Bioethics in the Laboratory: Perspectives on Embryonic Stem Cell Research

by John H. Dirckx, M.D.

Because biomedical research is phenomenally expensive and deals with critical issues of life and death, it has traditionally faced two major types of limitation: financial and ethical. Sometimes those two become entangled, as when funding is tied up by restrictions based on ethical principles. That is the case with human embryonic stem cell research, currently the focus of intense controversy because of sharply opposing viewpoints on the morality of its methods.

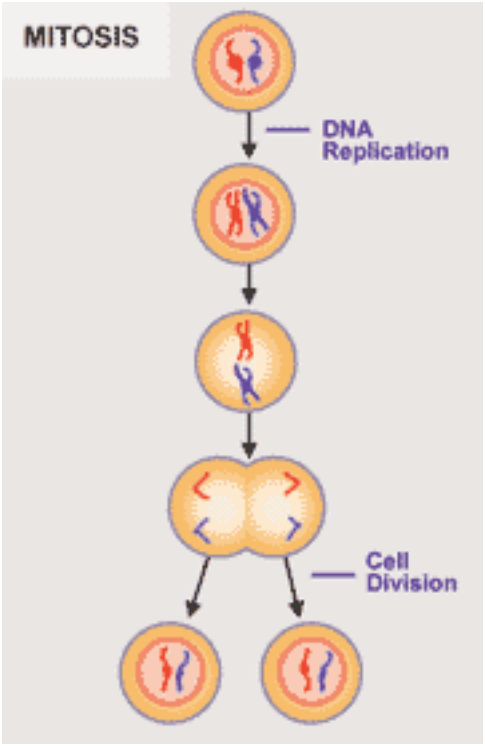
This article reviews basic cell biology, explains the nature of stem cells and their role in biomedical research, and describes the ethical dilemmas faced by those involved in such research.

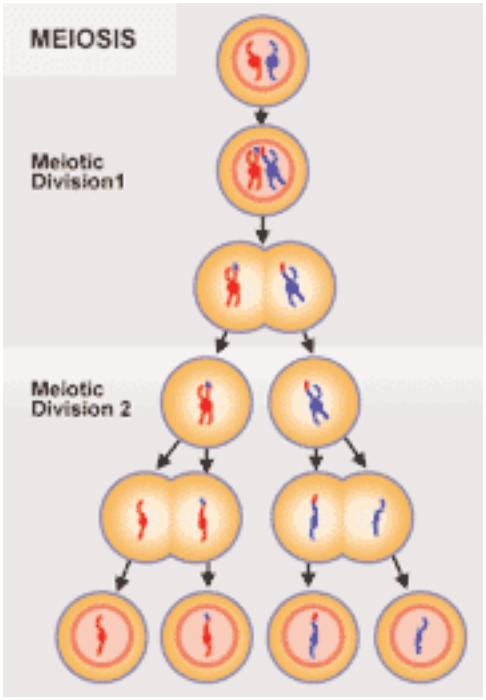
One of the most fundamental concepts in biology is that living things are made up of microscopic units called **cells**. Since the 19th century it has been axiomatic that all cells—from the solitary one that constitutes the entirety of a bacterium or ameba to the countless trillions that make up a human body—are derived from preexisting cells and that, despite their broad structural and functional diversity, they all follow the same basic morphologic and biochemical blueprint.

The cell is isolated from its physical and chemical environment by a membrane that serves as a selective barrier, actively pumping water and an immense variety of substances in or out to maintain internal composition and equilibrium. Chemical substances inside the cell (inorganic ions, proteins, lipids, carbohydrates, and other substances) are suspended in a fluid medium, the **cytoplasm**, along with organelles such as the nucleus, the endoplasmic reticulum, ribosomes, and mitochondria.

The **nucleus** is a compact mass of nucleoprotein that determines the unique identity of each cell and directs its functions and activities. Genetic material is stored in the nucleus in the form of long coiled strands of DNA, which are called chromosomes. In the nucleus of a somatic (body) cell, the chromosomes occur in pairs, one of each pair having been contributed by each parent. Nuclei containing chromosomes that are thus paired are said to contain the **diploid** (i.e., 'double') number (in human beings, 46 chromosomes or 23 pairs).

