Despite scores of cloned animals, the process is fraught with problems. Many researchers are going back to the lab to find out why

Clones: A Hard Act to Follow

Cumulina. Cupid. Peter. Webster. Diana. Dotcom. Dolly. Once the realm of science fiction, cloned animals are now becoming almost commonplace. In the past 4 years, cows, mice, goats, and pigs have joined sheep in an expanding menagerie of cloned mammals. Just around the corner, to judge from the press releases and headlines, looms the next brave new world of biotechnology, with herds of identical cattle, sheep, and goats producing bucketfuls of drugs in their milk, pigs designed to grow spare parts for humans, primates and other animals custom-cloned to study human diseases, and even replacement parts cloned from a patient's own cells. Indeed, given the seemingly endless string of birth announcements, a logical question is, "Will humans be next?"

Not likely, say numerous experts in the field. What the press accounts often fail to convey is that behind every success lie hundreds of failures—some so daunting that many would-be cloners have put efforts to create live animals on hold and are going back to the lab to study why cloning sometimes works but far more often fails. Despite years of effort, "we're in the same bind that we've always been in. A majority of [would-be clones] do not make it to term," says Robert Wall of the U.S. Department of Agriculture (USDA) in Beltsville, Maryland. "We have no explanation; it's more art than science," adds Jean-Paul Renard of the National Institute of Agricultural Research (INRA) in Jouy en Josas, France.

Indeed, even Ian Wilmut, the Scottish researcher who brought the world Dolly, hasn't cloned another animal in years; instead, he is trying to find out what makes cloning by nuclear transfer possible by studying how genes are reprogrammed. Although Wilmut, who works out of the Roslin Institute near Edinburgh, isn't throwing in the towel, he says enormous hurdles must be overcome before cloning becomes practical, much less profitable. First and foremost is the problem of efficiency, which remains at a less-than-impressive 2%; out of some 100 attempts to clone an animal, typically just two or three live offspring result. Even when an embryo does successfully implant in the womb, pregnancies often end in miscarriage. A significant fraction of the animals that are born die shortly after birth. And some of those that survive have serious developmental abnormalities, suggesting that something in the recipe is fundamentally wrong.

What's more, cloning, however arduous, is just the first step. If the goal is to create "bioreactors" that produce therapeutic proteins in milk, or pig pancreases the human body will not reject, then cloners need to insert foreign genes into the genome in exactly the right place—a process that has so far defied most efforts. "The issues are back in the laboratory rather than in the barnyard waiting for something to gestate," says Wall. Cloning veteran Jim Robl of the University of Massachusetts (UMass), Amherst, agrees. Since he and his colleagues at the biotech company Advanced Cell Technology (ACT) in Worcester, Massachusetts, cloned six transgenic calves, they have focused less on producing live offspring than on questions such as whether cloned animals are genetically older or younger than normal (*Science*, 28 April, pp. 586 and 665).

Proofs of principle. Dolly (*above*) showed that cloning could be done. Cupid and Diana show that gene targeting is possible.

To increase their odds of producing healthy clones, researchers are now probing fundamental questions of cell biology, such as what kinds of cells make the best donors or what environments are most conducive to the earliest stages of development. They are trying to figure out whether there is something inherently flawed in "asexual" reproduction in mammals—in other words, do we really need two parents? Researchers know that genetic competition between sperm and egg helps to modulate imprinting, a process that selectively silences certain genes early in development. That process may go awry in cloning, accounting for some of the developmental abnormalities.

Or does some problem lie in the "in vitro" component? Toward that end, teams are sorting out more mundane questions of how, exactly, to culture the growing embryo in the lab, and what concoction of hormones is necessary to ensure adequate development.

In all species, the basic hurdles are the same, but the details differ sufficiently that each species has gotten sidetracked at different points along the way to becoming a commercially or medically useful clone (see chart on facing page). Yet in the highly competitive world of animal cloning, researchers are loath—or sometimes forbidden—to share their tricks of the trade. Added to the normal passions, jealousies, and simple desires for credit that plague most high-profile research is the fact that much cloning research is done with corporate sponsorship—and with corporate requirements of secrecy. "Breakthroughs" are often announced long before the technical details are published in journals, making it hard for researchers to verify or extend the results. Even attracting scientists to a recent closed-door conference* at the Banbury Center at Cold Spring Harbor Laboratory in New York required a "major diplomatic effort," says molecular biologist Norton Zinder of The Rockefeller University in New York City, who helped organize the meeting to try to promote better communication among the cloners.

