

ALTERED NUCLEAR TRANSFER AS A MORALLY ACCEPTABLE MEANS FOR THE PROCUREMENT OF HUMAN EMBRYONIC STEM CELLS

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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 cloning for biomedical research (CBR)¹ and human embryonic stem cell (ES cell) 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.

¹Also termed therapeutic cloning, somatic cell nuclear transfer, or nuclear transfer for the procurement of ES cells. For the difficulty of terminology, see President's Council 2002, chap. 3.

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, which prohibits the use of federal funds for embryo-destructive research and is the legislative foundation of the President's executive order restricting funding to ES cell lines created before August 9, 2001 (President's Council 2004, chap. 2). While there are currently no federally legislated constraints on the use of private funds for this research, there is a consensus in the scientific community that without NIH support for newly created ES cell lines, progress in this important realm of research will be severely constrained.

In joining with fellow members of the President's Council on Bioethics in support of a moratorium on CBR in July 2002, I considered this recommendation not an admission of ambivalence on matters of policy, but a recognition of the difficulty of the moral issues involved and an affirmation of the need for further discussion and deliberation (President's Council 2002). Throughout our proceedings over the past three years, it has become increasingly apparent that without clear and distinct moral principles, grounded in scientific evidence and reasoned moral argument, no policy can be effectively formulated or enforced. Most specifically, the proposed limitation of 14 days for research on human embryos and the prohibition against implantation appear to be arbitrarily set and therefore vulnerable to transgression through the persuasive promise of further scientific benefit. Clearly, a more thorough and thoughtful consideration of the moral status of the human embryo is warranted. It is in the spirit of this continuing discussion that I offer the personal perspectives that follow.

As our science is changing, so is the nature of our moral dilemmas. Each advance forces us to think more deeply about what it means to be human. As the scientific focus on genomics moves on to proteomics and now to the early stages of the study of development, we are confronted with the challenge of understanding the moral meaning of human life in its dynamics of change, as both potential and process. Concerns about cloning are likely to be just the beginning of a series of difficult ethical issues related to embryo experimentation and medical intervention in developing life. In addition, advances in developmental biology will open more deeply the ethical dilemmas of human-animal hybridization, extra-corporeal gestation, and genetic and cellular enhancement. Driven by the vast range of research applications and opportunities for clinical interventions in disease and disability (especially the open-ended possibilities promised by regenerative medicine), this technology will be powerfully propelled into the forefront of medical science.

Given the complex course of science and the drive to its development, any moral assessment of CBR or human ES cell research must describe the central human goods it seeks to preserve, the range and boundaries of these values, and

the broad implications for science and society. Such an assessment should serve the dual purpose of helping to define the moral dangers while clearing the course for the fullest and most open future for scientific investigation and application.

MORAL PRINCIPLES

Although there are already numerous promising approaches for research on human development even without cloning techniques, I believe this technology could provide valuable tools for scientific inquiry and medical advance. In my judgment, the moral imperative to foster an increase of knowledge and new modes of therapeutic intervention weighs heavily in the equation of consideration. Nonetheless, I believe that, as they stand, current proposals for CBR and human ES cell procurement will breach fundamental moral goods, erode social cohesion, and ultimately constrain the promise of advances in developmental biology and their medical applications. However, there may be morally acceptable ways to produce ES cells through nuclear transfer (the technique used for CBR) that could both preserve our commitment to our fundamental moral principles and strengthen our appreciation of the significance of developing life. Such a technique would sustain social consensus while opening positive prospects for scientific advance in ES cell research.

The principle of valuing human life as the fundamental good serves as the cornerstone of law for our civilization. In no circumstance is the intentional destruction of the life of an innocent individual deemed morally acceptable. Even where a right to abortion is given, for example, it is based on a woman's right not to be encumbered—a right of privacy, not a right to directly kill the fetus—and if the fetus is delivered alive during an abortion, there is a legal obligation to resuscitate and sustain its life. This valuing of human life is indeed the moral starting point for both advocates and opponents of CBR. The principle of the inviolability of human life is the reciprocal respect that we naturally grant as we recognize in the other a being of moral equivalence to ourselves. Although different cultures and eras have affirmed this recognition in varied ways, I will argue that it is reasonable in light of our current scientific knowledge that we extend this principle to human life in its earliest developmental stages.

