Seeking Consensus: *A Clarification and Defense of Altered Nuclear Transfer*

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Since 1998, when human embryonic stem cells were first isolated, our nation has been locked in a conflict over federal funding of this new field of scientific research. Both sides in the debate are defending important human goods, and both of these goods—opening avenues for advance in medicine and protecting nascent human life—are important to all of us. A purely political solution will leave our country bitterly divided, eroding the social support and sense of noble purpose that is essential for the public funding of biomedical science. While there are currently no federal restrictions on the use of private funds for this research, there is a consensus in the scientific community that without federal support for newly created embryonic stem cell lines, progress in this emerging field of scientific inquiry will be seriously constrained.

In May 2005, acknowledging our national impasse over embryonic stem cell research, the President's Council on Bioethics published a white paper that outlines a series of proposals for obtaining pluripotent stem cells (the functional equivalent of embryonic stem cells) without the creation or destruction of human embryos.[1] One of these proposals, "altered nuclear transfer" (ANT) has stirred considerable public interest and affirmation, including several legislative proposals that would provide funding for its further exploration. There is substantial support among leading scientists, moral philosophers, and religious leaders for the view that ANT may offer a scientifically feasible and morally sound way forward on ES cell research.[2]

At the same time, there has been some confusion about ANT, leading to its mischaracterization in certain reports and published commentaries. In a recent cover story on stem cell research in *Time* magazine, ANT was described as a project that "would ensure that the embryo lives only long enough to produce stem cells and then dies."[3] But the whole idea of ANT is to produce pluripotent stem cells *without* creating an embryo. *Time*'s description has ANT violating the very moral principle it is intended to uphold.

Acknowledging the complexity of the scientific and ethical issues at the foundation of this proposal, and in the spirit of constructive dialogue, we seek in this essay first to offer a clear and accurate account of ANT, and then to respond to some of the more significant questions and concerns about it.

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Altered Nuclear Transfer: Non-Embryonic Sources of Pluripotent Cells

The President's Council report draws a critical distinction between *pluripotency*, the capacity of a cell to give rise to many if not all the different cell types of the human body, and *totipotency*, the capacity of a zygote or other cell to develop as a complete, integrated, living being. A naturally conceived zygote—the single-celled embryo brought into being by fertilization—is totipotent; embryonic stem cells are merely pluripotent.

ANT is a general concept that might take a variety of specific forms. The basic idea is to employ somatic cell nuclear transfer (SCNT), the technique often known simply as "cloning," but to alter it in such a way that pluripotent stem cells are produced *without the creation and destruction of human embryos*. In standard SCNT, the nucleus of a differentiated body cell is transferred into an egg cell that has had its own nucleus removed.[4] The egg cytoplasm then reprograms the transferred nucleus and, if all goes as planned, the newly constituted cell proceeds to divide and develop like a naturally conceived embryo. This is how Dolly the sheep was produced. In ANT, the adult body cell nucleus or the egg cytoplasm (or both) are altered *before* the nucleus is transferred into the enucleated egg so that the newly constituted cell will, from the outset, lack the integrated unity and developmental potential of an embryo, yet will nevertheless possess the capacity for a certain limited subset of growth sufficient to produce pluripotent stem cells.

The underlying idea of ANT is that alterations performed prior to nuclear transfer will allow the construction of cellular structures that can produce pluripotent stem cells, but are not embryos.

