Science

The Cloning of a Human Being and Other Developments in Stem Cell Science

A human being has been cloned and killed. In a paper with a title and an abstract conspicuously lacking the words "clone" or "cloning," scientists at the Oregon Health and Science University used an improved variant of somatic cell nuclear transfer (the technique used to clone Dolly the sheep) to clone a human embryo.[1] Recall that somatic cell nuclear transfer involves taking the nucleus of one cell (in this case, a cell derived from a human donor's skin) and transplanting the nucleus into a human egg cell (in this case, an egg cell donated anonymously by a twenty-three- to thirty-one-year-old woman) that has had its own DNA removed. The cloned embryos were then killed in the laboratory to obtain embryonic stem cells from them. The study revealed that the efficiency of human cloning is dependent on numerous factors, including the quality of the egg—where higher levels of ovarian stimulation appeared to correlate with lower egg quality—and the genetic constitution of the egg donor. One woman had what the authors described as "exceptional" oocytes because two of her cloned embryos were enough to generate an embryonic stem cell line. Extensive testing of the embryonic stem cells derived from the cloned human embryos showed that they were indistinguishable from those derived from fertilized human embryos. They could easily be transformed into different mature human cell types including nerve cells, liver cells, and heart cells.

Incidentally, I should note that this groundbreaking human cloning paper published in the prestigious journal *Cell* has been criticized for possible scientific misconduct because of images that were apparently duplicated.[2] The problems were

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[1] Masahito Tachibana et al., "Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer," *Cell* 153.6 (May 15, 2013): 1228–1238.

[2] David Cyranoski and Eryka Check Hayden, "Stem-Cell Cloner Acknowledges Errors in Groundbreaking Paper," *Nature News*, May 23, 2013, http://www.nature.com/news/stem-cell-cloner-acknowledges-errors-in-groundbreaking-paper-1.13060.

raised by an anonymous individual on PubPeer.com, a website where anyone can raise comments about published scientific papers.[3] The leader of the Oregon team has admitted that these errors reflect honest mistakes made in the rush to get the cloning results published. It is important to note that these flaws do not undermine the primary findings of the study.

Next, the standard protocol for reprogramming adult stem cells into pluripotent stem cells involves some standard reprogramming factors that were identified from embryonic stem cells. A recent report from China suggests that two critical reprogramming factors, named OCT4 and SOX2, can now be replaced by other molecules, called lineage specifiers, which have been implicated in the development of distinct tissues types within the adult organism.[4] Eight novel lineage specifiers could replace OCT4, while one novel lineage specifier could replace SOX2. Indeed, combinations of these specifiers could completely replace OCT4 and SOX2. This paper unearths a novel and unexpected strategy that will allow scientists to reprogram adult cells into pluripotent stem cells.

Finally, a research team led by Anita Bhattacharyya from the Waisman Center at the University of Wisconsin–Madison took skin cells from an individual with Down syndrome and reprogrammed them into induced pluripotent stem cells that were then transformed into Down syndrome nerve cells.[5] They used these iPS-derived neuron cells to show that Down syndrome brain cells are not as good at making connections among themselves and other nerve cells as are brain cells obtained from normal individuals. They only had 60 percent of the usual number of connections, which are called synapses. The research team also discovered that the extra copy of the genes located on the third copy of chromosome 21 found in patients with Down syndrome changed the activity of 1,500 genes elsewhere in the genome. These genes were involved in a cell's response to oxidative stress, the stress associated with high levels of oxygen. This discovery suggests that many of the symptoms associated with Down syndrome could be the consequences of oxygen stress. Moreover, despite the celebrity surrounding the human cloning paper, this study, among others, shows that the nuclear reprogramming of adult cells remains the easiest and cheapest technique for obtaining pluripotent stem cells.

Nature versus Nurture: Toward a Biology of Social Influence

The debate over the relative influence of nature versus nurture on human biology and behavior is an old one. The growing consensus among biologists is that the dichotomy is a false one: biological phenomena are shaped by both environmental and genetic factors that are difficult, if not impossible, to disentangle.

[3] The PubPeer comments on this article can be found at http://pubpeer.com/publications/F0CFE0360002C25DC0BEFE28987D70.

[4] J. Shu et al., "Induction of Pluripotency in Mouse Somatic Cells with Lineage Specifiers," *Cell* 153.5 (May 23, 2013): 963–975.

[5] J. P. Weick et al., "Deficits in Human Trisomy 21 iPSCs and Neurons," *PNAS* 110.24 (June 11, 2013): 9962–9967.