LIFE AS PROCESS

When looked at through the lens of science, it is evident that an individual human life cannot be described atemporally, but must be recognized in the full procession of continuity and change that is essential for its development. From conception, our unique genetic endowment organizes and guides the expression of our particular nature in its species and individual character. Fertilization initiates the complex integration and functional unity of a self-directing, developing organism that may live for a hundred years or more. In both character and

conduct, the zygote and subsequent embryonic stages differ from any other cells or tissues of the body; they contain within themselves the organizing principle of the full human organism.

This is not an abstract or hypothetical potential in the sense of mere possibility, but rather a potency, an engaged and effective potential-in-process, an activated dynamic of development in the direction of human fullness of being. For this reason, a zygote (or a clone) differs fundamentally from an unfertilized egg, a sperm cell, or later somatic cells: it possesses an inherent organismal unity and potency that such other cells lack. Unlike an assembly of parts in which a manufactured product is in no sense “present” until there is a completed construction, a living being has a continuous unfolding existence that is inseparable from its emerging form. The form is itself a dynamic process rather than a static structure. In biology, the whole (as the unified organismal principle of growth) precedes and produces the parts. It is this implicit whole, with its inherent potency, that endows the embryo with its human character and therefore its inviolable moral status. To interfere in its development is to transgress upon a life in process. The argument is sometimes made that potential should not be part of the moral equation, because of the low probability of successful development of the early embryo.² This, however, is itself an argument based on potential, in this case the lack of potential to develop normally. The fact that life in its early stages is extremely fragile and often fails is not an argument to lessen the moral standing of the embryo. Vulnerability does not render a life less valuable.

ACCRUED MORAL STATUS

The major alternative to the view that an embryo has an inherent moral status is the assertion that moral status is an accrued or accumulated quality related to some dimension of form or function. Several arguments have been put forward for this position.

²The argument based on probability fails because it does not acknowledge the continuity of essential nature that characterizes an organism across its various stages of development. Such an argument might hold some weight if one could argue that a given stage of development represents an emergent state in which a newly manifest property is in ontological discontinuity with the material from which it emerged. At first consideration, this seems true of all biological systems where the whole reveals properties unpredicted within the parts. The problem in this line of reasoning, however, is that these properties are exactly that to which the whole is ordered, and so are inherent powers, “actual” within the whole when seen across time. To know what a biological being is, we must observe it over time, understand it across its life span. It is the essence of life that it is ordered to employ these leaps to emergent states as an agency in development. New realities will emerge; this is established in the potency of the developing organism.

Gastrulation

One such accrual argument is based on the idea that before gastrulation (designated as the 14th day), the embryo is an inchoate clump of cells with no actuated drive in the direction of distinct development.³ It is argued that the undifferentiated quality of the blastocyst justifies its disaggregation for the procurement of stem cells, while the evident organization at gastrulation reveals an organismal integrity that endows inviolable moral status to all subsequent stages of embryological development. Scientific evidence, however, supports the argument that from conception there is an unbroken continuity in the differentiation and organization of the emerging individual life. The anterior-posterior axis appears to be already specified within the zygote, and early cell divisions (at least after the eight-cell stage) exhibit differential gene expression and unequal cytoplasmic concentrations of cell constituents, suggesting distinct cellular fates (Gardner 2001; Grabel et al. 1998; Piotrowska and Zernicka-Goetz 2001). This implies that the changes at gastrulation do not represent a discontinuity of ontological significance, but merely the visibly evident culmination of more subtle developmental processes (at the cellular level) driving in the direction of organismal maturity.