Many types of cells can multiply by splitting into two identical daughter cells. As a preliminary to cell division, the nucleus first forms two identical daughter nuclei by a process called **mitosis**, during which each chromosome splits in two—more precisely, generates a copy of itself. After mitosis nothing





Illustrations from Genetics, GlaxoSmithKline, **http://genetics.gsk.com/chromosomes.htm**

remains of the original nucleus except the two daughter nuclei, and after cell division nothing remains of the parent cell except the two daughter cells. (See illustrations, page 20.)

Many cells are adapted to perform highly specialized functions. In the human body, for example, muscle cells contract and glandular cells secrete mucus, enzymes, or hormones. Specialization of function often dictates differentiation of form. Thus, a nerve cell has unique processes (dendrites and an axon) that conduct nerve impulses to and from the cell body respectively. Some of the cells lining the respiratory tract bear hair-like cilia whose whipping action keeps the mucus film in constant motion and thus performs a cleansing function.

Gametes or sex cells (sperm and oocytes) differ from somatic cells in that the chromosomes in their nuclei are not paired. That is, each nucleus contains 23 single chromosomes (called the **haploid** number, from a Greek word meaning 'simple') instead of 23 pairs. That condition results from a type of nuclear splitting called **meiosis** or reduction division. Fertilization, the fusion of a male sex cell and a female sex cell, results in the formation of a **zygote** (fertilized oocyte) whose nucleus again contains the full diploid complement of 23 pairs of chromosomes.

A **stem cell** is a relatively immature or undifferentiated cell that has both the capacity of replicating by repeated cell divisions through many generations and the potential of differentiating into a more specific cell type. The range of this potential depends on the cell's composition and its degree of maturity. The specific line of development that an individual stem cell follows depends, at least to some degree, on the needs of the organism of which it is a part, as expressed to it by transcription factors and other biochemical signals.

While some of the cells belonging to a stem cell line mature and differentiate to take up specific functions, others simply keep on dividing so as to ensure a continuing supply of undifferentiated cells. In this way, for example, stem cells in bone marrow constantly replenish the body's stock of red blood cells, white blood cells, and platelets throughout life. Mesenchymal stem cells in connective tissues play a role in the growth, development, and repair of bone, cartilage, tendons, and ligaments.

The stem cell par excellence is the zygote (fertilized oocyte), because that one cell gives rise, through repeated division and differentiation, to all the numerous and various cells that compose the adult body, as well as to the fetal membranes and placenta. The zygote, and indeed all the cells making up a very early (1- to 4-day) embryo, are said to be **totipotent**, meaning that, given the proper biological environment and stimuli, they can develop into any type of human cell whatsoever. Each of those cells can become a complete human being, and if two or three of them mature simultaneously, the outcome will be twins or triplets.

As embryonic cells continue to divide, mature, and differentiate, each cell's range of possible development narrows. The inner cell mass of a 4-day embryo (blastocyst) contains **pluripotent** cells that can develop into any somatic cell of the mature fetus but not into placenta or fetal membranes, and hence cannot form a complete human being.

If stem cells could be artificially made to differentiate into more specialized cells, their introduction into a human host might permit the development, regeneration, or repair of deficient, abnormal, or injured tissues.

Among pluripotent embryonic cells are some that will eventually develop into gametes (sperm or oocytes, depending on the sex of the embryo). Because, throughout the first decade and more of life, human beings are sexually immature and therefore do not form gametes, these **germline** cells continue to propagate by mitosis just like somatic cells, and their nuclei contain the diploid number of chromosomes until some time after sexual maturity is attained. Only then does meiosis reduce their chromosomal complement to the haploid number.

With further specialization, embryonic pluripotent cells become **multipotent** cells. Although these too are stem cells, in that they can either continue to divide or develop into any of several types of mature cell, their range of differentiation is more limited. Multipotent nerve cells, for example, are genetically committed to produce various kinds of neuron but cannot develop into muscle or skin cells.

During the 19th century the pioneer microbiologists Rudolf Koch, Louis Pasteur, and others developed techniques for artificially propagating pure strains of bacteria, yeasts, and fungi in the laboratory. Later workers refined and adapted those techniques to permit culturing animal and human cells. Cell cultures play vital roles in modern laboratory medicine. For example, because viruses can survive and replicate only within living cells, a colony of such cells is an absolute necessity for culturing viruses.

