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Substance Ontology Cannot Determine the Moral Status of Embryos

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Assigning the appropriate moral status to different stages of human development is an urgent problem in bioethics. Many philosophers have attempted to assess developmental events using strict ontological principles to determine when a developing entity becomes essentially human. This approach is not consistent with recent findings in reproductive and stem cell biology, including the discovery of the plasticity of early embryonic development and the advent of induced pluripotent stem cells. Substance ontology should therefore not be used to determine the moral status of the embryo.

Keywords: *ontology, moral status, embryo, substance*

I. INTRODUCTION

When an ethicist attempts to assign the appropriate moral status to different stages of human development, she has several types of analysis at her disposal. Each of these types presents the ethicist with different problems. For example, capabilities-based ethics requires the ethicist to determine which capacities are relevant to moral status (e.g., a functioning central nervous system or viability outside the uterus) and then to determine when in development the relevant capacities appear. Consequences-based ethics requires the ethicist to balance the effects on individuals and society of extending or withholding rights and protections to different beings. Substance-based ethics posits that any entity that is a human being must be accorded high moral status and strong legal protections. Applying a substance-based analysis to determine moral status therefore requires the ethicist to determine when in development a being transits from nonhuman to human. Many ethicists and philosophers who take this third approach assume that an entity becomes a

human substance in one discrete event, termed a substance change, and they interrogate the biological events in early development to determine when the relevant substance change occurs. For the purposes of this discussion, I will refer to this philosophical or biological approach as strict ontology. I do not mean to imply that any belief in a human substance necessitates that one belong to this school. This discussion focuses on the problems inherent in strict ontology in light of recent discoveries in reproductive and stem cell biology.

Metabolism ensures that the precise atoms that constitute a human being are changing all the time, yet identity is not compromised. Identity is similarly maintained despite graduations, marriages, career changes, and so on. In contrast with these qualities that do not determine a being's identity or continued existence, strict ontology maintains that we can define for any entity a "substance sortal" that defines "the kind of thing an individual essentially is by means of a consistent set of persistence conditions that apply throughout its history. When a substance sortal no longer applies, the individual necessarily ceases to exist" (Brown, 2007, 592). A being's unchanging essence or nature determines its persistence conditions: "a thing cannot change its criterion of identity partway through its career" (Olson, 1999, 78).

To determine when an entity comes to exist, strict ontology requires that we begin by determining its substance sortal: what is most essential to that being. "When we speak of substance or nature or essence, we are drawing attention to a distinction between the kind of thing an entity is and the various characteristics that an entity might possess accidentally, contingently, or temporarily" (George and Tollefsen, 2008, 58). For example, a sapling could become a tree without changing its identity, but it would change its identity if it were made into a table (Brown, 2007, 600). A table is essentially or substantially different, according to strict ontology, from a tree. Similarly, "a wooden house is a potential pile of ashes; but that does not imply that any one thing could be first a house and later a pile of ashes" (Olson, 1999, 29) because the house must cease to exist in order for the pile of ashes to arise. In other words, according to Strict Ontology, "table," "tree," "house," and "ashes" are real, objectively definable, mutually exclusive, natural categories that obey well-defined ontological rules.

According to strict ontology, human is a substance concept, and if the embryo transits from not human to human during development, there must have been a substance change: one could not speak of the same being before and after such a change. A human being is always a human being as long as he or she exists. A prehuman could not be the same entity as a human. It follows that if an entity now has a certain moral status, and has not undergone a substance change since it was at stage S, then at stage S it already had that moral status: "The argument is that the adult is identical to the embryo he or she once was because there are no essential differences in the kind of being one is between any two stages" (George and Lee, 2009a, 303).