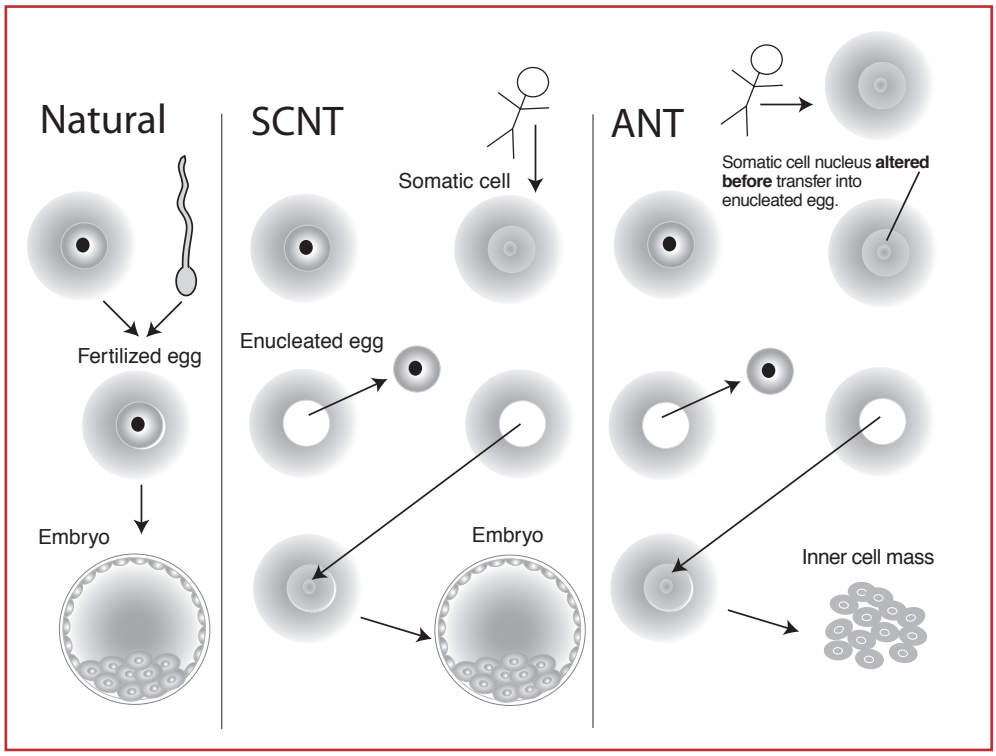
This process mirrors certain naturally occurring phenomena. In normal conception, fertilization signals the activation of the organizing principle for the self-development of the human organism towards developmental maturity. But without all of the essential elements—the necessary complement of chromosomes and the cytoplasmic factors for the proper pattern and sequence of gene expression—there can be no living whole, no organism, no human embryo. Recent evidence from infertility studies suggests that, in the natural reproductive process, incomplete or inadequate combinations of the necessary elements lead to many "failures of fertilization." Some of these naturally occurring failures of fertilization may still proceed along partial trajectories of organic growth without being actual organisms.

For example, certain grossly abnormal karyotypes (including haploid genomes, with only half the natural number of chromosomes) will form blastocyst-like structures but lack the basic organization of an embryo and thus lack an embryo's capacity for self-integration and self-directed development towards organismal maturity. Even an egg without a nucleus can undergo a series of cell divisions when it is artificially activated, yet it is clearly not an embryo—or an organism at all. The messenger RNA for the protein synthesis that drives these early cell divisions is generated during the maturation of the egg and then activated after fertilization. Like a spinning top, the cells have a certain biological momentum that propels a partial and unorganized trajectory of development, but unlike an embryo, they are not adequately constituted to establish the dynamic molecular interactions that characterize a coherent and self-regulating organism.

Some of these aberrant forms appear to be capable of generating ES cells or their functional equivalents. One evident example is a benign tumor, known as a teratoma, caused by spontaneous development of an activated egg. Mature teratomas generate all three primary embryonic cell types as well as more advanced cells and tissues, including partial limb and organ primordia—sometimes even hair, fingernails, and fully formed teeth. These chaotic, disorganized, and nonfunctional masses are like bags of jumbled puzzle parts, entirely lacking the unified structure and dynamic character of an organism. Biomedical science has never considered these growths to be embryonic human beings, nor have defenders of the dignity of embryonic human life in debates about abortion or embryo-destructive research. Everyone acknowledges that they are not organisms, but tumors; yet they generate cells with the functional characteristics of embryonic stem cells.

The disorganized character of teratomas seems to arise, not from changes in the DNA sequence, but from genetic imprinting, an epigenetic modification that affects the pattern of gene expression (keeping some genes turned off and others on).[5] In natural reproduction, the sperm and egg have different but complementary patterns of imprinting, allowing a coordinated control of embryological development. When an egg is activated without a sperm, the trophectoderm (the outer layer in a natural embryo) and its lineages fail to form. In the absence of the complementary genetic contribution of the male, the activated egg is inadequately constituted to direct the integrated development characteristic of human embryogenesis.

Examples such as this show how even a small genetic or epigenetic change can affect the entire balance of an



Growth without Life. In altered nuclear transfer, targeted alterations to the somatic cell nucleus (or to the egg cytoplasm) preclude the integrated organization and potential for development of an embryo.

enormous network of biochemical processes necessary for embryogenesis. An organism is a dynamic whole, an interactive web of interdependent processes that express emergent properties not apparent in the biochemical parts. Within this dynamic, self-sustaining system is the very principle of life, the organizing information and coordinated coherence of a living being. With the full complement of essential elements, an organismal system subsumes and sustains the parts; it exerts a downward causation that binds and balances the parts into a patterned program of integrated growth and development. Partial organic subsystems (cells, tissues, and organs) that are components of this larger whole, if separated or separately produced, may temporarily proceed forward in development (though not in the direction of a mature member of the species). But without the coherent coordination and robust self-regulation of the full organism, they are, in reality, nothing more than fragmentary cellular structures whose trajectory of growth is distinct from that of an embryo.