Twinning

Another argument for accrued moral status is that as long as an embryo is capable of giving rise to a twin, it cannot be considered to have the moral standing of an individual. There is the obvious objection that as one locus of moral status becomes two, it does not diminish but increases the moral moment. But perhaps more substantially, this argument actually supports the notion that crucial dimensions of individuation (and their disruption that results in twinning) are already at work in the blastocyst, the stage at which most twinning occurs. Monozygotic twinning (a mere 0.4% of births) does not appear to be either an intrinsic drive or a random process within embryogenesis. Rather, it is a disruption of normal development by a mechanical or biochemical disturbance of fragile cell relationships that provokes a compensatory repair, but with the restitution of integrity within two distinct trajectories of embryological development

³The differentiation of the trophoblast, which is evident by day four, is sometimes considered as distinct from the embryo itself. However, in light of current scientific evidence, it should be recognized as an inextricable component of the embryo, involved in a multitude of dynamic interactions essential for embryogenesis. The fact that it participates in the formation of the extra-embryonic membranes that are left behind at birth does not make it less central to the embryonic being and its development. Throughout the continuum of human life, cells, tissues, and organs are reabsorbed, transcended, and transformed: examples include the umbilical vein and arteries (which become supporting ligaments), neural cells (more than half of which are culled by apoptosis), and immune organs such as the thymus (which shrivels in an adult). We do not just develop and then age, but undergo a continuous transformation and fuller manifestation of our organismal nature present within the earliest embryo.

(da Costa et al. 2001).⁴ In considering the implications of twinning for individuation, one might ask the question from the opposite perspective. What keeps each of these totipotent cells from becoming a full embryo? Clearly, crucial relational dynamics of position and intercellular communication are already at work establishing the unified pattern of the emerging individual (Wang et al. 2004). From this perspective, twinning is not evidence of the absence of an individual, but of an extraordinary power of compensatory repair that reflects more fully the potency of the individual drive to fullness of form.

Implantation

Some have argued that the implantation of the embryo within the uterine lining of the mother constitutes a moment of altered moral status. Implantation, however, is actually a process that extends from around the sixth or seventh day to about the 11th or 12th day, when the uteroplacental circulation is established. This complex circulatory exchange extends the earlier relationship between mother and embryo in which physiological conditions, including the diffusion of essential nutrients and growth factors, sustained the life and nourished the development of the pre-implantation embryo. Although these early conditions can be artificially simulated, as with in vitro fertilization (IVF), the delicate balance of essential factors and their effect on development is evidence of the crucial contribution of the mother even in the first week of embryogenesis (Fernández-Gonzalez et al. 2004). Changes in the intricate interrelations between mother and infant cannot be viewed as an alteration of moral status, but as part of the ongoing epigenetic process all along the continuum of natural development that begins with conception and continues into infancy. This continuity implies no meaningful moral marker at implantation.

Function

Arguments for a change in moral status based on function are at once the most difficult to refute and to defend. The first and most obvious problem is that the essential functions (even their minimal criteria and age of onset) are diverse and arbitrarily assigned. Generally they relate to the onset of sentience, awareness of pain, or some apparently unique human cognitive capability such as consciousness. But if human moral worth is based on actual manifest functions, then does more of a particular function give an individual life a higher moral value?

⁴The fact that these early cells retain the ability to form a second embryo is testimony to the resiliency of self-regulation and compensation within early life, not the lack of individuation of the first embryo from which the second can be considered to have “budded off.” Evidence for this may be seen in the increased incidence of monozygotic twinning associated with IVF by blastocyst transfer. When IVF embryos are transferred to the uterus for implantation at the blastocyst stage, there is a two- to tenfold increase in the rate of monozygotic twinning, apparently due to disruption of normal organismal integrity.

And what are we to make of the parallel capacities in animals that we routinely sacrifice for food and medical research? Furthermore, what becomes of human moral status with the degeneration or disappearance of such a function? While we might argue that our relational obligations change along with changes in function, such as those that occur with senile dementia, we would not sanction a utilitarian calculus and the purely instrumental use of such persons no matter how promising the medical benefits might be. The diagnostic requirements of "brain death" for removing organs for transplantation, far from being a justification for interrupting a developing life before "brain birth," actually point to the moral significance of potential and the stringency of the criteria for irreversible disintegration and death.