* Mammalian Cloning, Biology and Practice was held 12 to 15 March in Cold Spring Harbor, New York.

CREDITS: (TOP) ROSLIN INSTITUTE; A. D'ARCY/PPL THERAPEUTICS

Dolly and friends

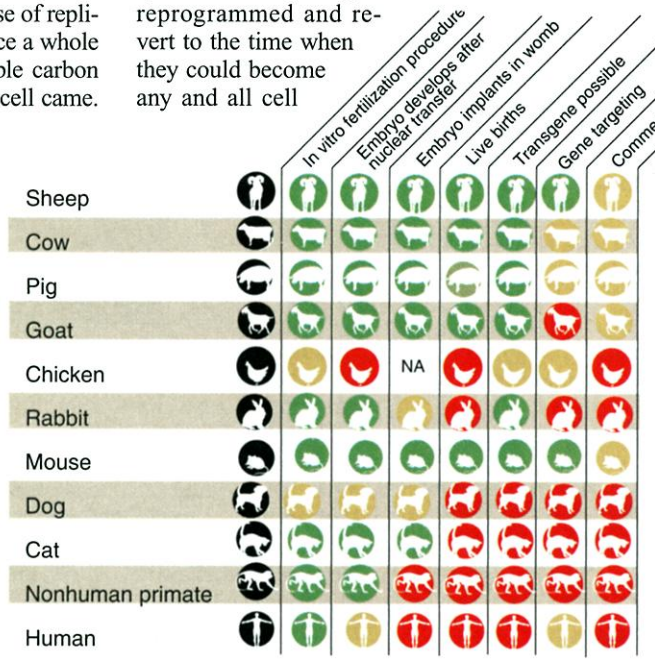
With new clones being announced almost monthly, it is easy to forget just how mind-boggling the process really is. Take a single adult cell, whose fate is supposedly sealed, and send it back in time, so to speak, unsealing the genetic instructions contained in that cell's nucleus. Then ask that nucleus, once inserted into another cell, to set that cell on a course of replication and differentiation to produce a whole new animal—one that is a veritable carbon copy of the adult from which that cell came. There's no true biological "mother" or "father" involved.

Through the past half-century, researchers had dabbled with this bold endeavor, transferring nuclei from a variety of cell types and sources (see this month's "Pathways of Discovery" essay on p. 1775) into cells whose own genetic material had been removed. Sometimes the nuclear transfer experiments seemed to work. In cows, for example, when nuclei from relatively undifferentiated embryonic cells were put into ripened eggs ready for fertilization, offspring could result. But cloning from an adult or even a fetal cell, which would have begun to differentiate, seemed impossible.

To pull off their paradigm-altering experiment, Wilmut and Keith Campbell at the Roslin Institute spent years painstakingly manipulating both the donor cells and the receiving eggs, developing the finesse to make nuclear transfer work with differentiated cells. Ultimately, they teamed up with PPL Therapeutics of Midlothian, Scotland, which wanted to make herds of identical sheep that carried a human gene for a therapeutic protein.

The Roslin group thought they might succeed where others had failed if they could synchronize the cell-division cycle of donor cells with that of the egg. They did this by depriving the donor cells of nutrients, which caused them to shut down most genetic activity. At the same time, they worked out better ways of removing the DNA from the ripened oocytes and, after fusing the oocyte with the donor cell, of triggering cell division as if the egg had been fertilized.

Wilmut, Campbell, and their Roslin colleagues first tried their idea out with embryonic cells, which were presumed to be more malleable. Their success in cloning two lambs in 1996 prompted them to try the same approach using older cells—eventually, cells that they had cultured from an adult ewe's udder. That the experiment worked with the mammary cells was just short of miraculous: Dolly was the product of 434 attempts at nuclear transfer, all but one of which went bad. But that one lamb, reported in February 1997, was enough to set off a worldwide tizzy. Not only did Wilmut's work demonstrate, for the first time, that specialized cells might be reprogrammed and revert to the time when they could become any and all cell types, but it also implied that, if the process worked in sheep, then humans might be just around the corner.