In 1951 George and Margaret Gey at Johns Hopkins University established the first human tissue culture with cells taken from a malignant uterine tumor. This line (called HeLa cells after Henrietta Lacks, who died of the tumor in 1952) continues growing to this day and is represented by billions of cells in hundreds of laboratories around the world.

As recently as 1998, two independent researchers, James Thomson at the University of Wisconsin and John Gearhart at Johns Hopkins University, announced the successful propagation in laboratory culture of pluripotent stem cells harvested from human embryos. Because during normal embryonic and fetal development these cells can differentiate into virtually every type of cell present in the adult body, the ability to culture and manipulate them in the laboratory is widely believed to hold the key to positive interventions in many diseases and disorders that are currently untreatable.

Other suggested benefits of stem cell research include gaining further information on various reproductive issues (infertility, miscarriage, contraception), on embryonic and fetal development, and on the causes of congenital diseases (that is, those existing at birth, whether genetic or induced during intrauterine development).

If stem cells could be artificially made to differentiate into more specialized cells, their introduction into a human host might permit the development, regeneration, or repair of deficient, abnormal, or injured tissues. For example, normally functioning beta cells producing insulin might be introduced into the pancreatic islets of persons with type 1 diabetes mellitus. Patients with Parkinson disease might be helped by the insertion into their central nervous systems of neurons producing normal amounts of dopamine. Healthy cardiac muscle cells might be substituted for tissue that has been damaged by myocardial infarction, and new skin might be generated for burn victims.

Of crucial importance in the preceding paragraph is the recurring word *might*. No one knows at present whether the goals and promises of embryonic stem cell research are realistic and humanly attainable or whether they pertain to the realm of fantasy and science fiction. The answer to that question can be sought only through intensive and expensive research, and that is where funding and moral issues complicate the picture.

Gearhart started his cultures with germline cells derived from the primordial reproductive tissues of aborted early embryos. Thomson, in taking somatic cells from embryos that had been produced by in vitro fertilization at an infertility clinic, damaged them lethally.

There is no societal consensus in this country as to the ethics of destroying a human embryo in order to preserve or enhance the life of one or more other human beings. Although the U.S. Supreme Court decision in the case of *Roe v Wade* (1973) withdrew the status of personhood from human embryos and fetuses and made abortion legal at the federal level, many religious groups and individuals consider abortion to be morally wrong and, in effect, homicide.

There is a subtle juridical distinction between the sources of cells used by Gearhart and Thomson. An aborted embryo is essentially cadaveric tissue, in that its expulsion from the uterus of the mother terminates its life by making its survival and further development impossible. Gearhart's removal of cells from embryos that had already been aborted was therefore not responsible for their destruction. In contrast, Thomson's dissection of living embryos that had been created by in vitro fertilization did indeed directly destroy them.

Those who date human personhood from the moment of conception object not only to abortion but also to in vitro fertilization, which involves the artificial creation of human embryos that will never have the opportunity for implantation and that will eventually be destroyed. Most pro-life advocates, adducing the principle that a good end cannot justify an evil means, would therefore ban both of the ways in which embryonic stem cell lines have been started and all research activities based on them.

The use of germline cells instead of somatic cells to start colonies of embryonic stem cells raises ethical issues apart from the question of embryonic survival or destruction. A line of such cells, once established, could theoretically mature and differentiate into gametes that could be used for in vitro fertilization. Some bioethicists have expressed concern that permitting such research could open the door to germline manipulation in the name of eugenics, with the ultimate aim of genetic "enhancement"—modifying the germline to select genetic traits deemed positive or advantageous by the researcher.

To date, the National Institutes of Health (NIH), a federal agency, has provided the chief financial support for research on embryonic stem cells. President George W. Bush, responding to conservative pro-life positions and his own personal convictions, has limited eligibility for federal support of embryonic stem cell research to work conducted with the 19 cell lines that had already been established as of 9:00 p.m. on August 9, 2001. Embryonic stem cell research that is not funded by the federal government is not subject to federal restrictions but is illegal in many states.