From a scientific perspective, there is no meaningful moment when one can definitively designate the biological origins of a human characteristic such as consciousness. Even designations such as "the nervous system" are conceptual tools, reifications of the parts of what is actually an indivisible organismal unity. Zygote, morula, embryo, fetus, child, and adult: these are conceptual constructions for convenience of description, not distinct ontological categories. With respect to fundamental moral status, therefore, as distinguished from developing relational obligations, the human being is an embodied being whose intrinsic dignity is inseparable from its full procession of life and always present in its varied stages of emergence.

A BRIGHT LINE AT CONCEPTION

If the embryo has an inherent moral status that is not an accrued or accumulated quality related to some dimension of form or function, then that moral status must begin with the zygote (or clonote). Anything short of affirming the inviolability of life across all of its stages from zygote to natural death leads to an instrumental view of human life. Such a revocation of our most fundamental moral principle would reverse a long and overarching trend of progress in moral awareness and practice in our civilization. From human sacrifice, to slavery, child labor, women's rights, and civil rights, we have progressively discerned and prohibited practices that subject the individual to the injustice of exploitation by others. The reversal of such a basic moral valuation will extend itself in a logic of justification that has ominous implications for our attitude and approach to human existence. This is not a mere "slippery slope," where we are slowly led downward by the ever more desirable extension of exceptions to moral principle. It is, rather, a "crumbly cliff," where the very utility of abrogating a basic moral prohibition carries such convenience of consequence that the subsequent descent is simply practice catching up with principle.

The inviolability of human life is the essential foundation on which all other principles of justice are built, and erosion of this foundation destabilizes the social cooperation that makes possible the benefits of organized society. Medi-

cine is especially vulnerable to such effects, since it operates at the intrinsically moral interface between scientific technique and the most tender and sensitive dimensions of personal reality in the vulnerable patient. As we descend into an instrumental use of human life, we destroy the very reason for which we were undertaking our new therapies; we degrade the humanity we are trying to heal.

The promise of stem cells lies beyond simple cell cultures and cell replacement therapies. The 14-day marker will not hold up to logical argument.⁵ The technological goal is to produce more advanced tissues, organs, and possibly even limb primordia. Producing such tissues may require the complex cell interactions and microenvironments now available only through natural gestation. Embryonic development proceeds within the context of a highly refined spatial and temporal niche of organized complexity of positional cues, signal diffusion, and cell-cell contact between cellular lineages of diverse types (Nishimura et al. 2002). The benefits of implanting cloned embryos (either into the natural womb or possibly an artificial endometrium) so as to employ the developmental dynamics of natural embryogenesis seem self-evident. The implantation of cloned embryos for the production of patient-specific tissue types to bypass problems of immune rejection would further extend the logic of the instrumental use of developing life. The public pressure that has already been brought to bear on the politics of stem cells and cloning by patient advocacy groups has provoked such a sense of promise that it may propel the argument for allowing implantation of cloned embryos. Different people may have different limits to the duration of gestation they find morally acceptable, but in light of the current sanction of abortion up to and beyond the end of the second trimester, it is difficult to argue that creation, gestation, and sacrifice of a clone to save an existing life is a large leap in the logic of justification.

ALTERED NUCLEAR TRANSFER

While maintaining a bright line at conception safeguards our most fundamental moral principle, the challenge remains to find an acceptable method of drawing on the great medical promise of CBR and ES cells while precluding their use in ways that degrade the dignity of human existence. Some proponents of CBR

⁵The designation of 14 days as the moral boundary for embryo experimentation is in the category of a "received tradition," almost a superstition in the sense that it is a belief in a change of state without a discernible cause. The validity of this designated moral marker has not been reexamined in the light of recent advances in our understanding of developmental biology. As a moral marker of ontological change, 14 days makes no sense. Even if one disagrees with the discussion above, the date should be set earlier: implantation is complete by the 12th day, the onset of gastrulation occurs as the primitive streak between the 12th and 14th days, and twinning is rare after the ninth day. Furthermore, it is worth noting that 14 days is not of current scientific relevance, since stem cells can be procured at the four- to five-day stage and, with present technology, human embryos can sustain viability in culture for only eight to nine days.