The underlying idea of ANT is that small but precisely selected genetic or epigenetic alterations performed prior to nuclear transfer will allow the laboratory construction of cellular structures that are capable of producing pluripotent stem cells, but are not embryos. They are biologically (and therefore morally) equivalent not to embryos, but to teratomas and other fragmentary and unorganized growths.

ANT has sometimes been misrepresented in the popular media as a procedure that prevents implantation of a cloned embryo;[6] in fact, neither the President's Council report nor the writings of any of the advocates of this project have ever made such a proposal. The alterations in ANT are not destructive operations on an existing embryo, nor do they involve the creation of an embryo with an intrinsic deficiency. The inability to establish the trophectoderm cell lineage is not reasonably considered a defect within a part, but rather a failure in the formation of the whole.[7] This "deficiency" is more properly understood as an "insufficiency," not a defect *in* a being (like not having a limb), but an inadequacy at such a fundamental level that it precludes the coordinated coherence and active disposition for development that are the defining characteristics of an embryonic organism.

Possible Forms of ANT

CDX2. Of the range of forms ANT might take, one much-discussed possibility involves deleting or silencing a gene essential at the most primary level of coordinated organization. As described in a January 2006 paper in the journal *Nature*, stem cell biologists Rudolf Jaenisch and Alexander Meissner have provided proof of principle for the scientific feasibility of this approach in a series of elegant mouse model experiments in which they procured fully functional pluripotent stem cells.[8] Using the technique of RNA interference, they were able to reversibly silence the functional expression of a gene called *Cdx2* before the nucleus containing the gene was transferred into an enucleated egg. Research reported just a month later in the journal *Science* suggests that it may be possible to achieve the goals of ANT through the preemptive silenc-

ing of *Cdx2* in the egg, in this case too before the act of nuclear transfer.[9] This study, by University of Missouri biologist Michael Roberts, showed that, in mice, maternally derived messenger RNA for *Cdx2* is present in the egg and asymmetrically distributed in the first cell division after fertilization. The asymmetric distribution of *Cdx2* directs the cells at the two-cell stage to form distinct cell lineages. One of the cells at the two-cell stage goes on to become the trophectoderm and forms the outer layer of the embryo (and later the extra-embryonic membranes, including the placenta). The other cell forms the "inner cell mass" (ICM) which is the source of cells used to culture embryonic stem cells. By the selective silencing of *Cdx2*, the authors were able to produce an unorganized mass composed exclusively of cells with the character of those of the ICM.

ANT-OAR. Another form of ANT, known as "oocyte-assisted reprogramming" (ANT-OAR), involves the activation of genes that are expressed in pluripotent cells, but not in totipotent cells. In standard SCNT, factors in the egg cytoplasm reset the nucleus of the body cell to the totipotent state of a naturally produced zygote. As the cells divide and differentiate into different kinds of cells, the type of cell they differentiate into is determined by transcription factors that act as molecular switches to activate whole programs of gene expression. The gene *Cdx2* is a master regulator that produces just such a transcription factor, one that is essential for the differentiation of the trophectoderm. ANT-OAR focuses on genetic manipulation with factors that positively define the pluripotent cells of the ICM but are not present in early embryos. The concept is to create a cell with an epigenetic state that is immediately distinct from that of an embryo. For example, embryos do not contain the powerful pluripotent stem cell-specific transcription factor *Nanog* until several cell divisions have taken place, and even then *Nanog* is expressed only in ICM cells, not in the cells of the trophectoderm. A cell producing Nanog from its inception thus cannot be properly considered an embryo. In ANT-OAR, master regulator genes such as Nanog would be overexpressed in the body cell nucleus before it is transferred into the egg cytoplasm. The egg cytoplasm would then help reprogram the altered nucleus *directly* to the epigenetic state of a pluripotent cell, bypassing entirely the totipotent state of a human embryo.

Recent scientific evidence suggests that the functional silencing of *Cdx2* and the activation of *Nanog* and another pluripotent transcription factor, *Oct4*, are in fact complementary approaches to promote the specific pattern of gene expression characteristic of pluripotent stem cells. *Cdx2* and *Oct4* appear to regulate each other: blocking *Cdx2* promotes the expression of *Oct4* and *Nanog*, and the overexpression of *Oct4* represses *Cdx2*.[10] In natural embryogenesis, this mechanism is used to establish the distinct development of both the ICM and the trophectoderm, the organism's two earliest and essential differentiated cell lineages. In ANT, the proposed alterations to this fundamental mechanism will ensure that only a single cell lineage, the ICM, develops. The product is the biological (and thus the moral) equivalent of a complex cell culture.