Green light? Cloners have had varying degrees of success with different species.

Several colleagues were skeptical, suggesting that perhaps Dolly was the product of a rogue fetal or undifferentiated stem cell and not a true mammary gland cell. But others were in awe and rushed off to try to replicate the results in a variety of animals, from mice to cows. Within a year, companies like ACT and the newly formed Infigen in DeForest, Wisconsin, made public their own successes with cattle, and cloning became a household word.

Meanwhile, Campbell moved over to PPL and set out to take the next step: putting foreign genes into the donor cell's DNA. It didn't take much work to add new DNA to the cultured fetal cells, select those that took in the new genes, and fuse them with enucleated eggs. It worked, as evidenced by the birth of Polly and five other lambs bearing the gene for human factor IX, published in December 1997. Yet even though Polly expressed the new gene—that is, made the protein encoded by the gene—she was just an interim success. Campbell had inserted the gene into a random location in the donor cell's genome, which meant he had little control over how active it would be. To guarantee high production of factor IX protein in sheep's milk—essential if the research was ever to yield a commercially viable bioreactor—Campbell needed to target the gene to a specific spot in the genome. But there was a problem. Until then, gene targeting had only worked in mice and, in one experiment, in human connective tissue cells—and decidedly not in livestock.

That would soon change. In 1997 David Ayares, a molecular biologist at a pharmaceutical company, joined PPL with one goal in mind: gene targeting. The big problem, he, PPL's Alex Kind, and their colleagues quickly surmised, was finding a way to insert the gene before the donor cells got too old. Most cells can divide only a limited number of times in culture, and gene targeting requires several steps in which DNA is inserted and the few select cells that incorporate it into their chromosomes are allowed to multiply until there are enough for the next modification. "By the time you go through and get a large enough population, it's really pushing the limits [of the cells' ability to divide]," says Robl of UMass.

PPL researchers on both sides of the Atlantic set out to improve the efficiency of each step. They also concocted different brews of growth factors that kept the cells healthier through more cell divisions.

In August 1999, at a meeting on transgenic animals, Ayares showed the fruits of this labor: slides of Cupid and Diana, two sheep clones, one containing a marker gene as a control, and one containing both the marker and a gene for alpha-1 antitrypsin, a potentially therapeutic human protein. Both genes were "knocked" into the sheep's genome in much the way gene targeting is done in mice—in other words, they were precisely inserted into the correct spot.

Ayares's announcement was "the most earthshaking news of the year," recalls Wall of USDA, as gene targeting in livestock no longer seemed to be an insurmountable problem. The work has not yet been published, however (it is scheduled for publication in *Nature*), and no one has yet repeated the results. Even so, Robl is confident that others will soon succeed. As for PPL, Ayares says the team has pulled off the same feat in cow and pig cells—and that it's just a matter of time before PPL will turn those cells into clones.

Cows: 200 and counting

Wilmut's success in part grew out of the failures of nuclear transfer in cattle. Wilmut and Campbell built on a technique that researchers had been using for years to "clone" cows from very early embryonic cells. Indeed, in the 1980s, a Texas ag-biotech start-up named Granada had built its business plan on cloning fast-growing cattle this way. At the time, recalls Ken Bondioli, now with Alexion Pharmaceuticals in New Haven, Connecticut, nuclear transfer in cattle had become routine. Although the efficiency was low, "we produced hundreds of calves," he says. But there was a catch. More than the usual number of pregnancies were proving problematic: Deliveries were difficult, and many calves died just before or after birth. "It took us some time to recognize this as a problem having to do with nuclear transfer," Bondioli explains. Eventually, their data led Granada scientists and others to characterize what they called "the large calf syndrome," the cause of which remains a mystery.

Nurture? No, nature. To the cloners' amazement, Second Chance (*right*) has the same gentle personality as the bull, Chance, from which he was cloned.