maintain that the laboratory creation of the cloned embryo makes it a “pseudo-embryo” or “artifact,” a product of human technological production. They point to the unnatural means of its creation and the low probability of successful development to birth evident in most animal studies. Although we have no experience with the gestation of cloned human embryos (and only a single study involving gestation of nonhuman cloned primates), one significant difference from natural fertilization is that animal cloning consistently produces a high percentage of defective offspring (Jaenisch 2004). Indeed, most of the products of cloning never make it past early developmental stages, and among those that do, many die during gestation. Some argue that this high rate of early failure of development means that all products of nuclear transfer should be considered as lacking the moral standing of a natural embryo. The problem with this assertion is that, at least in some cases, the cloned embryo appears to share the developmental potential of the product of natural fertilization.

Why some of the products of nuclear transfer proceed to develop while others do not is an important scientific question. The answer to this question is relevant to the search for a morally acceptable method for the procurement of ES cells and the proposal that follows. Evident abnormality during early development does not, of itself, preclude the formation of a whole and healthy offspring. IVF embryos often exhibit slower division rates and fewer cells at the equivalent stages of naturally conceived embryos (Barry Behr, Stanford University, personal communication). Likewise, at least in mice, intracytoplasmic sperm injection appears to disrupt the natural specification of cell fates and body axes normally associated with the point of sperm entry. In these cases, the capacity for regulation, for robust repair and restitution of the normal pattern of development, is evidence of the organizational integrity and unified principle of growth that characterizes a genuine organism. This capacity, together with the more fundamental powers of self-development and self-maintenance, is a crucial determinant in the moral status of any product of fertilization or nuclear transfer. To be rightly designated a human embryo with moral standing, an entity must have the organismal character of a living being.⁶ Clearly, many products of nuclear transfer lack these fundamental capacities of organisms, but since some are capable of integrated development, the fact of cloning alone does not establish a different moral status for the entity produced. Could we, however, use our advancing

⁶The word *organism* implies organization, an overarching principle of unity, a cooperative interaction of interdependent parts subordinated to the good of the whole. As a living being, an organism is an integrated, self-developing, and self-maintaining unity under the governance of an immanent plan. The philosopher Robert Joyce (1978) explains: “Living beings come into existence all at once and then gradually unfold to themselves and to the world what they already but only incipiently are.” To be a human organism is to be a whole living member of the species *Homo sapiens*, to have a human present and a human future evident in the intrinsic potential for the manifestation of the species typical form. Joyce continues: “No living being can become anything other than what it already essentially is.”

knowledge of developmental biology to create an entity that *consistently* lacks the qualities and capabilities essential to be designated a human embryonic organism? By intentional alteration of the somatic cell nuclear components or the cytoplasm of the oocyte into which the nucleus is transferred, could we truly create an artifact (a human creation for human ends) that is biologically and morally more akin to a tissue or cell culture?

There are several possible approaches that might allow the production of ES cells 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 become the functional equivalents of ES cells. In natural embryogenesis, ES cells are produced within a restricted area (the inner cell mass) of a blastocyst.⁷ Over the first few days of development, a series of cell signals induces the specific pattern of gene expression that characterizes ES cells 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 may be possible to bypass embryogenesis and directly induce this transformation in adult cells. For example, as suggested by Alan Trounson of Monash University, Australia, we may be able to reprogram the nucleus of a somatic cell by transplanting it into the cytoplasm of an existing ES cell (personal communication). 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 ES cells by harnessing partial organic trajectories apart from the full natural system of embryonic development. Using the techniques of nuclear transfer, 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) may be able to generate functional ES cells from a cellular system that lacks the intrinsic potential of an actual organism, but possesses the limited organic powers of a tissue or cell culture. 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.⁸

⁷It is important to note that ES cells may be a product of laboratory isolation and culture and may exhibit properties quite different from their natural counterparts within the developing embryo.