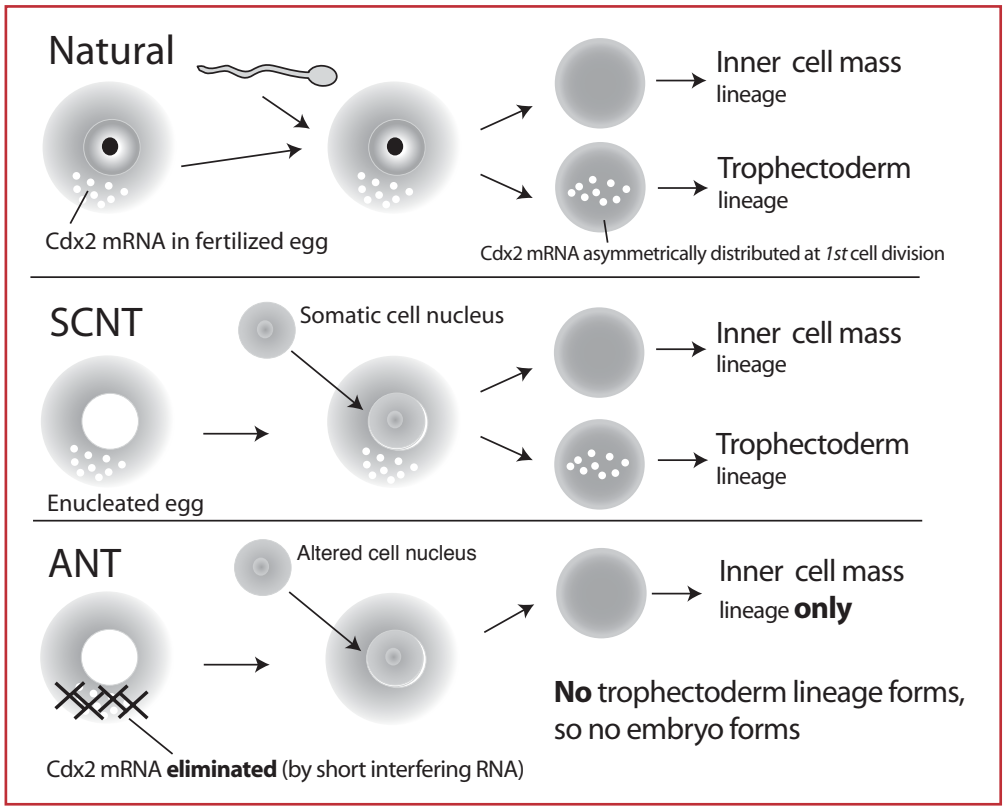
In each of the approaches described above, and any other technical variation of ANT, the crucial principle is the *preemptive* nature of the intervention. From the beginning, and at every point along its development, what is produced through ANT is not an embryo or a member of the species at any developmental stage. If such a limited biological construct were produced only for serious scientific purposes and were accorded the respect appropriate to all human tissues, this project would not compromise any fundamental moral principles.

Medical science should be a matter of unity in our national identity: No one should enter the hospital with moral qualms about how their therapies were developed.

ANT's Advantages

ANT, in its many possible variations, could provide important advantages that would help to advance pluriptoent stem cell research and its possible medical applications. Unlike research on "leftover" embryos in IVF clinics, ANT would allow the production of pluripotent stem cell lines with an unlimited range of genetic types for the study of disease, drug testing, and possibly the generation of therapeutically useful immune-compatible cells. Moreover, as a laboratory technique, ANT would unburden embryonic stem cell research from the additional ethical concerns of "left over" IVF embryos, including the attendant clinical and legal complexities in this realm of great personal and social sensitivity.[11]

ANT would also establish a broader moral precedent for future progress in developmental biology. A morally sound and scientifically reliable method of ANT would provide a valuable research tool to probe and explore other dimensions of cell development and differentiation, including gene expression cascades, imprinting, and intercellular communication. Furthermore, the basic research essential to establish and refine the technical procedures for ANT would advance our understanding of the molecular mechanisms of biological development and might serve as a bridge to other technologies, such as direct reprogramming of adult cells.



Cell lineages at first cell division. With ANT, the Cdx2-mRNA necessary for the trophectoderm, and therefore for embryonic development, has been removed from the egg.

Most significantly, stem cell research that relied on ANT would be open to federal funding and the advantages of ethical oversight, wide public support, and coordinated research collaboration on a nation level.

Qualms, Concerns, and Critics

The ANT proposal has received considerable criticism, some of which seems to have arisen from scientific misunderstanding, some for reasons related to politics, and some from a genuine concern over the moral problems that ANT seeks to resolve.