Unable to overcome the problem, the company shut down by 1991. Not until Wilmut and the Roslin group produced Dolly and ACT and Infigen had cloned calves with genes randomly inserted into their genomes did nuclear transfer in cattle seem attractive again, says Bondioli. Very quickly, cloning successes in Japan and New Zealand showed that nuclear transfer of fetal or even adult cells worked just fine in cows—or so it first appeared. But as the worldwide total of cloned cattle approaches 300, researchers are finding that they, too, are haunted by Granada's ghost: About a quarter and sometimes more of the calves that survive to birth are bigger than normal, and many of the frustrating spontaneous abortions involve fetuses that are unusually large. Even the normal-sized newborns frequently have lungs like those of premature babies. Others seem to have blood potassium levels high enough that "the calf should be dead," says Michael Bishop of Infigen. Adds Randy Prather of the University of Missouri, Columbia, "Just because you've got offspring doesn't mean they are normal."

In trying to sort out what goes awry, researchers are focusing on two areas. One is imprinting, the critical but poorly understood process by which the protein signal is determined by whether a certain gene came from the mother or the father (*Science*, 25 September 1998, p. 1984). Developmental abnormalities result when one copy of the imprinted gene turns on or shuts down inappropriately—a likely prospect in clones, as the whole genome comes from one donor cell rather than the typical two.

Another possibility is that problems arise because of the way the egg is handled before implantation—for instance, if the brew of hormones is not quite right, or if the jostling, poking, and prodding damages the egg in some imperceptible ways. One hint is that cattle conceived in test tubes tend to have some of these same abnormalities. Robl of UMass wonders if they are stymied by both problems: mishandling of the egg and a lack of reprogramming of the donor nucleus. "What we still have is a black box," he admits.

He and others are now systematically evaluating each step. For instance, Bishop and his colleagues at Infigen are keeping a comprehensive database of cell lines, the specific techniques used during nuclear transfer, care and feeding of the surrogate mother, and in utero growth rates in an effort to try to predict which calves will have higher risks of problems. Already they have noticed a correlation between a certain cell line and unusual fluid buildup around the placenta. The company is also using microarrays to assess which genes are active in a given cell to see if they can discover a connection between certain genes and cloning success. "In 10 years, I'm sure we'll look back and see how archaic we are," Bishop predicts.

PUBLISHED LIVE BIRTHS FROM NUCLEAR TRANSFER IN MAMMALS

Species	Cell type	Nuclear transfers (% embryos formed)	Live births/ Number transferred (%)	Transgenic donor?
Cow	Adult granulosa	552 (69)	10/100 (10)	No
	Fetal fibroblast	276 (12)	4/28 (14.3)	Yes
	Adult cumulus	47 (38)	5/6 (83)	No
	Adult oviduct epithelial	94 (21)	3/4 (75)	No
	Fetal fibroblast	174 (20)	2/7 (29)	No
	Fetal germ cell	85 (?)	1	No
	Adult fibroblast	? (?)	1	No
	Adult fibroblast	338 (30)	6/54 (11)	No
	Adult muscle	346 (21)	4/26 (15)	No
Goat	Fetal fibroblast	71 (68)	1/47 (2.1)	Yes
	Fetal fibroblast	54 (76)	2/38 (5.3)	Yes
Sheep	Adult mammary gland	227 (12)	1/29 (3.4)	No
	Fetal fibroblast	172 (27)	3/40 (7.5)	No
	Embryonic epithelial-like	385 (33)	4/87 (4.6)	No
	Fetal fibroblast	507 (13.6)	6/62 (9.7)	Yes
	Embryonic epithelial-like	128 (24.2)	2/31 (6.5)	No
	Embryonic epithelial-like	258 (17)	1/44 (2.3)	No
	Embryonic epithelial-like	176 (14.8)	4/26 (15.4)	No
	Embryonic epithelial-like	68 (11.7)	1/8 (12.5)	No
Mice	Adult cumulus	2468 (56)	16/1385 (1.2)	No
	Adult fibroblast	250 (39)	1/97 (1.0)	No
	Adult fibroblast	467 (38)	2/177 (1.1)	No

The not-so-impossible pig

This March at the Banbury Center meeting, Prather likely experienced one of the worst moments in his career. After almost three frustrating years of trying to clone pigs, he was close to calling that goal unachievable. Hiroshi Nagashima of Meiji University in Tokyo, another speak-

CREDITS: (TOP TO BOTTOM) INFIGEN INC.; J. WULFSON/TEXAS A&M UNIVERSITY; (SOURCE) A. COLMAN, PPL THERAPEUTICS

er that afternoon, had a similar tale of woe. But just before their session was to begin, Alan Colman of PPL Therapeutics made a startling announcement: "I hate to have to say this, given what's coming up, but we've got pigs." A press release about those five piglets, which included a randomly inserted transgene, made the headlines a few days later, although a peer-reviewed scientific paper has yet to be published—convincing most would-be pig cloners that their long-sought goal is now in reach.

