factors that we tend to overlook. One is whether a technical enhancement, despite its artificiality, will increase the interest or value of the activity. Fiberglass vaulting poles and neoprene racing suits have passed this test, but the development of ultralight racing bicycles has been banned because it places too much emphasis on technical innovation. Another consideration is whether we can realistically prevent or discourage an enhancement's use. High-tech nutrition, including "carbo-loading," is an artificial enhancement, but we do not ban it because it is too hard to control what athletes eat. An emphasis on nutrition shifts the locus of achievement from athletes to their cooks, but since the intervention is harmless and hard to monitor, we ignore it.

We will see that a multifaceted decision process applies to all of our thinking about genetic enhancement. We cannot reject an enhancement just because it's "unnatural" or because it poses questions of fairness or justice. Instead, we have to balance safety, fairness, justice, controllability, and the effects on parents, children, and society. Sometimes this balancing will prompt us to ban a type of enhancement. At other times, it will lead us toward deliberate genetic changes as a part of the everyday repertoire of medicine.

CHAPTER 2

How Will We Do It?

Mario Capecchi has invented a technology that could change our world. Seated in his fifth-floor office in the Eccles Institute of Human Genetics on the University of Utah campus, with the Great Salt Lake and the Oquirrh mountain range shimmering in the window behind him, he reviews the ways that genome research is expanding our ability to understand and alter human genetic inheritance. Capecchi's voice rises with emotion when he considers whether we should go beyond genetic selection to actively modify the human genome. "We're not close enough to understanding the issues to make wise decisions or predict the outcomes," he insists.

Capecchi's caution is shared by many other molecular biologists working at the forefront of genomic science today. But unlike many of these scientists, he has discovered a tool likely to make human gene modification a reality. The tool is homologous recombination, a utilization of the cell's own gene repair mechanisms to make site-specific gene targeting and gene alteration possible. Capecchi's work in developing homologous recombination has earned him scientific prizes around the world. Yet in 1980, when he first submitted an application to fund experiments testing the feasibility of gene target-

ing in mammalian cells, NIH reviewers rejected his grant proposal, labeling it "not worthy of pursuit." Capecchi persevered. When he resubmitted his proposal four years later, the reviewers not only enthusiastically approved his grant but also extended an apology: "We are glad that you didn't follow our advice."[1]

To understand the revolutionary importance of Capecchi's discovery/invention, it helps to tell the story of some of the most innovative gene therapy research today. All around the world, scientists are seeking ways of changing aberrant gene sequences that cause disease. Take the rare genetic disorder known as X-linked Severe Combined Immune Deficiency (X-SCID). Think of this as a genetically inherited, as opposed to a virally induced, form of AIDS. Infants born with X-SCID carry misspellings in the sequence of DNA letters in a gene (known as IL2RG) that codes for the proteins that make up the interleukin-2 receptor. These receptors are needed for the proper functioning of key cells of the immune system. Because of X-SCID patients' DNA defect, they have very few T cells and lack the antibody and natural killer cells needed to fight infection. A generation ago, children with X-SCID died before the age of two; they survived longer only if they were kept in sterile environments, like David Vetter, the "boy in the bubble."[2] Today, most children with X-SCID are able to survive thanks to matched bone marrow transplants from close relatives. But bone marrow therapy fails for some of these children, and they face an inevitable downward course.

In 2000 a team of researchers at the Necker Hospital for Sick Children in Paris tried a new approach. Working with ten children with X-SCID who had not responded to bone marrow therapy, they removed a sample of blood cells from each child and exposed the cells to an otherwise harmless retrovirus that had been altered to carry a corrected DNA sequence for the IL2RG gene. Retroviruses are able to insert their DNA into the cells they infect. The genetically modified

blood cells were then transfused back into each child and allowed to multiply. Within a short period of time, nine of the children were producing enough healthy immune cells to fight off infections. This was the first major success in gene therapy. Unfortunately, it was not a complete success. Within a year or two of the treatment, two of the ten children developed leukemia (a third later contracted the disease).[3] Research showed that in each case, the corrected IL2RG had misinserted itself into each child's genome, usually falling into a region known to activate a gene related to leukemia. It was as if I had mistyped this sentence and accidentally pushed a repeater key (xxxxxxxxxxxxxxxxxxxx) that triggered cancer. Although this may have happened in only a few of the millions of transformed cells transfused back into each child, those cancer-prone cells had a tendency to proliferate and soon outnumbered normal cells. Fortunately, leukemia is usually treatable in children. To date, only one of the children in this experiment has died, and the others, who would probably have succumbed to X-SCID by now, are doing well. On balance, the Necker experiment remains a success.