⁸The mouse study by Chawengsaksophak and Rossant (2004) did not involve ANT, but it did demonstrate that ES cells may be procured where a gene essential at a fundamental level of embryogenesis is knocked out. As discussed below, whether an entity with such a dramatic disruption of development should be characterized as a “disabled” embryo or as a non-embryonic entity is an important consideration. Nonetheless, ANT could involve an intervention or complementary interventions at an even earlier and more fundamental level. Defining the moral boundary will be a crucial step in the implementation of this project.

For practical implementation of ANT, a working definition of the term “human embryonic

**FAILURES OF FERTILIZATION AND
PARTIAL DEVELOPMENT**

The activation of an egg by the penetration of a sperm (or the equivalent events in nuclear transfer/cloning) triggers the transition to active organismal existence, with its potential for development toward the adult human form. But without all of the essential elements (the necessary 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 incomplete combinations of the necessary elements—"failures of fertilization"—are the fate of many, perhaps most, early natural initiations in reproduction. ANT proposes the artificial construction of a cellular system mimicking these natural examples, one that lacks the essential elements for embryological development but contains a partial developmental potential capable of generating ES cells.

Many naturally occurring failures of fertilization may still proceed along partial trajectories of organic growth without being true organisms. For example, grossly abnormal karyotypes, such as trisomies of chromosome 1, will form a blastocyst but will not implant (Boué, Boué, and Gropp 1985). Even an enucleated oocyte, when artificially activated, has the developmental momentum to divide to the eight-cell stage, but clearly is not an organism. The mRNA 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 contain a certain biological momentum that propels a partial trajectory of development, but unlike a normal embryo they are unable to bootstrap themselves into becoming an integrated and self-regulating organismal entity.

Some of these aberrant products of fertilization that lack the qualities and characteristics of an organism appear to be capable of generating ES cells or their functional equivalent (Byrne, Simonsson, and Gurdon 2002). Mature teratomas are neoplasms that generate all three primary embryonic germ cell types, as well as more advanced cells and tissues, including partial limb and organ primordia. Yet these chaotic, disorganized, and nonfunctional masses entirely lack the structural and dynamic character of organisms. Teratomas may occur as benign ovarian tumors that are, at least in some cases, derived by spontaneous and disorgan-

organism" might be any entity, regardless of its source or means of production, which, when provided the support and nurture of a natural gestational environment (or its technological equivalent), has the intrinsic potential to express the minimal manifestations of form and function that characterize a human organism. This still leaves open the discussion of the exact definition of such minimal developmental potentiality, but affirms that the moral status of such an entity is related to its intrinsic nature, not its mode of creation or present location. It is important to recognize, however, that such criteria of minimal developmental potentiality are only of secondary concern for ANT, where the most practical and (morally uncontroversial) technique may involve an alteration at a far more fundamental level. For a discussion of the defining criteria of a human organism, see Ashley 2001; Grisez 1989; Huarte and Suarez 2004.

ized development of activated eggs. They generally have a complete karyotype (46XX), and they produce a diversity of cell and tissue types that suggests that they may proceed through a developmental process similar enough to natural embryogenesis to produce pluripotent stem cells. In fact, through intentional parthenogenetic activation of monkey eggs (which mimics teratoma formation), Vrana et al. (2003) were able to coax them to a blastocyst-like stage and harvest ES cells. Serious scholars and scientists, including the geneticist and Dominican priest Nicanor Austriaco (2002), have made moral arguments supporting such a source of human ES cells. Furthermore, there are already patent applications for such a procedure.

The disorganized character of teratomas appears to arise not from changes in the DNA sequence, but from genetic imprinting, an epigenetic modification that affects gene expression. 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 trophoctoderm and its lineages fail to develop properly. The differentiation of the trophoctoderm and the inner cell mass (which forms the ES cells) is considered the first globally coordinated divergence into distinct cell lineages. The trophoctoderm is necessary for the cross-inductions that are the foundation for all further coordinated and organized growth of the embryo. Later it contributes to the formation of the extra-embryonic membranes, but earlier in development it is crucial for both embryo structural integrity and the development of a normal inner cell mass. In the absence of the complementary genetic contribution of the male, the activated egg is simply inadequately constituted to direct the integrated development characteristic of human embryogenetic process.