Pigs are one of the hottest commodities in cloning, as many scientists believe they are the key to xenotransplantation. Because of their size, pig organs are considered most likely to be compatible with humans and could thus satisfy the unmet need for replacement organs, such as hearts or pancreases. Moreover, some researchers think pig tissue, transplanted, say, in the brain, might be a source for much-needed chemicals that the human body fails to make, such as dopamine, whose loss contributes to Parkinson's disease. So it was no surprise that several animal scientists redirected their research toward cloning pigs once Dolly burst on the scene.

The problem is that until 1998, few scientists had tried to work with immature pig eggs or to grow pig embryos in the lab. Pigs differ from cows and sheep in that they are born in litters, and unless there are at least four viable fetuses in the womb, the pregnancy fails. That

Bringing home the bacon. Researchers want to clone pigs to provide replacement parts for humans, but cloning has been impossible—until now.

CREDITS: (LEFT TO RIGHT) GLENN/BLACKSBURG, VA; S. O'NEILL/NEXIA BIOTECHNOLOGIES INC.; OREGON HEALTH SCIENCES UNIVERSITY

means that a day's work has to yield at least several viable embryos if the cloning experiment is to have any chance of success.

Ayares says PPL spent more than a year trying to clone pigs with the techniques the company and Wilmut had used for sheep. Each attempt failed. Pig embryos proved too fragile, and the cells often broke apart during nuclear transfer or handling. In those rare instances when the researchers were able to add the donor nucleus to the egg and then activate development, the embryos never made it to the blastocyst stage. Then 2 years ago, company scientists junked that approach and hit upon an entirely new—and apparently successful—one that Ayares will not discuss until the scientific paper comes out—other than to say that he is confident that PPL can clone more pigs when they want to. Next time around, he says, the company plans to genetically modify the donor cells to make pig organs more acceptable to the human immune system—a key step toward making xenotransplantation a reality.

Prather, however, is withholding judgment until he sees more piglets. "I think they got lucky," he says of PPL.

Goats galore. Clones Webster and Peter carry a gene for making spider silk protein.

Filling out the barnyard

Cloners are testing the waters on other livestock, with mixed success. Goats, it seems, are easy. In 1999, two companies reported—one through a press release and the other in *Nature Biotechnology*—that they had successfully cloned goats. And this year, Nexia Biotechnologies Inc. of Montreal announced the birth of two goats, Webster and Peter, that carry a gene from arachnids that codes for the spider silk protein. This spring, says a company spokesperson, Nexia mated the two bucks with normal females; by year's end they expect the female offspring will be churning out milk chock-full of spider silk protein. The company plans to extract the protein and spin it into light, high-strength fibers for use as sutures or in bullet-proof vests or automotive and aerospace components.

On the other hand, despite their natural fecundity, rabbits have so far defied efforts to produce them in the lab. "We can get a lot of cloned embryos," says Renard of the National Institute of Agricultural Research in France, but all pregnancies abort after transfer to a surrogate mother. He suspects that the problem may be in the earliest cell divisions in the embryo. Renard and his colleagues will keep trying, however, as transgenic rabbits produced by cloning could be valuable tools for studying cardiovascular disease.

In terms of bioreactors, it would be tough to beat the chicken—or more precisely, its egg, says USDA's Wall—which is why several companies are now trying to clone chickens. A typical egg costs about 2 cents to make, and if cloners can insert a therapeutic gene and get it to express in the egg white, commercial technology already exists for separating the whites from the yolks. But the eggs themselves present cloners with a distinct problem: their huge size, says Leandro Christmann, a reproductive biologist at AviGenics in Athens, Georgia. And size does matter. To remove DNA from mammalian eggs with diameters of roughly 100 micrometers, you simply put the fairly transparent egg under a microscope and suck out the DNA with a pipette. But in chickens, the egg yolk is far too big and opaque. Just figuring out how to "see" the chicken egg nucleus has been a challenge, Christmann says. Even so, the start-up says it has made progress in developing or adapting the technology to take that egg through all the necessary steps, prompting AviGenics president Carl Marhaver to predict that within a year, "we will be able to produce the first cloned bird."

