Cloning Human Embryos

By KATHY A. FACKELMANN

Exploring the science of a controversial experiment

On October 13, 1993, researchers Jerry L. Hall and Robert J. Stillman entered the annals of reproductive history with the announcement that they had "cloned" a human embryo.

Ethicists raised the specter of a society straight out of Aldous Huxley's *Brave New World,* complete with scores of duplicate human babies. A Vatican theologian denounced the research effort as "perverse."

Meanwhile, infertile couples worried that a backlash could curtail funding for such experiments. The announcement put a "strange spotlight on infertility treatment," notes Diane Aronson, executive director of Resolve, an organization of infertile couples based in Somerville, Mass.

As for the scientific significance of the experiment, conflicting views abound. Many researchers working with human embryos consider the work laudable, noting that it may help infertile couples conceive a child. Others say "cloning" techniques may lead to an improved method of genetic diagnosis. Yet researchers working with animal embryos call the findings "ho-hum" and almost trivial in nature.

"Any graduate student could have done the experiment," says George E. Seidel Jr. of the Animal Reproduction Laboratory at Colorado State University in Fort Collins.

Almost all fertility specialists agree, however, that inaccuracies fueled the public response to the announcement. Most blame the news media and ethicists for conjuring up frightening scenarios of the future. At the same time, some fertility specialists admit that the research community muddied discussions of "cloning" by using technical terms in an imprecise manner.

This analysis attempts to elucidate some of the unexplored scientific issues raised by the first report of "cloning" a human embryo (SN: 10/30/93, p.276).

The word "clone" comes from a Greek word that means "twig" and suggests the practice of slicing off a piece of a plant and rooting it. Gardeners routinely use this practice to duplicate a favorite shrub.

One modern definition of "clone," as found in *Merriam-Webster's Collegiate Dictionary,* is "an individual grown from a single somatic cell of its parent and genetically identical to it." All body cells except those that give rise to sex cells are somatic.

Scientists have never taken such a nonreproductive cell from an adult human — or any other adult mammal — and fashioned an identical clone. Indeed, such a feat remains in the realm of fiction, at least for now.

Why? Adult cells are differentiated, or specialized, to perform a specific function. Differentiation is the developmental process by which unspecialized embryonic cells take on their mature role in the body. Once the process is complete, there's no turning back to an unspecialized state. An adult skin cell, for example, can't transform itself into an undifferentiated cell.

The experiment reported by Hall and Stillman, both at the George Washington University Medical Center in Washington, D.C., fell far from Webster's definition of cloning. The two scientists duplicated very young embryonic cells, not adult cells, points out Seidel. Such cells have yet to specialize, he notes.

In addition, the research Hall and Stillman described last October at the joint meeting of the American Fertility Society and the Canadian Fertility and Andrology Society is quite different from the popular notion of cloning. For example, in the movie *Jurassic Park,* scientists used somatic cells that had been trapped in amber to create replicas of long-extinct dinosaurs.

Thus, when Hall and Stillman used the word "cloning" to describe their research, many people reacted as if the pair had fashioned an exact copy of an adult human, points out Howard W. Jones Jr., honorary chairman and one of the founders of the Jones Institute for Reproductive Medicine at the Eastern Virginia Medical School in Norfolk. "*Jurassic Park* is simply science fiction," he says.

To understand what Hall and Stillman actually did accomplish, one must first consider the backdrop, including a long history of cloning by animal researchers.

In the 1940s and 1950s, embryologists took young embryos from rats and successfully separated each embryo into its few constituent cells. At that time, researchers knew that the egg starts to divide after fertilization, forming genetically identical cells, or blastomeres. A tough outer covering, the zona pellucida, protects the fragile blastomeres.

During the 1970s, researchers relied on the same technique, known as blastomere separation, to produce identical twin mouse pups.

Such work showed that each blastomere has the ability to develop into any type of cell. While an adult skin cell can never turn into a heart cell, a blastomere can become a skin cell, a heart cell, or any other cell in the body.