This experience reveals a major problem in gene therapy research. Many of the delivery vehicles (or "vectors") used to carry corrected gene sequences into cells are nonspecific: they deposit their DNA payload randomly among the three billion paired nucleotide letters of the genome. Most of the time this is harmless and even beneficial. Like little freestanding protein factories, the inserted genes can function normally just about anywhere in the genome, remedying the targeted deficiency or curing the targeted disorder. But sometimes the genes or sequences misinsert and disrupt the functioning of normal genes. This is termed "insertional mutagenesis." As in the case of the X-SCID children in France, it can lead to an artificially induced form of genetic malfunction that may be as serious as or even more serious than the original disease.[4]

Against this background, we can better understand the revolutionary importance of Mario Capecchi's work. To find a more precise way of inserting genetic material into an organism's genome, Capecchi looked to a natural process that occurs when DNA becomes damaged in a cell. In such cases, the genetic machinery of the cell somehow has a way of identifying a misspelled sequence of DNA letters on one of the two paired strands of DNA in the double helix. It then cuts it out and replaces it with the correct version from the accompanying strand. (In the formation of sex cells, this same process of "homologous recombination" works to cut and paste large blocks of similar DNA from one strand to the other, allowing the organism to exchange similar traits from the maternal and paternal DNA strands of each chromosome.) To this day, it is not quite clear how the genetic machinery of homologous recombination works, but Capecchi was able to harness this process to permit direct, site-specific insertion of DNA sequences into the cells of selected organisms. Identifying a target DNA sequence in a bacterium, yeast, or mouse cell, he would prepare a similar sequence of his own and then use a variety of techniques to cause homologous recombination to occur. The result was a cell with the old DNA sequence snipped out, and the new one precisely inserted in its place. Apart from its opening and closing letters, this new sequence did not have to contain every DNA letter of the old one; substantial similarity (or "homology") was enough. Capecchi had thus invented a way of directly altering the DNA in cells without incurring the problem of insertional mutagenesis that besets most other forms of gene transfer technology.

Why didn't the French researchers use homologous recombination instead of retroviruses to insert the corrected sequence for the gene? The answer is that homologous recombination is very inefficient. If you expose a large population of cells in a petri dish to a replacement sequence, only a few of the cells (perhaps one in a million) will take up the new sequence. Retroviruses and the other viral vectors used in gene transfer are far more efficient, potentially infecting almost all the cells and changing the DNA in many of them. Viral vectors are not free of problems. In addition to insertional mutagenesis, they can cause severe immune reactions in some patients. In 1999 such a reaction killed an eighteen-year-old man, Jesse Gelsinger, in a gene therapy experiment at the University of Pennsylvania.[5] But the very low efficiency of homologous recombination makes even risky viral vectors an attractive alternative in gene therapy research.

In early January 2006, a few months after meeting with Mario Capecchi, I visited Theodore Friedmann in his office at the medical school of the University of San Diego. Friedmann is one of the leading scientists working on human gene therapy. He has served as chairman of the Recombinant DNA Advisory Committee (RAC), the NIH group that oversees and regulates all such research in this country. Because of his regulatory background and experience, Friedmann is even more cautious about the prospects for safe human gene modification than Capecchi. Shortly before he became chairman of the RAC, the Gelsinger tragedy occurred, and he wrote articles tracing the long road ahead for gene therapy research.[6] Yet I had barely settled into my chair in Friedmann's crowded office when he volunteered the opinion that the science is moving so fast that things he regarded as impossible just a few years ago are already happening.