A special issue of the *Proceedings of the National Academy of Sciences* contains a series of papers from the Arthur M. Sackler Colloquium, sponsored by the National Academy of the Sciences and the Canadian Institute for Advanced Research, that explore "biological embedding."[6] As Clyde Hertzman, a major player in the field, defines it, "biological embedding occurs when experience gets under the skin and alters human biological and developmental processes; when systematic differences in experience in different social environments in society lead to systematically different biological and developmental states; when these differences are stable and long term; and, finally, when they have the capacity to influence health, well-being, learning, or behavior over the life course."[7] Or to put it more simply, the study of biological embedding seeks to understand the consequences of social adversity on the development and biology of offspring, including human children. A sampling of the papers in this series illustrates the range of this maturing and productive field of research. For example, Bryan Kolb and colleagues examine the influence of experiential knowledge, including stress, parent–infant relations, and peer relationships, on the development of the prefrontal cortex in rats.[8] The prefrontal cortex is the forward part of the mammalian brain, which in humans is involved in the planning of complex cognitive and social behavior that is called command control. In another example, Russell Fernald and Karen Maruska investigate how social information changes the brain by looking at how social status alters the physiological, cellular, and molecular biology of the African cichlid fish.[9] Finally, Yang and colleagues used mice to study how these animals develop a preference for one type of music over another type during a critical period of their infancy.[10] To appreciate the potential influence of this area of research on our understanding of how nature and nurture interact in human development, consider that, in a sense, these studies are trying to understand why particular children are resilient to suffering and misfortune while others founder in the face of severe childhood adversity.

In another example that illustrates the intricate relationship between nature and nurture, a research team from Michigan State University, the University of Alberta, and the University of Guelph in Canada has shown that female squirrels living in crowded woods improve their offspring's chances of survival by accelerating their offspring's rate of growth.[11] By playing recordings of squirrel territorial vocalizations in the woods, the scientists were able to create the illusion of a large population

[6] For a summary and overview of the series of papers, see W. T. Boyce et al., "Toward a New Biology of Social Adversity," *PNAS* 109, suppl 2 (October 16, 2012): 17143–17148.

[7] C. Hertzman, "Putting the Concept of Biological Embedding in Historical Perspective," *PNAS* 109, suppl 2 (October 16, 2012): 17160–17167.

[8] B. Kolb et al., "Experience and the Developing Prefrontal Cortex," *PNAS* 109, suppl 2 (October 16, 2012): 17186–17193.

[9] R. D. Fernald and K. P. Maruska, "Social Information Changes the Brain," *PNAS* 109, suppl 2 (October 16, 2012): 17194–17199.

[10] E. J. Yang et al., "Critical Period for Acoustic Preference in Mice," *PNAS* 109, suppl 2 (October 16, 2012): 17212–17220.

[11] B. Dantzer et al., "Density Triggers Maternal Hormones That Increase Adaptive Offspring Growth in a Wild Mammal," *Science* 340.6137 (April 18, 2013):1215–1217.

of squirrels. Pregnant females reacted to the increased vocalizations by producing more stress hormones. This in turn accelerated squirrel pup growth. Strikingly, however, the pups born during these simulated high-density population time periods did not live as long as their peers born outside these time periods. The environment during pregnancy had so critically altered their biology that they had a diminished life expectancy.

A Scientific Study of the Influence of Markets on Morals

How do financial transactions affect moral judgment? In a fascinating series of experiments described in the journal *Science*, two economists, Armin Falk from the University of Bonn and Nora Szech from the University of Bamberg, asked several hundred human subjects to decide between saving the life of a mouse or receiving money.[12] In the first scenario, subjects faced a binary choice of either saving the life of the mouse or receiving ten euros. In the second scenario, one seller and one buyer bargained over killing a mouse for a total gain of twenty euros that the two parties could then split between themselves. Finally, in the third scenario, seven buyers and nine sellers bargained over the price of killing the mouse. The study revealed that, among individuals participating in the market system of negotiation between buyers and sellers (second and third scenarios), the willingness to kill the mouse was substantially higher than among individuals who were simply asked to choose between the mouse and the money (first scenario). Moreover, it also showed that in the multilateral market scenario (third scenario), prices for the mouse's life deteriorated tremendously. Therefore, the authors conclude, "The point of this study is not to question market economies in general. . . . However, focusing on the causal effects of institutions, we show that for a given population, markets erode moral values. We therefore agree with the statement quoted at the beginning that we as a society have to think about where markets are appropriate—and where they are not." [13]