The next landmark occurred in 1979, when Steen Willadsen, then at the Institute of Animal Physiology in Cambridge, England, detailed a blastomere separation procedure for use on larger animals — in this case, sheep. He published his findings in the Jan. 25 NATURE.

Willadsen described removing very young embryos from ewes. With an ex-

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tremely fine needle, he poked a hole in the zona pellucida, then sucked the blastomeres out. To provide some protection, he coated each "naked" blastomere with a gelatinous material called agar.

When Willadsen transferred these agar-coated blastomeres to the womb of a ewe, they began to divide. Proof of the experiment's success came with the birth of several sets of identical twin lambs.

The NATURE paper represented a large leap forward, recalls Seidel. Soon after, other researchers employed blastomere separation to create twin lambs and calves.

Before long, the scientific terminology began to get messy. Although blastomere separation involves the isolation of embryonic cells, some scientists referred to the technique as a type of cloning, Seidel says.

The potential for confusion increased when scientists developed another reproductive technique — nuclear transplantation — also considered a type of cloning. This procedure, too, requires unspecialized embryonic cells and so far cannot be done with adult cells, Seidel says.

Nuclear transplantation works this way: Scientists obtain an embryo that has developed to the stage where it consists of 32 blastomeres. They separate these blastomeres, which contain identical genetic material. They then use an electric current or some other method to coax each blastomere to fuse with an egg cell whose nucleus has been removed.

In theory, nuclear transplantation could yield hundreds of identical high-volume dairy cows or other domestic animals with blue-ribbon qualities, Seidel says. In reality, the technique has fallen far short of that goal, he adds.

Hall and Stillman started their experiment with 17 very young human embryos slated for discard at an infertility clinic. All had started dividing and consisted of two to eight blastomeres. Using an enzyme called pronase, the duo dissolved the zona pellucida and separated the blastomeres. Next, they coated each blastomere with a synthetic shell made of a material derived from seaweed. They allowed those cells to develop in a laboratory dish, noting that some of the blastomeres divided a few times and then died.

Blastomeres from the two-celled embryos did best of all. Some made it to 32-cell divisions, a stage at which they could be transferred to the womb, Hall says.

Stillman says the method they used goes by a variety of names, including "twig cloning," "embryo twinning," or simply "cloning." Yet Seidel points out that their technique is probably most accurately described as blastomere separation.

Many scientists now regret this widespread lack of rigor in describing such complex methods. The public confusion over Hall and Stillman's research graphically illustrates the importance of using terms that describe exactly what was done, comments Mary C. Martin of the University of California, San Francisco (UCSF). "I think we should be very strict in our terminology," adds Robert G. Edwards, the *in vitro* fertilization pioneer whose work, along with that of Patrick Steptoe, led to the world's first test-tube baby in 1978.

In one sense, the Hall-Stillman experiment was designed to fail. The researchers used polyspermic embryos, which result when more than one sperm penetrates an egg. Such abnormal embryos have too much genetic information and cannot survive. Hall and Stillman turned to these flawed embryos, which had been slated for routine disposal, because testing normal human embryos in such a preliminary experiment would have been unethical, they said.

In another sense, the pilot study proved a success. It suggested that if one applied the same methods to normal human embryos, one could obtain viable blastomeres that would develop in the uterus, Seidel says.

Furthermore, there's no reason why such methods wouldn't work if carried to their logical conclusion: the transfer of such artificially coated blastomeres to a woman's womb and the birth of identical twins or triplets, he adds.

A review of the animal research shows that scientists have had the technical expertise to clone a human embryo for years. Why didn't they forge ahead? Most scientists cite ethical reasons for the *de facto* moratorium on "cloning" human embryos.

Why did Hall and Stillman break through that barrier? At a news conference in Washington, D.C., last October, Hall said they did the experiment to spur an ethical debate on the value of cloning human embryos.

The text of their scientific abstract, however, doesn't mention that as a goal. It states, "This technique could be useful to patients who have difficulty producing sufficient numbers of embryos for transfer." In fact, Hall now says that he considered the ability to create identical twins a scientific challenge — one that could provide substantial benefits to infertile couples.

