

This commissioned working paper was discussed at the Council's December 2004 meeting. The views expressed here do not represent the official views of the Council or of the United States Government.

Altered Nuclear Transfer as a Morally Acceptable Means for the Procurement of Human Embryonic Stem Cells

William B. Hurlbut, M.D.
Program in Human Biology
Stanford University

Introduction

With the sequencing of the human genome and our increasing knowledge of the molecular mechanisms of basic cell functions, we are entering an era of rapid advance in the field of developmental biology. Current scientific interest in embryonic stem cells is a logical step in the progress of these studies and holds the hope of providing important research tools as well as possible therapeutic applications.

The ethical controversy surrounding human embryonic stem cell (ESC) research arises from the fact that to obtain these cells living human embryos must be disaggregated and destroyed. Many Americans oppose such embryo destruction, believing that there is an implicit dignity and inviolability in the individual continuity of a human life from fertilization to natural death. Many others, however, believe that the benefits of advances in biomedical science outweigh these moral concerns.

The present conflict over the moral status of the human embryo reflects deep differences in our basic convictions and is unlikely to be resolved through deliberation or debate. Likewise, 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. These concerns are already encoded in the Dickey Amendment that prohibits the use of federal funds for embryo-destructive research and is the legislative foundation of the President's executive order restricting funding to ESC lines created before

August 9, 2001. While there are currently no federally legislated constraints on the use of private funds for this research, there is a consensus opinion in the scientific community that without NIH support for newly created ESC lines progress in this important realm of research will be severely constrained.

Notwithstanding this apparently irresolvable impasse, there may be morally uncontroversial ways to obtain embryonic stem cells. Drawing on our increasing understanding and control of developmental biology it may be possible to direct the organic powers of embryological development to generate ESCs even apart from the living human organism that is their natural origin.

Altered Nuclear Transfer

There are several possible approaches that might allow the production of ESCs without the creation and destruction of a human embryo. The ideal solution, one that many scientists believe will eventually be possible, would be the direct reprogramming of adult cells to the functional equivalence of ESCs. In natural embryogenesis ESCs are produced within a restricted area (the inner cell mass) of a 4-5 day old embryo (known as a blastocyst). Over the first few days of development, a series of cell signals induces the specific pattern of gene expression that characterizes ESCs and gives them their pluripotency, their capacity to subsequently produce all the cell types of the human body. With an understanding of the exact molecular nature of these signals it should be possible to bypass embryogenesis and directly induce this transformation in adult cells. Unfortunately, it may be many years before our scientific knowledge and control of these factors will make this approach feasible.

More immediately, there may be ways to obtain ESCs by harnessing partial organic trajectories apart from the full natural system of embryonic development. Using the techniques of somatic cell nuclear transfer (SCNT), but with the intentional alteration of the nucleus before transfer, we could construct a biological entity that, by design and from its very beginning, lacks the attributes and capacities of a human embryo. Studies with mice already provide evidence that such a project of Altered Nuclear Transfer (ANT) could generate functional ES cells from a system that is not an organism, but is biologically (and morally) more akin to the partial organic potential of a tissue or cell culture.¹

This proposal shifts the ethical debate from the question of when a normal embryo is a human being with moral worth, to the more fundamental question of what component parts and organized structure constitute the minimal criteria for considering an entity a human organism.

The Paradigm of Systems Biology

The moral argument for Altered Nuclear Transfer is grounded in the emerging science of systems biology. According to this radical revision of our prevailing reductionistic views, an organism is a living whole, a dynamic network of interdependent and integrated parts.

There are essential subsystems of growth (cells, tissues and organs), but a living being is more than the sum of its parts, and the parts are dependent on the integrated unity of the whole. Fully constituted, the organism is a self-sustaining and harmonious whole, a unified being with an inherent principle of organization that orders and guides its continuity of growth. In the human embryo, this principle of organismal unity is an engaged and effective potential-in-process, an activated dynamic of development in the direction of the mature human form. Incompletely constituted or severed from the whole, subsystems with partial trajectories of development may temporarily proceed forward with a certain biological momentum. Ultimately, however, they fail to rise to the level of the coordinated coherence of a living organism and become merely disorganized cellular growth.