Friedmann was particularly impressed with reports about the work of Aaron Klug and his lab at Cambridge University. Their research aims at making sure that the machinery of homologous recombination finds its target. The key is a specially engineered protein called a "zinc finger nuclease" (ZFN) that can be tailor-made for any identifiable DNA sequence. ZFNs search out the desired DNA sequence and home in on it like a guided missile, increasing the efficiency of homologous recombination a thousandfold.[7] This is the first,

but certainly not the last, breakthrough in a research direction that will someday give us the power to change DNA in living cells without fearing that we will be disturbing other features of the genome.

For two hours, Friedmann and I wended our way through the complexities of gene therapy research and the prospects of gene doping in athletics. In his role as a scientific advisor to WADA, the World Anti-Doping Agency, Friedmann has argued for the need to develop reliable assays for gene alterations if we hope to control the misuse of genetics in sports. As we ended our conversation, Friedmann reiterated his belief in the complexity of the human genome and his worries about the harmful effects of gene modifications. The science in some areas is moving faster than our ability to use it carefully, he said. But he acknowledged that the pace of developments surprises him.

Homologous recombination is not the only route to targeted gene modifications. Some researchers are tinkering with the idea of human artificial chromosomes (HACs) as a way of getting new DNA into the genome.[8] Chromosomes are the relatively isolated islands of DNA across which the three billion pairs of nucleotide letters of the genome are distributed. There are forty-six of them in the human genome. By adding a new island to this geography, scientists can insert new genes along with the upstream "promoter" regions that turn them on and off. Since the DNA on these new islands would not integrate into the normal forty-six chromosomes, HACs could, in theory, be introduced into embryos without fear of disrupting existing genes on the other chromosomes. Although artificial chromosomes have proved functional in bacteria and yeast cells, there are problems with this approach. Extra chromosomes in human beings are often associated with disease, and it is not clear that this would not also be true with HACs.[9] In addition, for successful reproduction, chromosomes must be matched with similar chromosomes from a sexual partner. Unlike with targeted gene changes in existing DNA, therefore, someone who received new genes via artificial chromosomes would not be able to transmit them to the next generation unless his or her mate had the same artificial chromosomes. In some cases, this one-generation limit on gene changes might be desirable, but in general the application of homologous recombination seems to be the best way of achieving gene modifications in the future.

The ability to change DNA in human cells is only one of the complex skills we will need to accomplish human gene modification. Another is the ability to understand what each part of the genome does. Before we modify genes, we must know what we want to change. Then we need the ability to inject modified gene sequences and cells into a living individual to achieve specific and long-lasting physical effects. Genomic science is making astonishing advances in all areas of the gene modification process.

In the past few years, researchers have made enormous progress in learning how DNA creates and shapes the human body, and the pace of learning is increasing exponentially. A major reason is the Human Genome Project. In 2003, the HGP made available on publicly accessible Web sites the entire sequence of nucleotide letters, the three billion As, Cs, Ts, and Gs that make up the human genome. Now scientists interested in finding a gene or understanding the meaning of a specific stretch of DNA do not have to waste months of laboratory time sequencing DNA on their own. Instead, a simple computer search locates targets of interest. Each new discovery of the meaning of parts of the genome is added to the list of annotations that accompanies the public sequence. Knowledge builds on knowledge to accelerate the pace of discovery.

Almost as important as the HGP map is the sequencing in 2002 and 2003, respectively, of drafts of the genomes of the mouse and the chimpanzee. Both mammals share a great deal of genetic sequence

with us. On a letter-by-letter basis, the mouse's genome is identical to ours in nearly 85 percent of the parts of the sequence that code for genes, which are the functional units that make proteins and other key building blocks of the body; the chimpanzee, our closest evolutionary relative, shares perfect identity with 96 percent of all of our DNA and 98–99 percent identity in gene coding regions.[10]