Embryo vs. Fetus

For the first 8 weeks after conception, the developing human being is called an **embryo**, and from 9 weeks to birth, it is called a **fetus**. It is therefore incorrect to refer to the work discussed in this article as "fetal stem cell" research. This is not a mere quibble about semantics. Because federal statutes and regulations currently allow certain kinds of transplant research involving **fetal tissue**, the persistent and widespread use of the wrong term for embryonic stem cell research could create a false frame of reference, favoring legal loopholes that might subvert the purpose of legislators.

Gearhart and his colleagues took primordial germ cells from the gonadal ridge and mesentery of 5- to 9-week embryos and cultured them on a feeder layer of mouse fibroblasts (partially differentiated connective tissue cells) that had been exposed to gamma irradiation to prevent them from proliferating. The cultures were enriched with nutrients and growth factors including fetal calf serum, leukemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF), and forskolin.

Because the primordial germ cells had not yet undergone differentiation into gametes through meiosis, they had the full (diploid) number of 23 pairs of chromosomes. And although they would ultimately have served a reproductive function, they were still pluripotent cells at the early embryonic stage at which they were harvested. As these cells grew and multiplied in vitro, they spontaneously formed variable numbers of **embryoid bodies.** These are ill-defined and unpredictable mixtures of partially differentiated cells that represent all three of the embryonic germ layers—endoderm, mesoderm, and ectoderm. Cells isolated from embryoid bodies were used to start colonies of partially differentiated cells.

Thomson obtained his starter cells from frozen spare blastocyst-stage embryos produced by in vitro fertilization (IVF), a technique designed to enable infertile couples to have children. In this procedure, a sperm and an oocyte are artificially combined in a laboratory setting rather than within the female reproductive tract. The resulting zygote is then implanted in the uterus of the woman.

Because the outcome of any individual fertilization is uncertain, it is standard procedure to fertilize several oocytes at the same time. After one or more of these have been implanted in the uterus, the remainder are frozen and stored for future use, which may include either a later uterine implantation or research. Embryos that have not been used by an arbitrary expiration date are destroyed.

Thomson took cells (blastomeres) from the inner cell masses of 36 fresh or frozen human embryos at the blastocyst stage. The protective outer covering (trophectoderm) of each embryo was first destroyed by the application of antibody. Although this process did not damage the blastomeres, it made further development of the embryo as an organism impossible. The cells were cultured on feeder layers of embryonic mouse fibroblasts like those used by Gearhart, but without the addition of LGF, bFGF, and forskolin.

As with Gearhart's cultures, Thomson's cells proliferated and displayed some spontaneous differentiation. When injected into immunosuppressed laboratory rodents, these human embryonic stem cells formed teratomas (tumors) containing a variety of cell types, an indication of pluripotentiality. Embryoid bodies did not form, but clumps of homogeneous-looking cells were isolated and used to start new cultures. After several repetitions of this procedure, several lines were started with single cells from these cultures.

A **telomere** is a repeating sequence of double-stranded DNA at either end of a chromosome. As cells divide and differentiate throughout the lifespan of an organism or cell line, the occasional failure of a telomere sequence to be replicated during mitosis leads to gradual shortening of chromosomes. This genetic erosion plays an important part in normal aging and sets a natural limit on the number of times that such cells can undergo mitosis. It also accounts for the fact that differentiated somatic cells in a laboratory culture eventually stop dividing, a phenomenon called replicative senescence.

A few types of cell, however, can propagate indefinitely without suffering this attrition of telomeres. At a critical stage in mitosis, these cells express the enzyme **telomerase**, a reverse transcriptase that not only prevents or delays the loss of DNA at telomeres but actually adds DNA sequences. Telomerase expression is a feature of some normal cells (germ cells and bone marrow stem cells) and of many tumor cell lines (including HeLa cells).

Embryonic stem cells in laboratory cultures also display high levels of telomerase activity. A cell line that is capable of indefinite propagation is said, by a slight stretch of language, to be "immortal." Although lines of embryonic stem cells maintained in laboratory culture are immortal because they produce telomerase, they are also genetically unstable and become more so with the passage of time. Pluripotency is gradually lost.

Advocates of human embryonic stem cell research base their hopes and claims on the assumption that these cells can be artificially induced to differentiate into any of the more than 200 types of normal cell found in the mature human body. Investigators working with embryonic stem cells have thus far reported only limited success in inducing embryonic stem cells to differentiate into heart muscle cells, pancreatic islet cells, nerve cells, and hematopoietic precursor cells (marrow cells capable of differentiating into blood cells).