If primates, then humans?

Perhaps no area of cloning research evokes more curiosity than primates. Although researchers aren't attempting primates as a dry run for humans—their goal is to create identical animals to study such diseases as hepatitis—their progress is likely to shed light on when it might be technically possible to clone people.

Those worried that some crazed scientist might ignore the ethical and legal sanctions against human cloning experiments and

Lonesome twosome. Cloned from embryonic cells, Neti and Ditto are still the only cloned primates, despite years of effort by several groups.

plow right ahead can rest assured: It won't be easy, at least according to Tanja Dominko, a reproductive physiologist at Oregon Health Sciences University in Portland.

When she arrived in Oregon in 1997, prospects looked fairly bright. Oregon's Don Wolf had just succeeded in using nuclear transfer to produce two monkeys, Neti (Nuclear Embryo Transfer Individual) and Ditto, a year earlier. As Granada had done with cows in the 1980s, Wolf had used nuclei from embryonic cells—far easier to work with than adult cells. But 300 attempts and no pregnancies later, the picture "is not as rosy," Dominko says. Cloning primates "is not just around the corner." Neither she, working with Oregon's Gerald Schatten, nor Wolf's team working one floor below, has been able to replicate Wolf's early success.

Once Dominko realized what she was up against, she tried to determine whether the problem was with nuclear transfer or the in vitro procedures. She attempted "mock" nuclear transfers, in which she and her colleagues went through the cloning procedure

Profits From Precious Pets

Face it, dog owners are suckers when it comes to their pets. Likewise, cat enthusiasts. So it should be no surprise that well before the first dog or cat is cloned, scientists-cum-entrepreneurs are already cashing in on this unconditional affection. Some four companies have set up shop storing tissue from people's favorite pets until the time is ripe to clone them. Each offers advice and a retrieval kit that veterinarians can use to collect skin, mouth, blood, or mammary cells from a living or even recently deceased animal. The companies culture the tissue until there are several million potential donor cells for nuclear transfer. Then the vials of cells are slowly frozen and stored in liquid nitrogen freezers until science advances to the point when Fido can be reproduced from scratch.

The boom in frozen-tissue storage started in 1998, after an anonymous millionaire, hoping to clone his pet dog Missy, awarded Texas A&M University animal scientist Mark Westhusin $2.3 million to develop the necessary techniques. Shortly thereafter, PerPETuate Inc. started up in Farmington, Connecticut, and about the same time, Canine Cryobank, a San Marco, California, company already in the business of transporting frozen semen of high-priced pets and show dogs, began freezing tissue for future cloning as a sideline. Richard Denniston, an animal scientist at Louisiana State University in Baton Rouge, started Lazaron BioTechnologies there, in part because his department was getting so many calls from pet owners requesting this service, he says. The last one on the market, Genetic Savings and Clone, is connected with the Texas A&M group working on cloning Missy in the much-publicized Missyplicity Project. Its doors opened for business earlier this year, in part because "these other companies started to cash in on our investment," says Westhusin.

Each company charges between $300 and $2000 for the tissue-retrieval kits and cell preparation procedures and then tacks on storage fees adding up to about $100 per year. Storage fees are likely to add up: Right now, even the most optimistic cloning enthusiasts think dog and horse cloning is still several years away. Prospects for cats look somewhat brighter, although significant challenges remain.

Priceless pet. Missy prompted her owner to fund dog cloning research.

Consider Missy. Three years into the Missyplicity Project, Westhusin knows all too well how tough his task is. The reproductive biology of dogs makes them more difficult to manipulate than any of the animals cloned to date. "They go 6 months between ovulation cycles, so you have to have a huge kennel of animals if you expect to [try cloning] on a daily basis," says Jim Robl, a reproductive biologist at the University of Massachusetts, Amherst. Thus, even though Westhusin is now working with 60 dogs—some as donors, others as surrogate mothers—when he does have an egg ready for nuclear transfer, the chances are that he won't have any females primed to receive an embryo, should he succeed in making one.