Interestingly, an inverse failure of formation characterizes development driven only by genetic elements from the male, where the complementary contribution of the female is missing. In complete hydatidiform moles an egg missing its nucleus is fertilized by one or more sperm. This time, lacking the maternal genetic contribution with its complementary imprinted genes, there is an overgrowth of trophoctoderm with no apparent ES-like cells and little or nothing in the way of fetal parts.

Recent evidence suggests that in their development both of these disorganized growths may proceed to the blastocyst stage. They may appear on visual inspection to be growing normally, but they carry an intrinsic insufficiency at the molecular level that renders them incapable of forming the body axes and essential infrastructure characteristic of human embryogenesis. (Clearly, the method and level of analysis we use will influence our interpretation of the identity and moral valuation of a thing. This highlights the importance of evaluating products of fertilization and nuclear transfer not simply by visual observation but also against the molecular signature that characterizes natural embryos.)

The exact cause of the aberrant and disordered growth of these “failures of formation” is not fully understood, but studies with parthenogenetic mice pro-

vide a remarkable window into the organizing (or disorganizing) role of a single genetic alteration. Using a technique similar to ANT, Japanese scientists produced a fully formed mouse by combining chromosomes from two oocytes, but with a single modification of an imprinted region to simulate the necessary male contribution (Kono et al. 2004). With this one change in genetic regulation directly affecting expression of just two genes, instead of disordered growth, normal offspring were produced. This simple restoration of the male/female complementarity of gene expression resulted in changes in the downstream gene expression of over a thousand other genes.

SYNTHETIC AND SYSTEMS BIOLOGY

This striking example of our increasing power to intervene and alter natural processes points to a coming era of challenging ethical dilemmas through advances in developmental biology. With new tools from cytology to synthetic biology, we are gaining control not just of component parts and their partial trajectories of growth, but of the principles and dynamics of organismal systems. Beyond highlighting our increasing powers over developmental biology, the parthenogenetic mouse points to another level of advance in our understanding: our new appreciation of systems biology, in which we see how even an alteration in a single gene can affect the entire balance of an enormous network of biochemical processes within the cell.

Systems biology offers us a renewed appreciation of an organism as 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, an organism is a self-sustaining, 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 activated dynamic of development in the direction of the mature human form. If severed from the whole, partial subsystems may temporarily proceed forward in development, but without the environment of their organismal system, they will ultimately become merely disorganized cellular growth. ANT proposes that small but precisely selected alterations will allow the harnessing of partial developmental trajectories apart from their full natural context in order to produce ES cells.

Cdx2

There are numerous potential approaches to such a project, involving the alteration of genes necessary for early intercellular signaling, cell differentiation, or integrated patterning of development. Of course, there must first be a thorough discussion to decide what level of alteration would be consistent with both the scientific and moral goals of this project. For the sake of discussion, one possi-

bility might be the alteration of *Cdx2*, a gene essential for the differentiation of the trophoctoderm (which, together with the formation of the inner cell mass, reveals the first globally coordinated segregation of cell lineages). This may not be an acceptable final solution, but examining it as a specific example could allow us to consider the necessary criteria for scientific success and moral acceptability.⁹ ANT must not be simply identified with *Cdx2* alteration, however, for the general proposal encompasses a wide range of alternative procedures.

