SCIENCE

Stem Cells

Moral Controversies and Moral Alternatives? Last year, the President's Council on Bioethics published a white paper that summarizes an ongoing conversation on the possibility of identifying moral alternatives to the destructive human embryo research that is currently required to isolate pluripotent embryonic stem (ES) cells (http://bioethics.gov/reports/white_paper/index.html). This past quarter, several papers have been published that try to make these moral alternatives a reality.

First, in an exciting advance reported in the prestigious journal *Cell*, Takahashi and Yamanaka describe their efforts to identify a minimal set of mouse molecules that could be used to directly reprogram an adult somatic cell into a pluripotent stem cell ("Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors," *Cell*, August 25, 2006.) In their study, the Japanese team show that four factors, including Oct3/4, Sox2, c-Myc, and Klf4, are sufficient to force mouse skin cells to become pluripotent stem cells, which they called induced pluripotent stem (iPS) cells. These cells manifest numerous morphological and growth characteristics associated with pluripotent stem cells derived from embryos. Significantly, transplanting these iPS cells into immune-compromised mice generated tumors that were composed of the major cell types of the developing organism. Furthermore, when these cells were injected into mouse embryos, they were able to contribute to mouse embryonic development, the hallmark sign of a pluripotent stem cell. The authors conclude that their data "demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors." Given the similarity between human and mice cells, this study raises the possibility that patient-specific pluripotent stem cells could eventually be derived directly from a patient's adult cells without the need to create or destroy human embryos.

Next, in a paper that generated headlines throughout the world including front page coverage in the *New York Times*, Klimanaskaya et al. claim that they have successfully derived human ES cell lines from individual cells called blastomeres, which had been extracted from human embryos ("Human Embryonic Stem Cell Lines De-

rived from Single Blastomeres," *Nature,* August 23, 2006). News reports from the mainstream media erroneously claimed that the team from Advanced Cell Technologies had successfully derived these human ES cell lines without the destruction of the human embryos, implying that this approach could be a moral alternative to the destructive embryo research currently required to obtain ES cells. This was patently false. In fact, the paper shows that all sixteen human embryos used in the study were destroyed to retrieve ninety-one blastomeres. (Disturbingly, the paper may have contributed to the false reports in the media, because its first figure suggests that the embryos biopsied in the study were allowed to continue embryogenesis until they became hatched blastocysts.) Moreover, the team from ACT was able to establish only two ES cell lines from these individual cells, and only when they had cultured the individual cells in close proximity to each other. In the end, the paper shows that this approach in its present form is unlikely to be used to efficiently derive ES cells from human embryos without destroying them. One would need to biopsy fifty embryos to obtain enough individual blastomeres to generate one ES cell line! The paper also shows that the claim that scientists successfully derived ES cell lines from individual human blastomeres is premature—they have not shown that these individual cells can become cell lines *when they are not co-cultured with other embryonic human cells*.

Will scientists eventually be able to do this? This is not clear, especially in light of a paper published by a scientific team in Singapore that reports that they were unable to derive any stem cell lines from sixty-six pairs of eight-cell-stage human blastomeres ("Unsuccessful Derivation of Human Embryonic Stem Cell Lines from Pairs of Human Blastomeres," *Reproductive Biomedicine Online*, August 2006). The Singaporean scientists conclude: "The results showed that it might not be possible to derive hESC lines directly from paired blastomeres. A minimum number of blastomeres in close contact with one another may be required to successfully generate an hESC line as blastomeres, like ICM and hESC cells, may be 'social' cells."

Fertilization- versus Cloning-Derived Embryonic Stem Cells? Cloned animals generated by nuclear transfer technology often die early in development because of abnormal gene activity. However, proponents of so-called therapeutic cloning would like to derive patient-specific ES cells from cloned human embryos. This has raised an important concern: Would these patient-specific ES cells derived from cloned embryos be abnormal like the cloned embryos from which they were harvested?