Having the mouse genome in hand is particularly important for helping us understand just what a gene or DNA sequence does. The mouse, a small and fast-breeding laboratory animal, can be used to explore gene function and dysfunction. Over the past few decades, researchers have become experts in producing made-to-order mice with specific DNA sequences either "knocked in" or "knocked out." They begin with a population of undifferentiated stem cells culled from mouse embryos and apply gene modification techniques, such as homologous recombination or viral infection, to make a genetically altered or "mutant" cell. A factor conferring resistance to an antibiotic is attached to the inserted gene to identify the small number of stem cells in which the desired gene change occurs. When the cells are dosed with the antibiotic, only those cells carrying the resistance factor *and* the desired DNA sequence survive. These modified stem cells are then injected back into a few-days-old mouse embryo, and, as the embryo develops, the mutant cells proliferate alongside its normal cells. What results is a "chimeric" mouse, with both normal and mutant cells permeating its body (the term is drawn from the Chimera of Greek mythology, a blend of a lion, a goat, and a serpent). The final step is to mate this chimeric mouse with another like it. Since some modified cells find their way into eggs and sperm, two chimeric mice can produce an embryo with two copies of the mutant gene in all of its cells. If dark coat color genes are linked with the modification, the pure mutant mouse shows up in the laboratory when two mottled chimeric mice give birth to a dark brown pup.

Scientists call this a "transgenic" animal, one that has had foreign DNA stably integrated into its genome. Depending on the gene modification, some of the mouse embryos produced in this way are never born, because the modification interrupts normal development. This can be instructive in illustrating the importance of the gene sequence involved and the lethal implications of its modification. But even more useful are changes that slightly alter the physical or behavioral functioning of the resulting animal. A mutant "knockout" mouse lacking the key genetic information needed to form cell membrane components for conducting sodium ions, for example, will develop a condition very much like cystic fibrosis. Since the mouse and human genes in this region are very similar, this offers scientists a way of understanding the corresponding role of the analogous DNA misspelling in human beings, and it also yields a line of transgenic mice that can be used to test new drugs and therapies for treatment of the human disease. In addition to physiological changes, genetically influenced cognitive and behavioral phenomena can be studied. Using knockin-knockout technology, scientists have produced, among other things, lines of mice displaying the features of Alzheimer's and Parkinson's disease, lines resistant to morphine addiction, and lines displaying hyperactivity and increased male-to-male aggression.

Long-lived, large, and strong-willed animals like chimpanzees do not furnish as good a test bed for trying out gene modifications. The importance of the chimpanzee genome lies elsewhere—in its even greater similarity to the human one. A mouse shares perfect identity with 85 percent of our DNA in gene coding regions compared with the chimpanzee's 96 percent. By studying the subtle variations in sequence between chimpanzees and ourselves, scientists are gaining new insight into the specific genetic features that have made us human.[11] For example, an international team led by the evolutionary biologist Gregory Wray of Duke University recently found that the func-

tioning of a gene that codes for the protein prodynorphin (PDYN) differs substantially in chimpanzees and humans. PDYN is a precursor to a number of endorphins (opiatelike molecules involved in learning, the experience of pain, and social attachment and bonding). Although chimpanzees and humans have the same PDYN gene, a promoter sequence just upstream from the gene's coding region is far more active in human beings. Similarly, scientists have found significant differences in the FOXP2 gene in the two species. This gene is associated with speech acquisition. Researchers studying a large British family, many of whose members have barely intelligible speech, found that affected family members have mutations in the FOXP2 sequence that make it more like the chimpanzee gene than the human one.[12]

Studying the chimpanzee genome more closely, therefore, may shed light on just what happened in evolution to form our species. Recent evidence suggests that our early human ancestors not only coexisted with chimpanzees but may also have interbred with them for hundreds of thousands of years until we finally diverged.[13] Learning precisely how, at the genetic level, we are similar to or different from our nearest animal relative might make it possible to accentuate such distinctive human characteristics as symbolic thinking and our ability to reason morally.[14] In the distant future, our understanding of just how we finally emerged from earlier primate species could lead to the emergence of a new, transhuman species, one as far beyond us as we are beyond chimpanzees.