Failures of Fertilization and Partial Development

The activation of the egg by the penetration of the sperm (or the equivalent events in nuclear transfer/cloning) triggers the transition to active organismal existence. But without all of the essential elements (a full complement of chromosomes, proper chromatin configuration, the cytoplasmic factors for gene expression, etc.), there can be no living whole, no organism, and no human embryo. Recent scientific evidence suggests that such a 'failure of fertilization' is, in fact, the fate of most early natural initiations in reproduction. The artificial and intentional construction of a biological entity lacking any of these essential

elements, yet bearing a partial developmental potential (similar to that in the aberrant products of fertilization), may make it possible to procure ES cells without producing a human embryo.

There are natural biological precedents for entities that lack the qualities and characteristics of an organism, yet are capable of generating ES cells. Teratomas are germ cell tumors that generate all three primary embryonic germ layers as well as more advanced cells and tissues, including partial limb and organ primordia. Yet these chaotic, disorganized, and nonfunctional masses lack entirely the structural and dynamic character of organisms. Likewise, failures of fertilization due to abnormal complements of chromosomes or improper chromatin configurations (imprinting) may still proceed along partial trajectories of organic growth without being actual organisms. Trisomies of chromosome number one, for example, will grow to the blastocyst stage but will not implant. Even an enucleated oocyte, when artificially activated, has the developmental momentum to divide to the eight-cell stage.

These natural examples of partial generative potential (described by some as pseudo-embryos), together with other observations of early embryonic processes, have led to a diverse array of suggestions for ways that ES cells might be produced without the moral ambiguity of the creation and destruction of full human embryos. These suggestions include the use of aneuploidies, polyploidies, viable cells from embryos in arrested development, parthenotes, and chimeras of human nuclear material and animal oocytes. Each presents its own particular technical challenges and raises unique and unfamiliar moral considerations.

The scientific prospects for ANT remain largely unexplored, but as stated by Rudolph Jaenisch in testimony to the President's Council on Bioethics (July 2003), they are within the reach of our current technology. There are numerous potential approaches involving alteration of the genes necessary for early intercellular organization, formation of extra-embryonic structures, or the primary patterning of organogenesis. One possibility is the alteration of 'cdx2,' a gene essential for differentiation of the trophectoderm (the tissues that ultimately form the placenta). In experiments with mouse models, when this gene is not expressed there is only a partial and disorganized developmental process

resulting in a visibly abnormal blastocyst. Nonetheless, there is the formation of an inner cell mass from which functional ES cells have been harvested. For ANT, this gene might be temporarily silenced (using RNA interference) by altering the somatic cell nucleus or the cytoplasm prior to transfer, so that once the ES cells have been procured the gene could be re-expressed to allow fully potent ES cells.²

The limited biological entity created by such a procedure would fail to establish even the most fundamental features of organismal infrastructure, and would be incapable of implantation. It would have no inherent principle of unity, no coherent drive in the direction of the mature human form, and no claim on the moral status due to a developing human life. Rather, such a partial organic potential would more rightly be designated a biological 'artifact' (a human creation for human ends). The fact that some part of such a constructed entity will carry a certain momentum of development is morally analogous to the fact that we can grow skin in a tissue culture and may one day grow whole organs or limbs. Lacking crucial elements in its fundamental constitution, such an entity would never rise to the level of a living being.³ When the overarching integration of essential parts and functions is not present (or, as in the 'brain dead' organ donor, no longer present), there is no living organism and therefore there is no being with human moral status.

Ethical Harnessing of Partial Developmental Potential

The intention in creating such an intrinsically limited "artifact" would not be one of reproduction (and disaggregation), but simply the desire to draw on natural organic potential through technological manipulation of biological materials. This intention is in keeping with the purposes of scientific research and medical therapy in which many "unnatural" manipulations are used for human benefit.

The crucial principle of any approach, however, must be the preemptive nature of the intervention. This process does not involve the creation of an embryo that is then altered to transform it into a non-embryonic entity. Rather, the proposed genetic alteration is accomplished *ab initio*, the entity is brought into existence with a genetic structure insufficient to generate a human embryo. From the beginning and at every point along its development it cannot be designated a

living being. No human embryo would be created; hence, none would be violated, mutilated or destroyed in the process of stem cell harvesting. If such a limited biological entity were accorded a certain cautionary respect (as with all human tissues), even though not the full protection of human life, this project would not compromise any fundamental moral principles. Moreover, such techniques could be developed using animal models and confidently extended to work with human cells without engaging in research that involves the destruction of human embryos.⁴

Over the course of the previous century we contended with ethical controversies over blood transfusion, tissue and organ transplantation, and the transfection of human genes into experimental animals. In this century we will be confronted by a series of even more challenging ethical questions related to the dynamic systems of developmental biology. Just as we have learned that neither genes, nor cells, nor even whole organs define the locus of human moral standing, in this era of developmental biology we will come to recognize that cells and tissues with 'partial generative potential' may be used for medical benefit without a violation of human dignity.