Two recent papers have addressed this question. Brambrink et al. performed molecular and developmental tests to compare mouse ES cells derived from cloned embryos with ES cells derived from normal fertilized embryos ("ES Cells Derived from Cloned and Fertilized Blastocysts are Transcriptionally and Functionally Indistinguishable," *Proceedings of the National Academy of Science USA*, January 24, 2006). The authors showed that these cell lines are indistinguishable: they have comparable developmental potentials and they also express (that is, turn on) similar genes at similar levels. These conclusions were reproduced by Wakayama et al., who created one hundred fifty ES cell lines derived from cloned mouse embryos and showed that these cell lines were comparable to ES cell lines derived from normal embryos ("Equivalency of Nuclear Transfer-Derived Embryonic Stem Cells to Those Derived from Fertilized Mouse Blastocyst," *Stem Cells,* September 2006). Again, both types of cells expressed similar

genes, suggesting that ES cell lines derived from both cloned and fertilized blastocysts may have an identical therapeutic potential. The scientific teams propose that the process of harvesting stem cells selects cells that have successfully corrected the genetic abnormalities associated with nuclear transfer, allowing them and only them to grow.

Awareness in the Vegetative State?

Last year, during the controversy surrounding the death of Terri Schiavo, the Florida woman who had been in a so-called persistent vegetative state (PVS) for many years, numerous medical experts interviewed by the mainstream media argued that PVS patients are irreversibly unconscious. Two papers published this past quarter challenge this claim.

First, Owen and coworkers at the Medical Research Council Cognition and Brain Sciences Unit in Cambridge (U.K.) used functional MRI (fMRI) to examine the brain function of a young woman who had sustained severe head injuries in a traffic accident ("Detecting Awareness in the Vegetative State," *Science*, September 8, 2006). PVS describes a disorder where patients who emerge from a coma appear to be awake but show no signs of awareness. The authors' fMRI scans showed that the language-processing regions of the young woman's brain became active when words were spoken to her. Furthermore, sentences that contained ambiguous words such as "creek/creak" produced an additional response in the language regions of the brain, similar to that observed with normal volunteers trying to understand the same sentence. In contrast, no similar brain activity was seen when the patient was exposed to non-speech sounds. These findings indicate that this PVS patient retained some ability to process language. In another test to directly assess the patient's awareness, the scientists told the patient to imagine herself either playing tennis or visiting all of the rooms of her house, starting from the front door. This led to the activation of a different set of brain areas involved either in motor function or in planning movements. Significantly, the patient's neural patterns were indistinguishable from those observed in healthy volunteers performing the same imagery tasks. The scientists conclude that these results suggest that their PVS patient had made an intentional decision to follow their instructions. In other words, despite her PVS state, this patient was consciously aware of herself and her surroundings!

Next, Clauss and Nel report that they successfully used zolpidem, a drug often sold under the brand name Ambien to treat insomnia, to wake three PVS patients—two who had been injured in motor vehicle accidents and one by near drowning; all three had been in a PVS for at least three years ("Drug Induced Arousal from the Permanent Vegetative State," *NeuroRehabilitation*, January 2006). After being given zolpidem every morning, the patients in the study could "interact, make jokes, and speak on the phone." Once the drug wore off, however, the patients returned to a PVS state. After three to six years of daily use, drug efficacy did not decrease, and there were no long-term side effects.

In sum, these two striking reports reveal that we do not really understand the PVS state. The studies open up the possibility that PVS patients may not be as "vegetative" as many have thought and that this condition may not be wholly irreversible. At a minimum, these papers should compel us to place a moratorium on

efforts to withdraw ordinary care from PVS patients, since it is clear that we cannot really know if they are in a truly permanent or a temporary nonresponsive state.

On Sperm, Sperm Stem Cells, and Interspecies Sperm Transplantation

Preserving sperm is one strategy for conserving animal species and strains of animals valuable for biomedical research or agricultural breeding. Two papers this quarter report advances in this technology that have ethical implications.

First, Ogonuki and coworkers have shown that sperm can be successfully isolated from frozen reproductive organs or from frozen whole bodies of male mice ("Spermatozoa and Spermatids Retrieved from Frozen Reproductive Organs or Frozen Whole Bodies of Male Mice Can Produce Normal Offspring," *Proceedings of the National Academy of Science USA*, August 30, 2006.) The team simply froze either the epididymides (the sperm collection tubules found alongside the testes), the testes, or the whole bodies of male mice for up to fifteen years and showed that they could generate normal offspring from these sources by microinseminating thawed sperm using in vitro fertilization (IVF) techniques. The study concludes that freezing either male reproductive organs or whole bodies is the simplest way to preserve male germ (that is, reproductive) cells. The Japanese team also conjectures that the restoration of extinct species could be possible if a male individual—a mammoth, for example—is found in permafrost.