Diverting resources. Some critics of ANT (and other such proposals) have called these efforts unnecessary—a "distraction " and a "waste of time."[12] The most obvious response to this assertion is that many of our fellow citizens (ourselves among them) have deeply held moral objections to any research that destroys a human embryo. They point to the biological continuity of human life from conception to natural death and consider the principle of the "inviolability of life" a fundamental good and moral cornerstone of our civilization. Yet even those who disagree with this moral valuation of nascent human life have good reasons to support the search for "alternative sources." First, there is the political reality that under current federal law, new ES cell lines are not likely to receive federal funding for at least two and half more years. Even if a change in the composition of the Congress were to allow an override of the president's veto of legislation allowing the federal funding of ES cell lines procured from IVF embryos, Congress would probably not extend that approval to lines derived through the intentional creation and destruction of cloned human embryos. Without an alternative source, there is no near-term prospect for federal funding of a full range of ES cell lines of specific genetic types.

Second, it is becoming increasingly clear that this whole debate is deeply dividing our nation, creating a patchwork of policies and eroding support for federal funding of biomedical research.[13] Medical science, however, should be a matter of unity in our national identity: No one should enter the hospital with moral qualms about how their therapies were developed.

Finally, with continuing advances in developmental biology, it is evident that the debate over ES cell research is just the first of many controversies we will face in this scientific arena. Parthenogenesis, human-animal chimeras, human body parts grown in laboratories—these and a wide range of other emerging technologies make it imperative that we define the boundaries of humanity with accuracy, clarity, and precision in order to ensure that our scientific practices as well as our laws and public policies protect human beings while avoiding confusion between human beings and entities that, while sharing some aspects of human biology, are not human beings. The social, scientific, and philosophical analyses necessary for the development and institution of ANT (or other alternative approaches) could help to establish a wider frame that

would allow scientific and medical advances in the emerging era of developmental biology. Clearly, the processes and the fruits of a constructive dialogue on these difficult and important issues would not be "a distraction" or a "waste of time."

Defining life. What do we mean by organism, and what degree of organization and intrinsic potential for development are the defining qualities of an embryo? And do the biological distinctions and laboratory interventions proposed by ANT provide the principles and practical method for a genuine solution? These questions are raised, at least by implication, in Insoo Hyun and Kyu Won Jung's article in this issue of the *Hastings Center Report*. Hyun and Jung assert that standard SCNT "might offer an alternative source for pluripotent stem cells similar to ANT" because it does not produce embryos that can "develop into human beings." They cite SCNT studies which indicate that the unique fragility of primate oocytes leads to a deficiency of an essential protein when the egg is enucleated, disrupting the mechanisms of cell division so severely that the resulting entities are "bioengineered embryo-like artifacts," not true human embryos.

The preemptive *nature of the intervention is crucial. What is produced through ANT is not a member of the species at any developmental stage.*

This is a provocative and interesting claim, but it faces two serious problems. First, the idea that SCNT cannot produce embryos is at this point largely a matter of conjecture. There have been so few studies of primate SCNT that it is simply too early to know what underlies the failures of current methodology. Further, it may turn out that the disruption can vary with the skill of the technician or in a manner out of the reach of predictable control and verification. The one report of success in producing a live-born primate using the nuclear transfer technique (in which the nucleus was from a cleavage stage blastomere rather than from a somatic cell) suggests that the fragility of the egg does not, in itself, always provide a principled barrier to natural development.[14]

The second and far more fundamental problem is that Hyun and Jung fail to offer adequate criteria for their claim that no embryo is created and destroyed. They only give hints of an implicit moral analysis. They affirm, for example, that "The crucial distinction is not between 'natural' human embryos on the one hand and 'artificial' blastocysts on the other. Rather, it is whether the latter have the potential to develop into human beings."[15] They suggest that the latter "may lack the inner biological organization necessary for gestation and development into members of the human species." If this is true, they say, "then it would be a misuse of language to even continue to call them 'embryos.'"[16]

At first, these statements may seem consistent with the scientific and moral foundations of ANT. However, a more careful reading would indicate otherwise. Drawing a distinction between "biological potential" and what they call "circumstantial potential," they state: "No ex-corporeal human blastocyst (fertilized or cloned) has even a remote chance of developing into a human being unless the choice is made to implant it into a woman's womb." They go on to suggest that "the term 'embryo' refers not to a single rarified entity but to a complex series of contiguous developmental events."

There is both insight and philosophical danger in drawing moral implications from a developmentally based definition of human life. Indeed, the intrinsic capacity for growth and development are the defining qualities of an embryo, the Greek root of the word embryo means "to swell or to grow." Yet this trajectory of change is not the embryo itself, but an expression of the intrinsic powers that are its defining nature.[17] These are materially grounded powers, yet they express something more than the mere matter through which they are manifest; though the matter changes, the individual perdures. In biology the whole, as the unified principle of life, precedes and produces the parts. We do not, as Hyun and Jung suggest, develop "into members of the human species." No living being, by self-directed development, can become anything other than what it already essentially is; we do not first come to be then become human beings. We begin as embryonic human beings and develop from the embryonic into and through the fetal, infant, child, and adolescent stages, and ultimately into adulthood, without changing from one natural kind of being into another.