Sexual Differences and Sexual Preferences

Males and females are different and they are different in different ways. In biology, these differences are called sexual dimorphisms. A paper published by researchers from the University of California at San Francisco reports that they have uncovered the function of sexually dimorphic brain cells: they regulate sex-typical behaviors in both sexes.[14] Specifically, the scientists describe differences between male and female adults in those brain cells containing a molecule sensitive to the human sex hormone progesterone. Moreover, when they genetically engineered female mice lacking the neurons that contain these molecules, they observed a dramatic reduction in sexual receptivity. In contrast, ablating these cells in males reduced mating and territorial aggression. These results demonstrate that sexual differences, including differences in sexual behavior, are biologically programmed.

[12] Armin Falk and Nora Szech, "Morals and Markets," *Science* 340.6133 (May 10, 2013):707–710.

[13] Ibid., 710.

[14] C. F. Yang et al., "Sexually Dimorphic Neurons in the Ventromedial Hypothalamus Govern Mating in Both Sexes and Aggression in Males," *Cell* 153.4 (May 9, 2013).

Despite the protestations of postmodern deconstructionists, the sexual differences between men and women, including gender-specific behaviors, are not mere social constructs.

Sex differences are also evident during the development of animals. In a fascinating study, Lan Jiang and colleagues have discovered that the DNA of sperm and egg carry different modifications that alter the activity of their genes.[15] These epigenetic modifications are called methylations, and their presence or absence can switch genes on or off. Egg DNA has significantly lower levels of methylation than its sperm counterpart. Strikingly, following fertilization, the embryo adopted the methylation pattern of the sperm, suggesting that embryos inherit not only DNA but also DNA modification patterns from their fathers—modifications that regulate the unfolding of the genetic program encoded in an animal's DNA.

Next, scientists at the Hospital for Sick Children in Toronto have published a study that reveals that the composition of the microbiome—the kilogram of single-celled microbes found on and in every mammalian organism, including every human being—differs between the sexes.[16] The team used cutting-edge sequencing technology to survey the gut bacteria in normal male and female mice and discovered that young animals had similar kinds of gut bacteria. However, they acquired distinct populations of bacteria once they underwent puberty. Surprisingly, the bacteria appeared to affect sex hormone levels in the animals, which in turn appear to regulate the organisms' immune responses. The study may explain why mammalian females, including human women, are more prone to autoimmune diseases than their male counterparts. This paper adds to the growing number of studies that reveal that the microbial cells living on and in an animal can influence the organism's weight, propensity for disease, and even sexual preference.[17]

Finally, referring to sexual preference, a study published this past quarter suggests that a single neurotransmitter found in the brain, called serotonin, can regulate sexual preference in mate choice among mice.[18] Serotonin is a hormone that influences, among other things, aggression, mood, sleep, and memory. According to this recent study, female mice bred to lack either serotonin or specific brain cells that usually release serotonin in various regions of the brain preferred to mount and sniff the genitals and heads of other females rather than males. Notably, they still responded to male sexual attention but preferred other females. In an earlier study, the same research team had shown that abolishing serotonin in male mice also

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[15] L. Jiang et al., "Sperm, but Not Oocyte, DNA Methylome Is Inherited by Zebrafish Early Embryos," *Cell* 153.4 (May 9, 2013): 773–784.

[16] J.G.M. Markle et al., "Sex Differences in the Gut Microbiome Drive Hormone-Dependent Regulation of Autoimmunity," *Science* 339.6123 (January 17, 2013): 1084–1088.

[17] A. Caricilli et al., "Gut Microbiota Is a Key Modulator of Insulin Resistance in TLR 2 Knockout Mice," *PLoS Biology* 9 (2011): e1001212; and G. Sharon et al., "Commensal Bacteria Play a Role in Mating Preference of *Drosophila melanogaster*," *PNAS* 107.46 (November 16, 2010): 20051–20056.

[18] S. Zhang et al., "Serotonin Signaling in the Brain of Adult Female Mice is Required for Sexual Preference," *PNAS* 110.24 (June 11, 2013): 9968–9973.

abolished these mice's preference for females.[19] Instead they mounted both sexes equally when given the chance. These findings suggest that an organism's sexual preference, in other words, its inclinations toward and its perception of a potential mate, can be understood as one dimension of the estimative sense described by the Aristotelian-Thomistic tradition. Finally, I must add that it is not clear if the results of these murine studies can be extended to explain human sexual preference, and any attempt to do so would be premature. Nonetheless, some online bloggers have already claimed that serotonin is an "anti-gay" factor. Others even propose that therapeutic interventions that can alter serotonin levels may be able to reverse homosexuality in the same way that altering serotonin levels can reverse depression.