Next, can sperm from one species be grown in males of another species? To answer this question, reproductive biologist Takashi Shinohara at Kyoto University in Japan and his colleagues began with rats that had been genetically engineered to produce a green fluorescent protein (GFP) such that all their cells were neon green ("Rats Produced by Interspecies Spermatogonial Transplantation in Mice and In Vitro Microinsemination," *Proceedings of the National Academy of Science USA*, August 30, 2006). They then transplanted sperm stem cells from these rats into the testicles of mice and allowed them to develop until the mice began producing green rat sperm. The scientists then collected the green sperm and used IVF to fertilize rats' eggs. The green rats that were born did not show any genetic abnormalities and were able to mature into fertile adults. This report opens up the possibility that mice may now be used to grow the sperm of endangered species or prize agricultural livestock in a cheap and efficient manner. It also raises the specter of surrogate fathers, human or nonhuman, who are used to grow human sperm for men unable to grow their sperm for themselves. In a sense, mice can now "father" human children.

Recent Biological Discoveries with Future Ethical Import

Tomorrow's ethical questions often arise from today's scientific discoveries. Here I highlight three papers that raise intriguing questions for bioethicists. They are only a sample of the many fascinating reports in the primary scientific literature that have moral implications for our society.

First, how are we to evaluate the moral dimensions of disordered behaviors that are associated with genetic predispositions? In a paper published in *Nature Genetics*, Dierick and Greenspan from the Neurosciences Institute in San Diego, California, describe their genetic studies of flies that had been bred for aggressive behavior ("Molecular Analysis of Flies Selected for Aggressive Behavior," *Nature Genetics*, August 13, 2006). The duo developed a procedure to select for increased aggression in flies, a procedure that generated flies with a fighting index more than thirty

times greater than normal. They then compared the genes of these aggressive flies with their normal counterparts and isolated a single gene, *Cyp6a20,* that increases the fighting frequency of flies when it is mutated. Their results suggest a genetic basis for aggressive behavior in these animals. Given the conservation of many genetic mechanisms between flies, mice, and men, it would not be surprising if single genes existed in humans that, when mutated, made individuals aggressive. How would this change or not change our perception of criminal behavior?

Next, several scientific teams have reported the discovery of the genetic switch that turns on puberty. Han and coworkers have shown that the activation of several neurons in the mouse brain by the molecule called kisspeptin led to sexual maturation in the animal ("Activation of Gonadotropin-Releasing Hormone Neurons by Kisspeptin as a Neuroendocrine Switch for the Onset of Puberty," *Journal of Neuroscience*, December 7, 2005). This discovery could lead to treatments for puberty disorders—about one in ten thousand children fail to go through puberty, while many others go through puberty prematurely, some at just two years of age. However, it could also lead to the manipulation of puberty. For instance, more specifically, should parents be allowed to either accelerate or delay the sexual maturation of their children for economic reasons? Or, more generally, should we as a society use drugs to reverse the trend in recent decades that has shown that the timing of normal puberty has advanced in Western societies? Why or why not?

Finally, Roozendaal and colleagues report that they have identified a process in the brain that is important for the memory enhancement that is associated with strong emotions ("Glucocorticoid Enhancement of Memory Requires Arousal-Induced Noradrenergic Activation in the Basolateral Amygdala," *Proceedings of the National Academy of Science USA*, April 25, 2006). Previous studies had shown that glucocorticoids, molecules released by the adrenal cortex, a gland located above the kidneys, during emotional arousal, play a key role in strengthening new memories, but exactly how these molecules functioned was unclear. The new paper suggests that they act by activating neuronal cells in the part of the brain called the amygdala, which secrete the neurotransmitter norepinephrine. Significantly, the team reported that blocking the activation of these neurons by the glucocorticoids prevented rats from acquiring the enhanced memories associated with strong emotion. This paper, along with numerous others in the field, suggests that we may be able to enhance or diminish memories in human beings, especially memories associated with emotionally charged events. As the President's Council on Bioethics recognized in its report, *Beyond Therapy*, this possibility raises numerous ethical questions surrounding the central role of memory in human and social identity. Should we help survivors of a trauma forget their experiences? If so, how would this affect our social understanding of history? For instance, how would it change our perception of September 11 if everyone who lived through that horrible tragedy were chemically induced to forget his or her memories? In other words, are there circumstances when bad memories are actually good?

REV. NICANOR PIER GIORGIO AUSTRIACO, O.P., PH.D.
Assistant Professor of Biology
Providence College
Providence, RI