Janet Rossant and her colleagues have shown *Cdx2* to be an essential component of early embryogenesis (Tam and Rossant 2003). The gene is expressed immediately after compaction (around the 16- to 32-cell stage) and is necessary for the differentiation of the trophoctoderm, the outer layer of cells that seals the embryo and controls the flow of water and ions to the inner cavity (Felix Block, University of Leicester, personal communication). Although the trophoctoderm cell lineage is crucial in the formation of the extra-embryonic membranes, it is properly considered part of the embryo, as it plays a central role in the interactive cellular inductions that generate all subsequent embryonic development. Studies confirm that a functional trophoctoderm is essential in embryogenesis. In mice, when *Cdx2* 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 (Chawengsaksophak et al. 2004). For the purposes of ANT, *Cdx2* might be deleted from the somatic cell nucleus prior to transfer. Once the ES cells have been procured, the gene could be re-installed to restore a full genetic constitution. Alternatively, the same goal might be accomplished through temporary gene silencing using RNA interference. Indeed, some combination of alterations in gene expression could be affected by the complementary employment of several systems of genetic knock-out and/or knock-down.

This technologically created, limited cellular system, from which the ES cells would be obtained, would fail to establish even the most basic features of human organismal infrastructure and would be incapable of implantation. A deficiency at the first complementary differentiation of cell types—the formation of the trophoctoderm and the inner cell mass—means the absence of the most funda-

⁹The ideal candidate would be a gene essential for the earliest expressions of organismal integrity, such that the “partial trajectory of growth” would lack the coordinated development of natural embryogenesis but be more akin to an “inner cell mass culture.” With the deficiency of a gene such as *cdx2*, which (by current evidence) is not expressed before the 16-cell stage, some might consider that the created entity is a “disordered” embryo. This is a serious concern and must be given careful consideration, but it is, of course, dependent on the definition of an embryo. It is possible, however, that this “deficiency” may be expressed earlier and at a more fundamental level of organization than that which produces a teratoma. While some might then argue that a teratoma is also a disordered embryo, a more convincing verdict would be that such an entity lacks the essential nature of a human embryonic organism and, as a pathological process, would be a proper target of therapeutic intervention at any stage in its development.

mental order. According to Dr. Maureen Condic, a developmental biologist at the University of Utah, "When [the] trophoblast does not form, subsequent development follows a chaotic pattern, suggesting that organismal development has not been "disrupted" in the absence of [the] trophoblast, but rather that an organism never existed in the first place" (Condic and Condic, in press).

The resulting cells 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 disorganized 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 in isolation. Lacking crucial elements in its fundamental constitution, such an entity would never rise to the level of a living being. 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.

ADVANTAGES OF ALTERED NUCLEAR TRANSFER

Unlike other proposals for ethical procurement, ANT would allow a uniquely flexible approach by providing a wide range of ES cell types that would have the full normal complement of human chromosomes, could be of specific genetic types for tissue compatible transplantation, and would not carry the danger of zoonotic contamination.

In addition, this technique would offer a far wider range of scientific and medical possibilities than ES cell lines derived from "leftover" IVF embryos, including generation of diverse and pre-designed ES cell lineages for disease modeling and pharmaceutical development. Indeed, in allowing controlled and reproducible experiments, ANT might serve as a temporary bridge to transcendent technologies such as direct nuclear reprogramming. Furthermore, in establishing a morally acceptable means for the procurement of ES cells, this important realm of scientific investigation would be opened to federal funding and the advantages of both broad public support and cooperative research collaboration on a national level.

ANT could also unburden ES cell research from the additional ethical concerns of the "leftover" IVF embryos, including the attendant clinical and legal complexities in a realm of great personal and social sensitivity. 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 morally dubious and expensive superovulation of female patients. These include the use of eggs left unfertilized from IVF procedures (nearly half of all eggs produced, some of which will fertilize with intra-cytoplasmic sperm injection or

nuclear transfer), xenotransplantation of human cadaveric ovaries or ovaries from oophorectomies transplanted into animals, in vitro maturation of ovarian tissue, and possible laboratory production of oocytes from ES cells.

ETHICAL HARNESSING OF PARTIAL DEVELOPMENTAL POTENTIAL

All cloning procedures where living embryos are produced should rightly be recognized as acts of reproduction, even if these nascent human lives are intended for disaggregation early in their development for projects of scientific research. The intention in creating an intrinsically limited “biological artifact” through ANT 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 employing ANT, 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.

Over the course of the previous century, we have 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 human embryonic stem 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 refinement 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.

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