In contrast to developmentally or circumstantially based criteria, the ANT proposal rests on clear biologically based criteria for moral standing. These are intrinsic to the entity itself and distinguish a human being, at any embryonic stage, from gametes, tumors, and other nonembryonic entities. Under such criteria, any organism (regardless of its location, mode of production, or stage of development) that has the innate powers (when provided with a suitable environment and adequate nutrition) to organize and develop as a human individual also has the inviolable moral standing of a human being.[18] Thus a defective embryo could develop *as* a human individual even though it did not successfully develop *into* a baby. The ANT entity would not develop *as* a human individual at all but would lack the requisite fundamental organization. Such a notion of organism is based on the concept that in a true embryonic organism, the intrinsic powers and ac-

tive potentialities are expressed in the coordinated unity and ordered unfolding of the species-typical form.[19] The very word "organism" implies organization—an overarching principle that binds the parts and processes of life into a coherent and coordinated whole. As a living being, an organism is a self-developing and self-maintaining unity under the governance of an immanent plan. In practical application, these criteria mean that if we create an embryonic human being—an incipient human life—in a laboratory dish, then it is not a resource to be used, but a distinct human individual with a moral claim for just and respectful treatment.

Even among those who accept these criteria, however, some have questioned whether ANT avoids creating and destroying a human embryo. One objection is grounded in the assertion that any act of nuclear transfer in which a "reasonably complete human genome" is placed into an enucleated egg directly mimics conception and must be considered the initiation of a human life.[20]

This comparison between nuclear transfer and natural conception fails to acknowledge the precise balance of material components essential for conception, as evidenced by the low rates of success not just in cloning but even in the natural reproductive process.[21] In particular, those pressing this comparison seem not to recognize the importance of epigenetics in setting the precise conditions for conception. Complementary epigenetic revisions are made during the differentiation of the male and female gametes, and soon after conception, erasure of methylation (a fundamental epigenetic change) occurs in the male-derived chromosomes of the early embryo.[22] The adult nucleus placed into the egg cytoplast (the egg cell without its own nucleus) is not the functional equivalent of what is formed by the union of sperm and egg.

Indeed, it is not the genome that distinguishes a distinct cell type; with a few exceptions every cell of the body has the same genome. Rather, it is the cytoplasmic factors and the epigenetic state that determine the pattern of gene expression and thereby establish the identity and development potential of a cell.[23] While every cell of the body shares the expression of many basic "housekeeping" genes necessary for the metabolic processes essential for life, every specialized cell type has its own distinctive and recognizable gene expression profile. Only one kind of cell has the epigenetic state of totipotency: only the single-celled human embryo has the epigenetic configuration constitutive of a nascent human organism.

The error of "DNA essentialism" is dramatically demonstrated in cloning by the way the cytoplasm reprograms the DNA of the adult cell nucleus and gives it an entirely different destiny than it had in the donor cell.[24] For conception of a new human embryonic organism to occur with nuclear transfer, a crucial balance of factors in the cytoplasm must erase specific imprints in the adult cell nucleus and set in motion the sequence of gene expression necessary for ordered embryonic development. The low rates of success in cloning (in all species) points to the difficulty of establishing these critical conditions.

ANT, as described above with the combination of alterations in *Cdx2* and *Nanog*, would not only preclude the establishment of the gene expression pattern of a totipotent cell, but would also initiate the transformation of the somatic cell nucleus directly toward the epigenetic configuration of a pluripotent stem cell. At every stage of cell division and growth the laboratory product of ANT would be verifiably distinct *in kind* (not merely in its prospects for survival) from a human embryo. These differences, at first detectable only at the molecular level, would become increasingly evident in both morphology and behavior.

A solution that sustains social consensus even amid the diversity of our fundamental moral beliefs would be a triumph for humanity as a whole.

Overseeing development and practical implementation. The scientific investigation and full development of ANT to the level of practical application will require additional ethical discernment and technical precision. There must be a high standard of reliability and regularity. A project such as this, where what must be avoided is not damage to an existing life but creation of a living human embryo, raises unfamiliar and difficult challenges. Clearly, both in development and application, a high degree of caution is required.