Conclusion

The moral distinctions essential to discern and define the categories of organism, embryo and human being will be vital as we go forward with scientific research involving ES cells, chimeras, and laboratory studies of fertilization and early embryogenesis. Advances in developmental biology will depend on clarifying these categories and defining the moral boundaries in a way that at once defends human dignity while clearing the path for scientific progress

At this early stage in our technological control of developing life, we have an opportunity to break the impasse over stem cell research and provide moral guidance for the biotechnology of the future. This may require a constructive reformulation of some aspects of moral philosophy, together with creative exploration of scientific possibilities, but any postponement of this process will only deepen the dilemma as we proceed into realms of technological advance unguided by forethought. We must initiate the cooperative dialogue that is essential to frame the moral principles that can at once defend human dignity

and promote the fullest prospects for scientific progress and its medical applications.

1. Personal communication with Janet Rossant, Mount Sinai Hospital, Toronto, Canada. Also, see "Cdx2 is essential for axial elongation in mouse development," Kallayane Chawengsaksophak, et al. PNAS May 18, 2004, vol 101, no 20, 7641-7645.

2. Although the trophoctoderm is the source of the extraembryonic membranes, it is properly considered part of the embryo. Indeed, studies confirm that normal trophoctoderm is essential for normal embryogenesis. Janet Rossant, a world authority on early embryonic development has done important studies with chimeric mice made by combining cells from two different early embryos, one tetraploid and the other the source of the ES cells that are joined to the tetraploid cells. Referring to studies using tetraploid mice with gene knockouts in their trophoctoderm, she states "the analysis of mutations in candidate patterning genes in chimeric mice has played a key role in elucidating the importance of extra-embryonic tissues as sources of patterning signals in the early mouse embryo." More specifically, she says: "Embryological and gene expression studies have shown that the early patterning of the antero-posterior axis of the mouse embryo at around the time of gastrulation requires signalling and transcriptional activity in both the extra-embryonic and embryonic tissues." (Beddington and Robertson 1999). She goes on to cite several genes such as Hnf4 stating that her studies indicate that "this outcome indicates that Hnf4 in the extraembryonic tissues is crucial for normal gastrulation." Citing a series of other studies on genes essential for early development she concludes "Therefore, these studies showed that Amn, Nodal, Akd and Foxa2 function is essential in the extra-embryonic tissues for normal embryogenesis and that the early patterning events must involve a complex interplay between embryonic and extra-embryonic tissues." (Rossant, Mouse Embryo Chimeras: tools for Studying Mammalian Development, Development 130,6155-6163).

A deficiency at the first differentiation of cell type (the formation of the trophoctoderm) means the absence of the most fundamental order. But one could rightly argue that later deficiencies in axes formation, alignment of basic

body plan and organogenesis also reveal a level of disorder that precludes organismal existence. At gastrulation the primary pattern of the developing being is laid down and many aspects of interference at this level would indicate a fundamental failure in the minimal composition of organization and integration. However, for the purposes of the proposed project, the earlier incapacity in the most primary differentiation of trophectoderm seems the most morally defensible. It is important to recognize that this deficiency is almost certainly more fundamental than that which causes teratomas.

3. As Thomas Aquinas observed eight centuries ago, an animal's life is not like its image on a fresco which one can scrape off inch by inch; an animal is a whole, unified being that is either alive or not alive.

4. With increasing knowledge of genetics and developmental biology it is clear that many of the basic mechanisms of animal development have been conserved across millions of years of phylogenetic process. This means that, with some confidence, we can extend our knowledge of animal models (such as the mouse) to an understanding of the principles and patterns of human development. With reasonable caution, including extensive studies with non-human primates, the techniques described in this proposal could be developed for application with human cells.