Let's assume that in the foreseeable future we will vastly develop our ability to understand the meaning of genomic sequences and be able to modify them at will at the cellular level. How could we use these powers to produce genetically modified human beings? In replying to this question, we should recognize first that, to a limited extent, we are already producing human beings to order. Since the mid-

1990s tremendous progress has been made in refining and clinically applying the gene selection technology known as preimplantation genetic diagnosis. This combines in vitro fertilization (IVF) with molecular genetic analysis to permit parents to select early-stage embryos free of a known familial genetic disease. At present, researchers have found more than 1,250 disease-related gene mutations that can be identified and potentially avoided by means of PGD.[15]

Mark Hughes is one of the pioneers of reprogenetic and PGD research. Following a distinguished academic medical career at Baylor University, the National Human Genome Research Institute of the NIH, and Wayne State University, Hughes went into business for himself and now heads the Genesis Genetics Institute in Detroit, with one of the world's leading PGD programs. I served with Mark Hughes on the NIH's Human Embryo Research Panel back in the early 1990s and grew to respect his pioneering work in helping couples at risk for transmitting serious genetic diseases have healthy children. In moral terms, this seemed to me a great advance over the best existing alternative, amniocentesis. Amniocentesis involves the extraction of fetal cells from a pregnant woman's amniotic fluid at fifteen to sixteen weeks' gestation. If the fetus tests positive for the disease, the woman (or couple) then faces the difficult moral decision to terminate the pregnancy at a fairly advanced stage. PGD, in contrast, takes place several days after conception and before the embryo is ever transferred back to a woman's womb.

Hughes's research was just starting when I first knew him, so I decided to pay a visit to his laboratory in Detroit to see how far the technology has progressed in the past decade. The Genesis Genetics Institute's laboratories occupy a suite of offices in an urban medical center. As Hughes showed me around the facility, I learned that each day, Federal Express couriers deliver tiny vials filled with one or two cells that have been extracted from early-stage embryos in infertility

laboratories around the country. The embryos are produced by means of IVF for couples who know they are carriers of one or another severe genetic disease and who may already have had (and lost) a child suffering from the condition. This includes people with cystic fibrosis in their families, or Fanconi anemia, a fatal blood disorder, or, worst of all, Lesch-Nyhan Syndrome, one of the most terrifying diseases imaginable. Children born with it cannot process uric acid properly, so it builds up in their tissues. In the first year of life this leads to symptoms like severe gout, poor muscle control, and moderate retardation. Because of their metabolic problems, these youngsters compulsively bite their lips and chew their fingers. Eventually they have to be placed under restraint. Even so, they will gnaw their lips until, often as a result of infection and the kidney damage done by the disease, they die at a young age.

During the early 1990s, when Hughes was trying to refine the PGD technique for routine clinical use, the challenges were extraordinary. Researchers had already become skilled at using micromanipulators to extract a single cell (or "blastomere") from an eight-cell embryo, a technique known as "single cell blastomere biopsy." Since the embryo would soon die unless transferred to a womb, this left the researchers with at most a day or two to perform the genetic tests, and there was never much DNA to work with.

During my visit to the institute in Detroit, I was impressed with how much progress Hughes has made using PGD. His institute now performs hundreds of tests a year. Because of the expanding knowledge of the genetic basis of disease, he can test for dozens of disorders. As we walked through the facility, Hughes showed me a room with shelves holding banks of thermyl cyclers, each about the size of a desktop printer. These devices use a technique known as polymerase chain reaction (PCR) to amplify the tiny samples of DNA received by

the laboratory, making DNA sequencing much easier than before. In another area, two automated sequencing machines, each no bigger than a small photocopy machine, spew scrolls of paper with the precise nucleotide sequence from the region of interest in each embryo. By examining the color-coded rows of As, Cs, Ts, or Gs, Hughes and his colleagues can determine whether each embryo tested carries a harmful sequence. This is done rapidly enough to determine which of the several highly perishable embryos available to the parents can be used. Within a day, the results are faxed back to the infertility program. Each embryo's number is listed on a sheet. Next to it is a notation indicating whether it is affected or unaffected by the disease. Only unaffected embryos are used to start a pregnancy.

Almost all of the conditions tested for at the institute represent serious genetic disorders that the child-to-be faces. One exception is the immune-system or HLA (human leukocyte antigen) profile of some embryos. These embryos have been deliberately conceived so that the resulting baby can provide a matching bone marrow transplant for an older sibling suffering from a disease like X-SCID or Fanconi anemia. They are often called "savior children" because without them the existing child would die. The institute program routinely tests embryos for HLA status when parents request it. Defenders of testing believe that the goal of saving lives, even though it is the life of a sibling rather than the tested individual, makes this a legitimate medical use of PGD. Critics fear that it is a first step down the slippery slope to gene testing and gene enhancement for nonmedical reasons.