Women who produce one egg have a 10 percent chance of a successful pregnancy, Stillman explains. If researchers could multiply a single egg, the pregnancy rate would increase dramatically, he says.

Most fertility scientists see nothing wrong with that application of the re-

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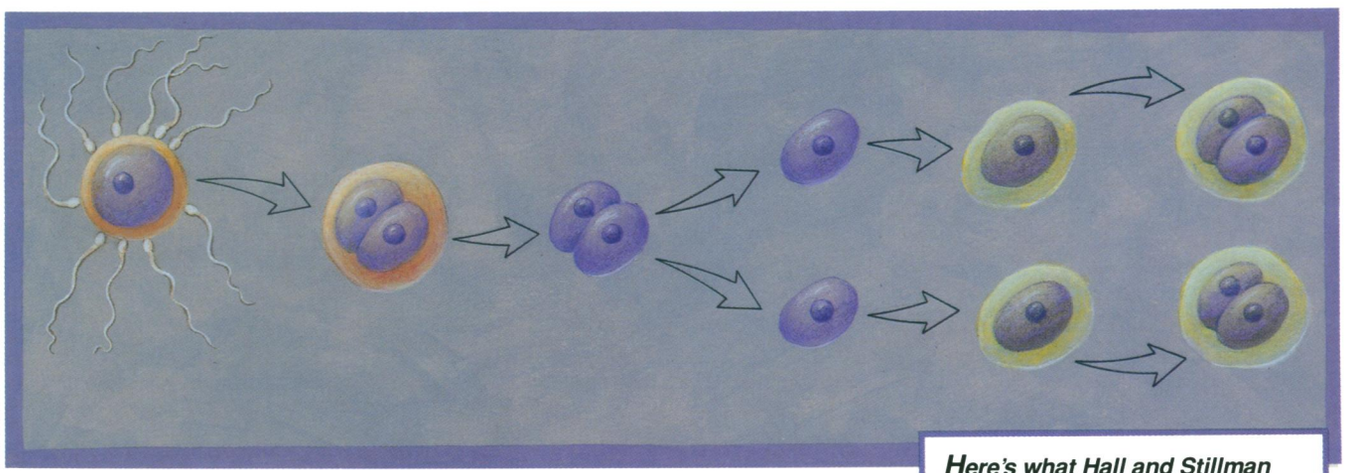

Taina Litwak

Here's what Hall and Stillman did: First, they allowed sperm and egg to unite in a petri dish. After the egg had been fertilized, it started to divide. Next, the team relied on an enzyme called pronase to strip off the natural zona pellucida (orange). Finally, they encased each blastomere in a synthetic zona derived from seaweed (green).

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search, noting that nature produces identical twins in much the same way: The two-celled embryo divides and eventually develops into two identical babies.

Indeed, says Gary D. Hodgen, president of the Jones Institute, if this reproductive technique can help infertile couples conceive a child, then this research would be "completely justified."

Researchers judging the scientific abstracts submitted for presentation at the joint fertility meeting had been impressed enough by Hall and Stillman's work to award it the top prize. The George Washington team's abstract, ranked blindly by two separate peer review panels, beat 90 others for the honor.

Scientists who attended the meeting, which was held in Montreal, reacted favorably to the abstract. "It was an important study," recalls UCSF's Martin. "It's a nice piece of work," concurs Edwards, who is now a professor emeritus at the University of Cambridge in England.

Some scientists say that one of the chief applications of the new method got lost in the media uproar. Lucinda L. Veeck, also at the Jones Institute, says that Hall and Stillman's technique would boost the efficiency of a new form of genetic diagnosis, one that can tell prospective parents whether a tiny embryo has inherited a serious disease, such as Tay-Sachs, cystic fibrosis, hemophilia, or muscular dystrophy.

With preimplantation diagnosis, couples with a family history of a serious genetic disease can find out an embryo's risk before it is transferred to the womb. The technique involves the now standard procedure of uniting a human egg and sperm in a petri dish. Once the fertilized egg begins to divide, researchers punch a hole in the zona pellucida and suck out a single blastomere. They then analyze the blastomere's DNA, searching for signs of an inherited disorder.