Such requirements raise a more general question: Can scientific protocols be designed and developed that will provide an adequate level of measurable predictability to ensure beyond a reasonable doubt that the entity created by ANT is not a human embryo? The scientific description and moral analysis given above would suggest this can be done. The premise that different cell types have unique and detectable "molecular signatures" representing their epigenetic state is well supported by current evidence, and there is rapid advance in the development of technological tools for detecting both quantitative and temporally relevant signs of such states.[25]

Ultimately, moving from studies in animal cells to research with human cells and wide practical implementation requires a judgment that the research is morally acceptable. However, as the philosopher Christian Brugger explains, there is a difference between absolute certitude

and moral certitude: "in the case of the scientific method we operate in the domain, not of doubtless certitude . . . , but of statistical certitude in which doubt is never ruled out; nevertheless, it is possible to have certitude beyond a *reasonable* doubt (i.e., *moral certitude*) that the entity created through ANT-OAR is not an embryo."[26] In support of such a conclusion is the high degree of similarity in fundamental developmental biological mechanisms across species. As Rudolph Jaenisch explains, "This underlying biology means that we can, with good confidence, extrapolate our findings in experimental animal systems to the biological mechanism of human development. Accordingly, the fundamental proof of principle necessary for the techniques proposed here should be obtainable without the destruction of human embryos."[27]

Maintaining Consensus amid Diversity

The current controversy over embryonic stem cell research challenges us to understand with clarity and precision the differences between embryos and partial and unorganized structures that, though capable of producing cells from which pluripotent stem cells may be cultured, are not embryos. An important benefit of this understanding, we believe, is the prospect of using nuclear transfer technology to produce structures that are biologically equivalent to teratomas, while eliminating any genuine risk of accidentally producing embryos.

Altered nuclear transfer offers a possible resolution to our current impasse over the procurement of pluripotent stem cells. It could at once respect human life at all stages and promote the fullest prospects for scientific progress and its medical applications. It could open federal funding for an unlimited range of new stem cell lines. It would also set a precedent for encouraging the creative use of science to meet ethical challenges and overcoming controversies arising among people of goodwill who find themselves in moral disagreement. A solution that sustains social consensus even amid the diversity of our fundamental beliefs would be a triumph for humanity as a whole.

Acknowledgement

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2. "Production of Pluripotent Stem Cells by Oocyte Assisted Reprogramming," at http://www.eppc.org/programs/bioethics/publications/programID.35,pubID.2374/pub_detail.asp.

3. N. Gibbs, "Stem Cells: The Hope and the Hype," *Time* (August 7, 2006): 41-46.

4. In some techniques, the whole body cell is simply fused with the enucleated egg.

5. Studies of benign ovarian teratomas (dermoid cysts) have demonstrated that they most commonly arise after meiosis I and failure of meiosis II and are therefore diploid, containing a full complement of forty-six chromosomes. U. Surti et al., "Genetics and biology of human ovarian teratomas. I. Cytogenetic analysis and mechanism of origin," *American Journal of Human Genetics* 47, no. 4 (1990): 635-43.

6. In this issue of the *Hastings Center Report*, Insoo Hyun and Kyu Won Jung similarly inaccurately characterize ANT as producing "entities engineered to develop like early embryos but lacking the biological capacity to implant and develop into human beings."

7. The trophectoderm is not simply the lineage of origin of the extra-embryonic membranes, it is an active participant in the comprehensive communication and cross-inductions that coordinate and control embryogenesis. The trophectoderm is the source of several chemical signals that are essential for development of the ICM to its next stage of differentiation. P.P. Tam and J. Rossant, "Mouse Embryo Chimeras: Tools for Studying Mammalian Development," *Development* 130 (2003): 6155-63.

8. A. Meissner and R. Jaenisch, "Generation of Nuclear-Transfer Derived Pluripotent ES Cells from Cloned Cdx2-Deficient Blastomeres," *Nature* 439 (2006): 212-21.

9. D. Kaushik et al., "Cdx2 Gene Expression and Trophectoderm Lineage Specification in Mouse Embryos," *Science* 311 (2006): 992 -96.

10. H. Niwa et al., "Interaction between Oct3/4 and Cdx2 Determines Trophectoderm Differentiation," *Cell* 123 (2005): 917-29.

11. The one remaining link with IVF, the procurement of oocytes, is a subject of intense scientific research and there appear to be several prospects for obtaining eggs without the expensive, morally dubious, and medically dangerous procedure of super-ovulation of female patients.

12. D.A. Melton, G.Q. Daley, and C.G. Jennings, "Altered Nuclear Transfer in Stem-Cell Research: A Flawed Proposal," *New England Journal of Medicine* 351 (2004): 2791-92; and D. Solter, "Politically Correct Human Embryonic Stem Cells?" *New England Journal of Medicine* 353 (2005): 2321-23.