Everyone has limits. In Hughes's case the limit is sex selection for nondisease conditions. Over a beer that evening following my visit to his lab, Mark was vehement about his refusal to provide tests for sex. "Sex is not a disease," he said. I was impressed by how much he, a world-class genetic researcher, remains a physician dedicated to pre-

venting or curing disease. As we shall see, not all PGD program managers share his understanding of the appropriate uses of their services.

Regardless of critics' objections, there are inherent limits to PGD as a tool for human gene modification. Even if Mark Hughes wanted to produce a baby with some novel genetic characteristic (which he does not), PGD limits him to the choices available in the embryos produced through IVF. In HLA testing, for example, on average only one in five embryos is likely to match the affected child. If a couple's IVF efforts yield five viable embryos, therefore, it is possible that none of them will have the appropriate constellation of HLA genes needed to help the sick sibling. The IVF-PGD process currently permits only selection, not modification. But research is now under way to take reprogenetic medicine beyond PGD to produce genetically modified human beings.

One approach involves a human application of the technology used in connection with transgenic mice. Beginning with a population of stem cells culled from a human embryo, researchers could use homologous recombination to alter the cells' gene sequence in a desired way. Cells that took up the change would then be injected back into that embryo or another one produced by the same parents.[16] Like the mice, the resulting child would be chimeric: some of its cells would have the good gene and some the bad. In many cases this would prevent the disease, because in recessive genetic disorders like X-SCID and cystic fibrosis, the cause is a nonfunctioning gene and a missing protein product. A chimeric child with at least some cells producing the missing protein might be very healthy. If you think it odd that a child could have two genetically different cell populations in its body—in effect two genomes—note that a low incidence of chimerism occurs naturally when two different early-stage embryos fuse in the

womb. A small percentage of human beings are chimeras. Usually this is not discovered unless a person undergoes genetic tests, when it is found that the individual has cells of one genetic identity in, say, the bloodstream and the cells of another in the sex cells.[17]

In some cases, chimerism will not work. The functioning of the gene may be harmful, so its presence in any cells in the body is unacceptable. This is true for some genetic conditions like the gene mutation that causes Huntington's disease. The genome of people with this condition reveals a long series of nonsense repeats of three DNA letters. For unknown reasons, this interferes with a gene responsible for key brain functions. In the fourth decade of life or later, Huntington's disease sufferers start an inexorable downward course marked by tremors, weight loss, depression, and, finally, total neurological collapse. How can we correct an embryo to produce an individual whose cells are free of this defect? The kind of forced cross-mating of chimeras used to produce transgenic mice cannot be done in human beings—at least for moral reasons—and even if it could, it would not benefit a couple seeking a healthy child.

Here is a role for human cloning (or nuclear transfer) technology. Although people tend to think of cloning in terms of its ability to create genetic copies of a living person, it is also a powerful tool for gene modification.[18] A researcher could begin with a line of stem cells derived from an embryo made from the parents' sperm and eggs. Homologous recombination could be used to repair the gene defect. But instead of injecting several of these cells into an embryo to make a chimera, the scientist could take one of the corrected stem cells and, using the tools of micromanipulation widely available in infertility laboratories, insert it into one of the mother's eggs from which the nucleus had been removed. This "reconstructed embryo" now possesses a full complement of forty-six chromosomes. Given a mild electric shock, it would begin to divide and grow just like an egg that had been

fertilized by a sperm. It could then be transferred back to the mother's womb for development until birth. This is how Dolly the sheep and many other cloned mammals have been produced. In this case, the result would be a human infant that has the corrected gene sequence in every cell of its body.