The Biology of Individuality and Twinning

What makes an individual an individual? This is certainly a philosophical question, recalling the ancient, medieval, and renaissance debates about the causes of individuation, but it is also a biological one. Two issues come to mind.

First, how do we explain the biological phenomenon of monozygotic (MZ) twinning, the genesis of two biological individuals who are genetically identical? The dominant postfertilization model attributes MZ twinning to the splitting of the embryo sometime during its first two weeks of development, where the timing of that split gives rise to the range of MZ twin types, from those who share a minimal amount of placental membrane during gestation to those who share bodily parts as conjoined twins.[20] In a recent proposal, Gonzalo Herranz at the University of Navarre has argued for an alternative model for MZ twinning that involves the single-celled zygote becoming two individuals at the moment after the first cell division, where later fusion of membranes or body parts gives rise to the known range of MZ twin types.[21] In a sense, according to this alternative, the larger whole—the multi-cellular embryo—is not split into two smaller wholes. Rather, a whole—in this case the single-celled zygote—becomes two wholes very early in its development. In my view, there is insufficient evidence to definitively choose one model for MZ twinning over the other. Therefore, it is important for bioethicists, especially those who reflect on the philosophical issues raised by MZ twinning, to realize that the biological mechanism behind MZ twinning remains unresolved.

Second, in spite of their genetic identity, how do we then explain the differences between MZ twins that inevitably manifest themselves throughout their lifetimes? In a recent study, a group of scientists examined the biological causality behind individuality by allowing a population of forty genetically identical mice to live

[19] Y. Liu et al., "Molecular Regulation of Sexual Preference Revealed by Genetic Studies of 5-HT in the Brains of Male Mice," *Nature* 472.7341 (April 7, 2011): 95–99.

[20] For an interesting review, see I. Blickstein, "Monochorionicity in Perspective," *Ultrasound Obstetrics and Gynecology* 27.3 (March 2006): 235–238.

[21] G. Herranz, "The Timing of Monozygotic Twinning: A Criticism of the Common Model," *Zygote*, published online June 5, 2013.

together in an enclosure filled with objects designed to encourage exploration.[22] They discovered that the individual mice that were more adventurous than their genetically identical littermates—those that explored their complex environment more than the other mice—grew more neurons than their less adventurous counterparts. These findings, the first that show a direct link between individual behavior and individual brain organization, suggest that brain plasticity, the ability of the brain to be organized and reorganized, can be uniquely shaped over time by the unique lived experience of the organism, even among individuals that are genetically identical. Clearly, as we already discussed above, organisms cannot be reduced to their genes. Rather, they are emergent systems shaped and directed by both nature and nurture.

A Morally Noncontroversial Replacement for the HEK 293 Cell Line

Finally, in recent years, there has been some controversy in the pro-life community surrounding the virtuous use of human cell lines obtained from the corpse of an aborted fetus.[23] One of these morally controversial cell lines is the human cell line HEK 293, which is commonly used in biomedical research laboratories throughout the world for the production of molecular tools.[24] Some Catholic bioethicists, and I count myself among them, have argued for the continued use of this cell line by pro-life scientists as long as they are clear about their pro-life convictions, because there have been no ethically acceptable alternative cell lines. An alternative now exists. The German company CEVEC Pharmaceuticals has recently developed a cell line, called CAP cells, from amniotic cells taken from a pregnant woman during a routine clinical procedure called an amniocentesis.[25] A master cell bank of these CAP cells has been established, tested, and certified according to European guidelines for the routine production of the molecular tools usually made from HEK 293. Pro-life researchers and other scientists of right conscience now have an alternative to the use of HEK 293 in their laboratories.

Rev. Nicanor Pier Giorgio Austriaco, OP

[22] J. Freud et al., "Emergence of Individuality in Genetically Identical Mice," *Science* 340.6133 (May 10, 2013): 756–759.

[23] Nicanor Pier Giorgio Austriaco, "Using Morally Controversial Human Cell Lines after *Dignitas personae*," *National Catholic Bioethics Quarterly* 10.2 (Summer 2010): 265–272.

[24] A. Wong, "The Ethics of *HEK 293*," *National Catholic Bioethics Quarterly* 6.3 (Autumn 2006): 473–495.

[25] See the CEVEC Pharmaceuticals website at http://www.Cevec.com/technology.