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Cells lines derived from human embryoid bodies differentiate spontaneously into many kinds of cell. Their differentiation can be partially directed by exposure to various growth factors, including retinoic acid, epidermal growth factor (EGF), bone morphogenic protein 4 (BMP4), activin-A, hepatocyte growth factor (HGF), and nerve growth factor (NGF). Cultures treated with retinoic acid differentiate into cells that resemble neurons and express neurofilament H. Cells in activin-A–treated cultures form a **syncytium** (a multinucleated mass of fused cells) resembling muscle. But to date only haphazard differentiation of stem cells has been achieved in vitro, and no one has ever produced a clone of normal and fully differentiated cells.

A clone is any aggregation of cells, ranging from a colony of a few dozen cells in a laboratory dish to a complete, mature organism such as a mouse or a sheep, that are all derived asexually from a single ancestral cell. A human being or an animal that was conceived and born in the normal way is not a clone, because it was produced sexually and its genetic makeup contains elements contributed by both parents.

But a colony, whether of bacteria or embryonic stem cells, that have all descended from a single ancestral cell is properly termed a clone. The fact that all of the cells in a given culture are known to be genetically identical is an enormous advantage in many kinds of laboratory work. Monoclonal antibodies, produced by clones of immune system cells (actually hybridomas formed by fusion of immune cells with established tumor cell lines), are widely used in diagnostic tests, in the manufacture of drugs and biologicals, in the therapy of certain inflammatory and malignant diseases, and in research.

Therapeutic cloning is a method intended to yield a pure strain of healthy differentiated cells—heart muscle cells, bone cells, or nerve cells—with which to replace diseased, damaged, or absent cells. The first step in therapeutic cloning is **somatic cell nuclear transfer**. Removing the nucleus from an oocyte deprives that cell of its genetic individuality but not of its character as a stem cell and its totipotentiality. If what remains of the cell—its membrane and cytoplasm containing supporting organelles and nutrients—is made to fuse with a somatic cell derived from the prospective recipient of the generated tissue,