Just getting started has been a challenge. Westhusin first tried the techniques he used with cows to prompt immature dog eggs to mature, but to no avail. At this point, he collects lots of immature eggs from spaying clinics, but only every once in a while does he get one to mature enough to be able to start dividing, assuming it had accepted the donor DNA. And when the researchers get a mature egg, there is no time to spare. No procedures exist for keeping the egg healthy once it has taken on its new DNA and begun to divide, so the Texas team must put it into a receptive female within hours of the nuclear transfer. Needless to say, "we have no clone pregnancies," Westhusin told *Science* in April.

Deep freeze. Pet cells are frozen for future use in cloning.

Cats, however, are another story. Even though no millionaire has tried to jump-start cloning his favorite kitty, cats are much more prolific, with a 2-month gestation time and frequent ovulations during a year. Would-be cat cloners can also draw on the wealth of knowledge about feline reproductive physiology, garnered mostly from research on endangered wild cats, says Cornell University's Jonathan Hill. As a result, researchers know what hormones will make female cats come into heat and also how to culture feline embryos outside the womb.

In March, Tatsuyuki Suzuki of Yamaguchi University in Japan set cat cloners' hearts aflutter when he announced he had used nuclear transfer to clone an embryo from a skin cell of a dead cat. At the time, he expected he would have a live cat clone by June. The Japanese team has since tried twice to implant eggs, with no success. Several groups in the United States have forged ahead, and at least one, Advanced Cell Technology (ACT) in Worcester, Massachusetts, has had no trouble establishing pregnancies, says ACT vice president of scientific development Robert Lanza. Of course, that's no guarantee he will soon have a healthy kitten clone, as establishing pregnancies is only part of the problem (see main text). Nevertheless, "we expect to be first," asserts Lanza, although even he is hesitant to predict when.

Whichever pet is cloned first, there is of course no guarantee that the animal will have the same loveable personality as the donor. And the cost is likely to be prohibitive, as high as $200,000 per animal. Eventually, the companies predict, costs should drop to a more modest $20,000, or perhaps even $5000 a pop. That might be a good deal for biomedical researchers seeking to clone dogs for use in studying human diseases. But that cost is still a good deal more than a trip to the pound.

–E.P.

With reporting by Dennis Normile.

CREDITS: (LEFT TO RIGHT) TEXAS A&M UNIVERSITY AND BIO ARTS AND RESEARCH; LAZARON BIOTECHNOLOGIES

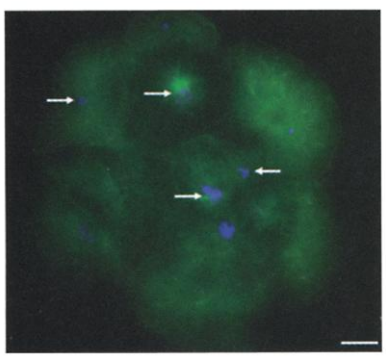
but didn't actually replace the egg's own DNA. Instead, they fertilized the egg in vitro after poking and prodding it the way they would have for a true nuclear transfer experiment, and then placed it in a female for gestation.

Those attempts didn't work well, suggesting to Dominko that the in vitro procedures were the problem. She then made "egg-friendly" improvements such as using sperm extract instead of harsher chemicals to prompt the egg to divide, which helped the subsequent nuclear transfer experiments. The team produced roughly 45 embryos by nuclear transfer this way, but none successfully implanted in a surrogate female monkey's womb.

Then Dominko, Schatten, and colleagues began looking at the transferred nucleus itself. Under a light microscope, the embryo's expanding cluster of cells, known as the blastocyst, looked just like those seen after successful in vitro fertilization. But a closer look at these dividing cells, with confocal microscopy, revealed "a whole gallery of horrors," says Schatten. The new nucleus seemed completely out of sync with the egg. Even the first cell division had gone awry, as the chromosomes didn't seem to have copied and separated as they should have. By the eight-cell stage, some cells had too much DNA, while a few seemed to have none at all.

Dominko and Schatten then took a closer look at the spindle, and in particular at the centrosomes, which help organize and guide the movement of DNA during cell division, making sure that each cell gets the right complement of chromosomes. In primates, they found, the incoming nucleus tends to leave behind one or both of its centrosomes. "The embryos we were making probably never had a chance," says Dominko. Given these results, which are still unpublished, Schatten and Dominko have all but given up on cloning by nuclear transfer until they develop a better understanding of these abnormalities. Instead, they have turned to embryo splitting, in which the early embryo is divided in two and gives rise to identical twins, as a means of generating like animals useful for research (*Science*, 14 January, p. 317). Dominko and Schatten don't know why primates are different from cows, but they are convinced that attempts to clone humans would run up against the same biological roadblocks.

