesitated to fund the work here. (Ironically, a U.S. foundation—the Ford Foundation—pays Edwards' salary through an endowment. The Ford Foundation is interested in Edwards' work on the human reproductive system in order to develop contraceptives rather than alleviate infertility.)

Next month, the National Ethics Advisory Board meets to decide whether to recommend that the moratorium be lifted. Many scientists, like Schulman, argue that there is no reason to continue to proscribe all aspects of this work. "For every year that we wait, thousands of infertile American women will, because of their ages, lose forever their opportunity to have children," he says. Others find that their concern for the suffering of infertile couples is outweighed by feelings of uneasiness about experiments involving human embryos.

Whatever the decision of the advisory board, it is unlikely that Louise Brown will remain the only baby conceived outside the human body. Already Western Europeans are working fervidly on the problems of implanting human embryos. It is virtually certain that we will soon know whether the technique is readily repeatable and whether the babies conceived in petri dishes are at increased risk of being abnormal.

—GINA BARI KOLATA