But researchers can't always get enough DNA from a single blastomere to make an accurate diagnosis, Veeck says. If they multiplied that single blastomere using Hall and Stillman's cloning method, researchers would have a bigger pool of DNA — and a better chance of predicting the future, she says.

The artificial zona pellucida was another scientifically notable aspect of the controversial "cloning" research, Martin says. She points out that Hall had won the top prize at the American Fertility Society meeting in 1991 for developing and testing the jelly-like coating on mouse embryos.

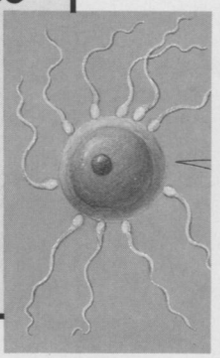
Without a protective "shell," fragile human blastomeres would die, never developing into an embryo, Hall says. Thus, the team's successful use of the seaweed-derived zona paved the way for more sophisticated experiments — such as the transfer of coated embryos to an infertile woman's womb, he says.

Hall envisions another use for the artificial zona: He believes the material could be used to repair damage to an egg's protective coating during test-tube fertilization.

Not everyone views that aspect of the report with enthusiasm. *In vitro* fertilization researcher Jacques Cohen says the development of a synthetic shell is just another "bell and whistle," not something really necessary to hold an early embryo together.

"You don't need an artificial zona," says Cohen of Cornell University Medical Center in New York City. Zona-free blastomeres taken from mouse embryos will continue to develop if they're left untouched in a laboratory dish, he says.

> ***"There were a lot of gimmicky tricks in this paper to make it look sexy."***
>
> **—Cohen**



Cohen speculates that Hall and Stillman received the top prize at the fertility meeting not because of their abstract's technical merits, but because the reviewers were unduly impressed with its fancy terminology, such as the use of the word "cloning."

"There were a lot of gimmicky tricks in this paper to make it look sexy," he says. In addition, he believes, the reviewers failed to take into account the vast animal research that had gone before. Indeed, the abstract seems little more than an updated version of Willadsen's work with sheep, an experiment reported 14 years earlier.

It comes as no surprise, then, that animal researchers have greeted this "advance" with muted enthusiasm.

The procedure was not as advanced as nuclear transplantation experiments being done routinely with animal embryos, says Willadsen, who is now a researcher in Calgary, Alberta. And Neal First, a reproductive biologist at the University of Wisconsin-Madison, calls the hullabaloo over the human "cloning" experiment "a lot of fuss over nothing."

Such comments could be construed as sour grapes, Willadsen acknowledges, adding that there will always be competition between animal researchers and those working in the human arena. Research with animal embryos can far outstrip the technical achievements of experiments with human embryos, he adds, largely because animal research is unfettered by the same ethical constraints as research on humans.

The news that Hall and Stillman had cloned human embryos raised a welter of complex scientific and ethical issues. Some scientists argue that the cloning report represents a significant advance that promises new hope for infertile couples. Others say the report simply rehashed the work that had already been done with animal embryos.

No one would deny, however, that one key aspect of the report was simply Hall and Stillman's use of human embryos. No scientist had dared cross that ethical boundary before, even though the technology had existed for years.

"The human embryo is considered the sacred sanctum," Willadsen says, adding that, despite the controversy, such work should go forward.

Veeck recalls the early days of *in vitro* fertilization when "busloads of angry protesters" opposed the practice of uniting human sperm and egg in a test tube. Yet work with *in vitro* fertilization went forward, helping thousands of couples deliver healthy children, she notes.

Many scientists worry that mounting ethical concerns triggered by the rapid-fire advances in reproductive medicine could bring such research to a halt. Indeed, Hall and Stillman's report was soon followed by the news that an Italian scientist had used *in vitro* techniques to help a 62-year-old woman become pregnant, a move that prompted French government officials to propose banning the procedure.

Edwards, who has had plenty of experience with such debate, argues that the very process of wading through a thicket of ethical questions will prove beneficial to society, forcing it to cope with almost undreamed of technical advances.

"You're always going to get these arguments, because early human life is a very precious thing," he says. □