Even Wolf on the floor below is now looking at embryo splitting, but he has not abandoned nuclear transfer. He's tried nuclear transfer with some 100 embryos, none of which has established a pregnancy. Indeed, his studies have revealed another source of failures: Embryos don't develop at the same rate in a lab dish as they do in the womb. It's important to keep trying, he argues, as clinical studies often require more than the two identical animals that can be produced by embryo splitting.



DNA disasters. Chromosomes (blue) fail to segregate properly in cloned monkey embryos, leaving some cells with too many and others with none at all.

CREDITS: (TOP TO BOTTOM) UNIVERSITY OF HAWAII; T. DOMINKO

Litters and litters of mice

Despite efforts by numerous labs to clone mice, this laboratory staple has proved remarkably elusive. Indeed, until recently, only one person in the world had been able to pull it off: Teruhiko Wakayama, who originally reported success with Ryuzo Yanagimachi at the University of Hawaii, Honolulu, in July 1998.

Even in Wakayama's skilled—and some say "magic"—hands, cloning is unpredictable and enigmatic. Fatal problems can crop up at every step. Even the temperature of the lab can make a difference: Slightly too hot or cold, and the technique won't work as well. When Wakayama first moved from Honolulu to Rockefeller University in November 1999, nothing seemed to go right. For the first few months, few of the embryos survived, says neuroscientist Peter Mombaerts, who helped lure Wakayama and his colleague Tony Perry to Rockefeller. One contributing factor, the team suspects, was that their brand-new incubator was producing toxic gases and killing the embryos.

Even without toxic incubators, other labs remained frustrated in their efforts to duplicate Wakayama's work, prompting some disbelief. Wakayama and Yanagimachi's procedure involves injecting the nucleus into the enucleated oocyte instead of fusing the entire donor cell with an electrical charge, and this feat requires the steady hand of a surgeon. "It requires very miniature handwork, and it has to go reasonably fast," explains Mombaerts. Adds Perry: Wakayama "certainly has the magic touch."

But now others working with Yanagimachi and at least three new labs are claiming to have the magic touch as well. Atsuo Ogura of the National Institute of Infectious Diseases in Tokyo and his colleagues have cloned mice with the Honolulu technique, and a description of their work was published this month in *Biology of Reproduction*. Renard says his group in France has also produced a few litters, with several more pregnancies under way. And after tutoring from the Hawaii team, postdoc William Rideout and graduate student Kevin Eggan in Rudolf Jaenisch's laboratory at the Massachusetts Institute of Technology have also successfully repeated the technique. Most agree that Wakayama has a knack for the injections, but "the technique is transferable," Jaenisch says.

Magic fingers. The microinjections required for cloning mice proved difficult to master.

That is good news for the cloning field as a whole, Jaenisch says. Scientists are eager to use cloned mice as a powerful lab tool—not the least to study cloning itself. Because mice are small and reproduce quickly, and because scientists know so much about their genetics and their development, researchers say the mouse offers the best hope for answering many of the questions that plague efforts to clone other species.

One of those other species, of course, is humans. For now, the serious obstacles to cloning every species, especially other primates, suggest that human cloning—even so-called therapeutic cloning to produce cell lines to be used in treating disease—may be a long way off. As for reproductive cloning, or actually creating a living replica, "it would be criminal at this stage in our abilities," says Zinder of Rockefeller. Most researchers concur. The U.S. National Bioethics Advisory Commission issued a report in 1997 saying that human reproductive cloning would be unethical for a variety of reasons, and the commission's chair, Harold Shapiro of Princeton University, says it is still "clinically and scientifically premature to produce human infants." Even if the technique were safe, he adds, it would be unethical to proceed without a clearer public consensus. Given the huge scientific unknowns, there should be ample time for sorting out whether human cloning would ever be acceptable should it, too, yield to the magician's touch.

–ELIZABETH PENNISI AND GRETCHEN VOGEL