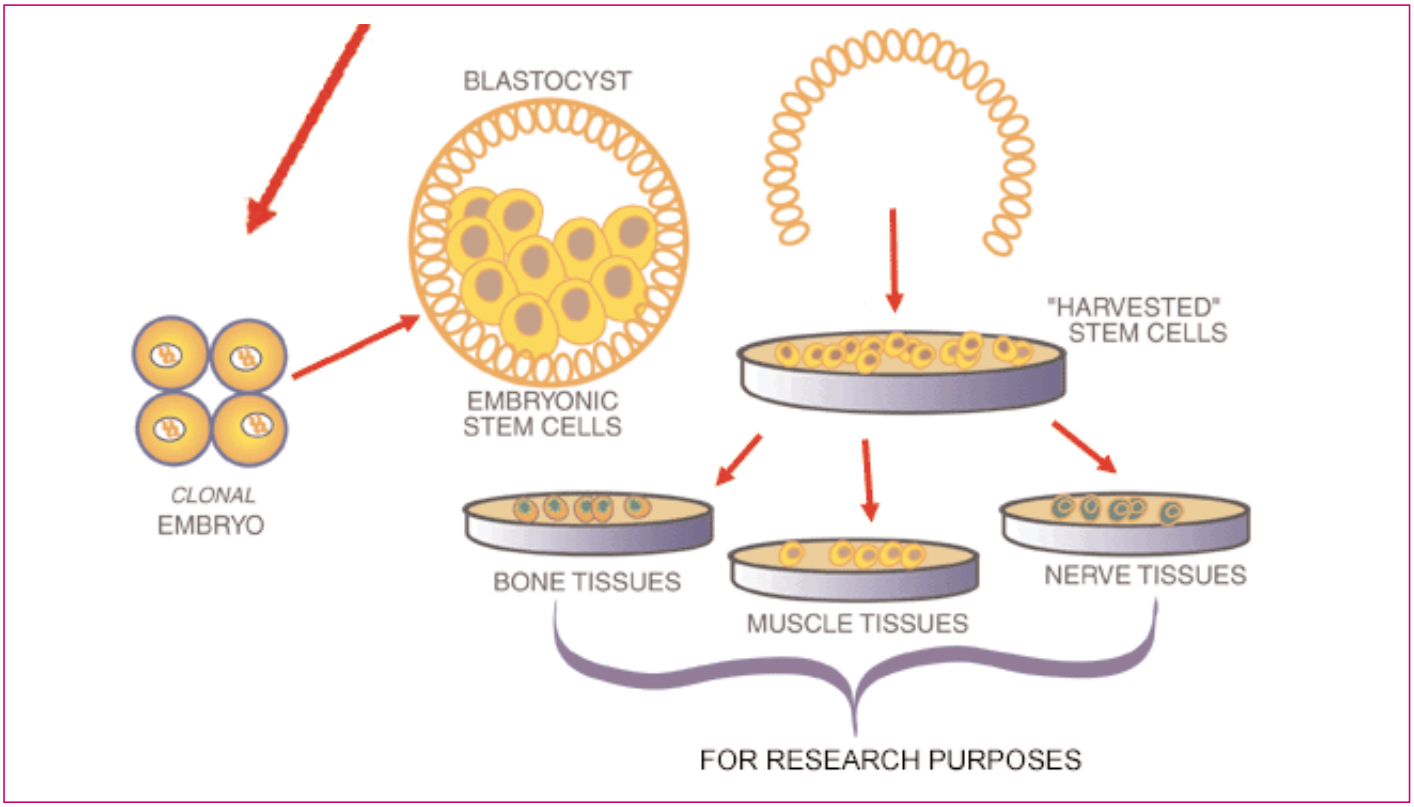

Illustration of nuclear transfer and therapeutic cloning from **http://www.genetics-and-society.org/technologies/cloning/research-science.html**

the resulting **chimera** (ki-mé-ra, named for a monster of Greek myth) has the genetic makeup of the donor cell but the developmental potential of a primitive germ cell.

Because the oocyte nucleus with its haploid number of chromosomes has now been replaced by a somatic cell nucleus having the diploid number of chromosomes, the resulting chimera has the same developmental potential as a fertilized oocyte. In 2004 a South Korean research group led by Woo Suk Hwang announced the successful creation of 30 human chimeras, which were permitted to develop as far as the blastocyst stage. Such a chimera could theoretically be a source of stem cells that are genetically identical to all the other cells in the body of the person from whom the somatic cell nucleus was derived. Grafts formed from such cells should be compatible with that person's tissues and hence unlikely to elicit rejection.

Pro-life advocates regard human chimeras created for purposes of therapeutic cloning as living human beings, and oppose both their creation and their destruction. Moreover, they point out that therapeutic cloning is just a step away from reproductive cloning, the artificial, asexual production of an entire organism from a single somatic cell. In 1997 Ian Wilmut and his colleagues at the Roslin Institute in the U.K. announced the birth of the sheep Dolly, the first mammal cloned asexually from a single cell of an adult animal. Since then, other workers have cloned animals belonging to other species.

If a human chimera resulting from nuclear transfer and intended for therapeutic cloning were to be implanted in a human uterus instead of being grown in a laboratory culture, it would have a substantial chance of developing into a mature fetus. No responsible scientist is likely to attempt such a feat, and to date, as far as is known, no one has cloned a human being.

Inserting a somatic cell nucleus of 46 human chromosomes into an enucleated oocyte from another human being is something like transplanting the hard disk of a Mac into the central processing unit of a PC, or maybe like moving the controls from the cockpit of a transatlantic jetliner to the bridge of an ocean liner. A chimera contains an admixture of nuclear DNA from the somatic cell with cytoplasmic mitochondrial DNA remaining in the denucleated oocyte. Although no one can accurately predict what that would mean to future generations, the likelihood is strong that it would introduce permanent deleterious alterations into the germline. Among cloned animals the incidence of spontaneous abortion and birth defects is higher than among products of natural reproduction, and these animals are subject to premature aging, impairment of immune response, and sudden and unexplained death.

For those and other reasons, reproductive cloning of a human being has been formally banned in more than 30 countries, including the U.S. A bill passed by the House of Representatives that would permit therapeutic cloning but would ban reproductive cloning and sentence violators to prison and impose fines as high as $1 million has still to be considered by the Senate.