Mammalian cloning is currently far too unreliable and risky a process to be used to accomplish gene modifications. It took almost 300 eggs and scores of cloned embryos to produce Dolly in 1997, and nearly a decade of trying has not produced much better numbers. Many cloned animals die during gestation or soon after birth as a result of subtle genetic errors, and cloned individuals may also suffer serious health problems. But, like all the technologies discussed here, cloning is moving forward. Its progress may converge with the other capabilities we are examining to forge an entire system for gene modification. In 2006, Ian Wilmut, the scientist who led the team that cloned Dolly and who opposes reproductive cloning, argued for the use of this technology to avoid the birth of children with genetic defects.[19]

A final approach to putting modified cells into babies is worth mentioning, even though it raises special problems of its own, because it is potentially very safe: the direct genetic modification of sperm or eggs. Just a few years ago, a team of Japanese researchers succeeded in injecting small DNA sequences directly into the testes of mice. When these males were mated with normal females, the result in 50 percent of the cases was transgenic pups.[20] The great advantage of this approach is that a male produces millions of sperm, making it possible to use one or another gene modification technique and then examine the large population of sperm cells for those in which the modified DNA has been taken up. Sperm modification provides a way of preventing the transmission of harmful genes in families where the father carries a disease-causing mutation. To avoid disease carried by

the mother, or to be certain that there is enough of a modified gene to confer a new, beneficial trait on the resulting child, the parents might also choose to modify the mother's eggs. The limited number of oocytes that a woman can provide, even when she is superovulated with powerful reproductive medications, complicates use of this technique. As we shall see, major developments are under way that promise to remove that limit.

One of the great problems facing all the approaches to gene modification mentioned so far, with the possible exception of sperm manipulation, is that they usually start with IVF. The careful insertion of new DNA sequences and the verification of their uptake usually require working with embryos, and that means gathering numerous eggs from a woman and arranging for in vitro fertilization under laboratory conditions. But IVF is a costly procedure usually resorted to only by infertile people who are willing to pay the eight to ten thousand dollars that clinics charge for a single cycle of drug stimulation, egg retrieval, incubation, and transfer of the embryos into the womb. Few people are able or willing to undertake this costly and uncomfortable procedure for gene modifications that produce only modest benefits for their offspring.

Within one or two decades, however, two new technologies could make IVF the way that many babies are conceived. The first technology is egg freezing; the second is in vitro oocyte maturation (IVM). Taken together, both technologies may change the way people start their families.

We still cannot easily freeze human eggs. We hear about frozen sperm and frozen embryos, and we assume that female reproductive cells can also be frozen. But eggs are among the largest cells in the human body—they can be seen with the naked eye as tiny dots the size of the printed period at the end of this sentence. The eggs are filled

with water. When they are frozen, the water forms crystals that disrupt the egg's delicate structures. Sperm and embryos also contain water, but these cells are much smaller (embryonic cells reduce in size as they multiply within the perimeter of the fertilized egg). The reduced amount of water is more easily suffused with the antifreeze (cryopreservant) fluids used in the freezing process.

Within the past few years, researchers in Italy and Japan have developed new techniques for egg freezing. Apparently, one of the keys is sugar. By adding just the right concentration of sucrose to the cryopreservant and by alternating several cycles of fast and slow cooling, Italian researchers have been able to produce eggs that, when thawed, are able to be fertilized at almost the same rate as fresh eggs and that have been used to start healthy, successful pregnancies in human beings. These techniques have now crossed the Atlantic and are being offered on an experimental basis in some of the leading infertility programs in the United States.[21]

Egg freezing is a boon to women suffering from cancer who have to undergo chemotherapy that may damage their ovaries and reproductive ability. It permits them to bank a supply of healthy eggs with which to have children after they recover their health. Many also see egg freezing as a great breakthrough for the millions of women whose career and reproductive decisions now take place to the noisy ticking of the biological clock. It is not uncommon today for young women seeking careers in medicine, law, and higher education to find themselves in their mid-thirties before they have the opportunity and freedom to conceive their first child. Embryo freezing cannot help these women: when they are young, they often have not married or found a partner. By the mid-thirties, however, normal aging causes a woman's eggs to undergo a steep decline in quality. This degradation can cause reduced egg viability, as well as birth defects like Down syndrome. Many infertility programs today work with women who, having

chosen to build careers or to wait until Mr. Right comes along, find that they experience problems when they try to conceive. While feminist critics rightly blame some of these problems on male-defined cultural patterns, including educational and business institutions that expect women to put in long years of apprenticeship before earning career success, it is unlikely that these institutions will change soon.