13. According to one recent poll, 56 percent of Americans express a distrust of science. "More Americans Than Ever Support Embryonic Stem-cell Research," Virginia Commonwealth University Life Sciences Poll, October, 24, 2005, at http://www.news.vcu.edu/news.aspx (last accessed September 14, 2006). Depending on how the questions are asked, generally 35-50 percent of Americans object to research in which embryos are destroyed to obtain ES cells. That figure jumps to 60-80 percent when the research involves the intentional creation and destruction of cloned human embryos to get patient specific ES cell lines. See United States Conference of Catholic Bishops, "New Poll: Americans Continue To Oppose Funding Stem Cell Research That Destroys Human Embryos," http://www.usccb.org/comm/archives/2006/06-109.shtml; "Split Remains," http://msnbc.msn.com/id/14527419/site/newsweek/ (last accessed September 11, 2006).

14. L. Meng et al., "Rhesus Monkeys Produced by Nuclear Transfer," *Biology of Reproduction* 57 (1997): 454-59.

15. The term "embryo" is generally defined as the human organism from fertilization to the end of the eighth week. "Blastocyst" is a structural description designating a fluid filled spherical form, and is generally used in embryology to indicate the stage between morulation and gastrulation. Blastocyst-like stuctures, however, are common in a range of tissues during organismal development, and therefore this term can be used without implying the presence of a living organism.

16. By their argument it would be even more inappropriate to call the entity produced by ANT an embryo or even a disabled embryo, since not only is altered nuclear transfer a form of SCNT, but the ANT entity has by design even less potential to develop into a human being.

17. This is the distinction between embryo and embryogenesis, the process by which the embryo develops: the embryo undergoes embryogenesis. The single-celled human embryo already carries within itself the program essential to establish placental connection with the mother and to direct its own development. Even apart from the womb, placentation and gestation may proceed in any well-vascularized tissue within the abdominal cavity.

18. See the personal statements of Robert George (joined by Alfonso Gomez-Lobo) and William Hurlbut in President's Council on Bioethics, *Human Cloning and Human Dignity: An Ethical Inquiry* (Washington, D.C.: President's Council on Bioethics, July 2002). On the question of what is the moral standing of a human being, and whether all or only some human beings have rights as persons, see P.L. and R.P. George, "The Wrong of Abortion," in *Contemporary Debates in Applied Ethics*, ed. A.I. Cohen and C. Wellman (New York: Blackwell Publishers, 2005), 13-26.

19. Nicanor Austriaco suggests that "*Philosophically*, an organism may be defined as a complete living substance that has its own internal principle of motion and change directed towards its natural perfection, and *scientifically* as a discrete unit of living matter that follows a self-driven, robust developmental pathway that manifests its species-specific self-organization"; N. Austriaco, "The Moral Case for ANT-Derived Pluripotent Stem Cell Lines," *The National Catholic Bioethics Quarterly*, forthcoming.

20. A.J. Walker, "Altered Nuclear Transfer: A Philosophical Critique," *Communio: International Catholic Review* 31, no 4 (2005): 649-84.

21. For example, a complete hydatidiform mole may result when an egg without a nucleus is "fertilized" by two sperm. This pathological failure of fertilization will divide and form a blastocyst-like structure, but it produces only an overgrowth of placental tissue with little or no fetal parts at all. As with a teratoma, the structure possesses a full human genome but lacks the complementary epigenetic factors of the male and female gametes.

22. R. Jaenisch, "Human Cloning—The Science and Ethics of Nuclear Transplantation," *New England Journal of Medicine* 351 (2004): 2787-91.

23. Rightly understood, the entire interrelated network of molecular parts (nuclear and cytoplasmic) determine the identity of the cell, but here we use the term "epigenetic" (somewhat broadly) to emphasize the functional relationship between cytoplasm and genome.

24. Of course, it is not our intention to proclaim an "epigenetic essentialism."

25. In particular, 1542 mouse genes with well-matched human homologs that are preferentially expressed in early embryos have been identified by the Green laboratory at the University of Otago in New Zealand; J. L. Stanton and D.P. Green, "A Set of 1542 Mouse Blastocyst and Pre-blastocyst Genes with Well-Matched Human Homologues," *Molecular Human Reproductin* 8 (2002): 149-66. This pattern of gene expression might provide a molecular signature of true embryos. Furthermore, 111 genes that are turned on and 95 genes that are turned off in human embryonic stem cells have been identified; M. Suarez-Farinas et al., "Comparing Independent Microarray Studies: The Case of Human Embryonic Stem Cells," *BMC Genomics* 6 (2005): 99. This pattern might provide a molecular signature of entities that would be uniquely classified as pluripotent stem cells. A comparison of these two gene patterns suggests that there is no overlap.

26. E. Christian Brugger, "ANT-OAR: A Morally Acceptable Means for Deriving Pluripotent Stem Cells. A Reply to Criticisms," *Communio: International Catholic Review* 32, no. 4 (2005): 753 -69.

27. Personal communication.