Debate by members of the United Nations on a global ban against all medical applications of human cloning continues at

the time of writing. All UN countries favor a treaty that would ban the creation of cloned human babies, but a U.S.-backed proposal put forward by Costa Rica that sought to extend the ban to therapeutic cloning encountered intense opposition from countries such as the U.K. and the Netherlands that want the right to pursue new medical treatments based on cloning.

Broad ethical principles on which most people of good will agree can yield widely differing interpretations when applied to specific moral questions and issues, especially when those questions and issues are unprecedented. The debate between advocates of stem cell research and their opponents has often become polarized along political, philosophical, and religious lines. Tolerance of embryonic stem cell research, of whose methodology the destruction of living embryos is an integral part, is seen as further erosion of respect for every human life that began with legalization of abortion and euthanasia during the latter part of the 20th century. Scientists conducting basic stem cell research have been depicted as irresponsible meddlers who seek to play God with utter disregard for possible adverse consequences. Conversely, opponents of stem cell research are often stereotyped as religious dogmatists or fanatics.

Some bioethicists have suggested a compromise position whereby embryonic stem cell research might be made morally acceptable. According to this view, even though a new life begins at conception, the primitive blastocyst lacks the complexity and organization required for true personhood, which begins only at the fetal stage. Additionally, some have held that a zygote produced in the laboratory, and to an even greater extent a chimera, differ so radically from a product of natural conception that they lack the moral and legal status of a human being. Strict pro-life advocates find these views impossible to reconcile with the undoubted fact that, from their earliest stages of existence, such organisms have the potential to develop into mature human beings.

Parthenogenesis ('virgin birth') is the production of a mature organism from an unfertilized oocyte. This process occurs naturally in some lower animals. Monkey oocytes have been induced in the laboratory to begin dividing so as to form embryos without being fertilized and without having gone through meiosis with reduction in their chromosomal complements. The morality of undertaking such experimentation with human oocytes for the purpose of starting embryonic stem cell lines is far from clear. Given that the resulting embryo has the theoretical potential of developing into a human being, its moral status is essentially the same as a zygote produced by nuclear transfer.

The climate of ethical debate over embryonic stem cell research and the relevant restrictions on federal support have prompted many researchers to seek alternative sources of pluripotent or multipotent cells. Although embryonic stem cell lines are theoretically the most versatile and useful for replacement or supplementation of diseased tissue, experimentation with such lines has thus far yielded no practical results. Meanwhile, other types of stem cells, not derived from embryos, have been used successfully in reparative (or regenerative) medicine.

Bone marrow appears to be particularly promising as a source of adult stem cells. . . . Umbilical cord blood is another readily available and ethically unobjectionable source of multipotent stem cells.

Adult stem cells are precursor cells, found in small numbers in adults, that give rise to specific tissue types, such as blood, muscle, and nerve. Bone marrow appears to be particularly promising as a source of adult stem cells. Until recently, transplanted marrow cells have been viewed only as a means of restoring marrow depleted by disease or by cancer chemotherapy or radiation. But experiments have shown that marrow-derived stem cells injected into animals with damaged heart, nerve, lung, and liver tissue can differentiate into cells that contribute to the repair of those organs. Umbilical cord blood is another readily available and ethically unobjectionable source of multipotent stem cells.

Adult stem cells are difficult to isolate. They grow slowly in culture and, because they do not produce telomerase, the cultures age and eventually die out. But because work with adult cells doesn't involve the destruction of embryos, ethical opposition and funding restrictions are not a problem.

Stem cell research became a major campaign issue in the 2004 presidential election. The defeated Democratic candidate, John Kerry, had promised, if elected, to reverse President Bush's 2001 policy restricting federal funding of such experiments to cell lines already established and to expand funding to $100 million annually.

Private investors have full freedom to support the development of new embryonic stem cell lines, and so do state governments. Proposition 71, passed in California in November 2004, authorizes the state to sell $3 billion in bonds and then dispense nearly $300 million a year for 10 years to researchers for human embryonic stem cell experiments, including cloning projects intended for research purposes. This funding initiative dwarfs all previous stem cell projects in the United States, whether privately or publicly financed. The issue specifically bans reproductive cloning.

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