Egg freezing is a partial solution to this problem. Once perfected, it could permit a young woman to put aside a store of eggs to use when she is in her thirties or forties. It is the age of the eggs—not the mother—that impairs their viability. Since freezing halts the aging, and since most women can safely bear a child into their forties, this is a very attractive way of stopping the biological clock. However, there is one serious obstacle: fertilization requires a ripe egg, but most women produce only one or two in each monthly cycle. To increase the supply of ripe eggs, infertility doctors must superovulate the woman by administering potent drugs, which cause most of the discomfort and costs of the egg retrieval procedure. How many women in their teens or early twenties are going to spend thousands of dollars and undergo weeks of drug stimulation to produce, at most, a dozen eggs? Since some of these eggs will not be fertilizable by the time they are thawed years later, the procedure represents a costly gamble.

Enter in vitro egg maturation (IVM). This technology, already being attempted with mouse eggs, mimics the process that takes place each month inside the ovaries when, from the stock of hundreds of thousands of immature eggs, the body chooses one or two to ripen for fertilization. When clinicians can mimic this process in vitro, the world of reproductive medicine will change overnight. Then, without the expense or difficulties of drug stimulation, a young woman could undergo a onetime, outpatient biopsy and put aside a small slice of ovarian tissue containing hundreds or thousands of tiny, immature eggs for freezing. When she is ready to start her family, a few of these

eggs could be thawed and matured in vitro to the point where they could be fertilized with her partner's sperm. If the procedure does not work, she would still have an ample supply of eggs on hand to try again.

Once egg freezing and IVM are available, many women will find these techniques attractive. It might become a rite of passage for mothers to take their teenage daughters to the doctor to put aside a store of eggs. As some have quipped, these technologies could lead to a world where sex is for fun and reproduction usually takes place in the laboratory. If even a minority of women avail themselves of these new opportunities, however, the door is opened on a brave new world of genetic modification and gene enhancement. In this world, many pregnancies will begin in a laboratory where clinicians have many eggs, sperm, and embryos for each couple. As selection via PDG becomes much easier and more routine, it opens the way to the deliberate modification of genetic material using some of the techniques explored here. Gene identification and targeting combined with egg freezing and IVM—all technologies not far from deployment—move us directly into the world of gene enhancement.

CHAPTER 3

Drawing Lines

Reginald Crundall Punnett, a British geneticist who worked during the first half of the twentieth century, spent much of his life studying sweet peas and domestic fowl. His name lives on not so much for that work but for a visual aid he invented that geneticists still use to communicate parents' chances of passing on a classic Mendelian trait (or disease) to their children. A Punnett Square consists of a large rectangle subdivided into four equal compartments, two above, two below. A mother carries two versions of a genetic trait, one on each of her two chromosomes. These versions are inscribed above the top two compartments, with the dominant trait—the one likely to manifest itself in the offspring if even a single copy is transmitted—to the left. The father's two versions are inscribed next to the two left compartments, with the dominant one above the other. Filling each box in the square with the corresponding maternal and paternal versions of a trait makes it easy to see the possible combinations of dominant or recessive gene variants that could crop up in any four of the parents' offspring.

Figure 1 is a classic Punnett Square for a genetic trait inherited in Mendelian fashion, in this case eye color. Here the mother and father are both hybrid for brown eyes; that is, each has a gene for brown and blue on the two chromosomes, but their eyes are brown because the

eggs could be thawed and matured in vitro to the point where they could be fertilized with her partner's sperm. If the procedure does not work, she would still have an ample supply of eggs on hand to try again.

Once egg freezing and IVM are available, many women will find these techniques attractive. It might become a rite of passage for mothers to take their teenage daughters to the doctor to put aside a store of eggs. As some have quipped, these technologies could lead to a world where sex is for fun and reproduction usually takes place in the laboratory. If even a minority of women avail themselves of these new opportunities, however, the door is opened on a brave new world of genetic modification and gene enhancement. In this world, many pregnancies will begin in a laboratory where clinicians have many eggs, sperm, and embryos for each couple. As selection via PDG becomes much easier and more routine, it opens the way to the deliberate modification of genetic material using some of the techniques explored here. Gene identification and targeting combined with egg freezing and IVM—all technologies not far from deployment—move us directly into the world of gene enhancement.

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