Strict ontology is also highly concerned with the individuality of substances; each substance must be distinct from all other substances and coordinated within itself. Therefore, for an embryo to be considered a human being, it cannot be just an aggregate of cells. Rather it must be a "unified causal system that is relatively isolated from its surroundings" (Smith and Brogaard, 2003, 49). For this reason, some ethicists attempt to determine when the embryo is anatomically separate from other entities (the mother or other embryos). Other ethicists focus their efforts on assessing when the embryo's development is internally determined toward the mature stage of a human being or when its behavior indicates that is a unified individual acting to achieve particular goals (Brown, 2007, 611; George and Tollefsen, 2008, 39: George and Lee, 2009a, 301–303). A second aspect of individuality according to strict ontology is that that one substance cannot be temporally continuous with two substances. When an amoeba divides or a human embryo splits to form identical twins, strict ontology would claim that one being ceased to exist and that two new beings appeared in its place (Olson, 1999, 93). Accordingly, many philosophers claim that the loss of an embryo's ability to twin marks a substance change (Olson, 1999; Wiggins, 2001, 72–73; Smith and Brogaard, 2003; Brown, 2007).

Not all philosophers would expect to find discrete developmental stages that objectively differ in their moral status. Because development is a continuous process, some philosophers claim that singling out any developmental event as ethically significant must be subjective (Green, 2001, 26). To answer the criticism that they are dividing the continuous process of development at arbitrary points, philosophers applying strict ontology arguments frequently argue that the embryo is different in a nonarbitrary way after a particular developmental event. For example, some philosophers point to the differences between sperm and eggs on the one hand compared with blastocysts and infants on the other as differences in substance (George and Tollefsen, 2008, 122–123). Unfortunately, although these differences are real, important differences distinguish all developmental stages. Strict ontology requires that we distinguish which of these differences are "accidental" and which are indicative of substance changes, but we shall see that these distinctions are themselves highly subjective.

I will show that recent genetic and molecular breakthroughs demonstrate that cell types and developmental stages are separated by limited, well-defined, subtle changes. These changes do not differ in their extent or mechanism from changes that strict ontology would classify as accidental changes. Biology is therefore no aid in identifying substance changes that separate these stages. Further, researchers have discovered modest, quantitative, reversible interventions that can alter the path of development dramatically. This effort has culminated in the production of induced pluripotent stem (iPS) cells, which can generate a human being from a skin cell or

neuron of an adult simply by altering the levels of several proteins in the adult cell and then placing the cell in a suitable context for further development. Strict ontology demands that iPS cell production be a substance change, but biology does not distinguish this process from many others that are not considered ontologically significant. In addition, there is no biological event distinguishing a stage at which embryonic development can be considered independent and internally determined. The analysis of strict ontology in light of modern developmental biology shows that this approach is based on false premises and should not be used to assign moral status to different developmental stages.

II. DEVELOPMENTAL EVENTS AND STRICT ONTOLOGY

Although assigning moral status to developmental stages based on substance-based analyses should be objective, philosophers applying strict ontology do not agree whether a substance change takes place after conception. Mark Brown states, "The biological facts, insofar as they demonstrate anything, imply that the human embryo is an intermediate life form bridging the ontological gap between gametes and infants" (Brown, 2007, 611). Brown explains that between fertilization and gastrulation, human development includes several substance changes and that therefore the early embryo is not the same entity as the infant (Brown, 2007, 609). Wiggins and Smith and Brogaard reason similarly (Wiggins, 2001, 239; Smith and Brogaard, 2003, 59). In contrast, George, Lee, Tollefsen, Damschen, Gomez-Lobo, and Schonecker do not believe that embryos undergo substantial changes after fertilization and they conclude that all humans have the same essential nature from fertilization onward (Damschen, Gomez-Lobo, and Schonecker, 2006; George and Tollefsen, 2008; George and Lee, 2009a). We must therefore examine early human development to evaluate these philosophers' arguments.

II.A. From Fertilization to the 16-cell Stage

Human conception without medical intervention requires the fusion of an egg and a sperm. Both the egg and the sperm are differentiated, specialized cells. Each has unique properties distinguishing it from all other cell types. On their own, eggs and sperm, which each contain only one complete set of genes, do not produce any of the body's other cell types, which require two complete sets of genes. Following sperm–egg fusion in the oviduct, the sperm and egg nuclei move toward each other over the course of a day or so. On the way, the genes in both nuclei are replicated so that when they fuse, they form a single nucleus carrying four complete copies of the genome (two maternal and two paternal). At that point, the single-celled embryo divides, generating two identical daughter cells, each containing

two copies of each gene (one maternal and one paternal). Each of these daughter cells is capable of generating all the tissues of the developing embryo along with its placenta and extraembryonic membranes. This capacity is called totipotency. The analysis of reproduction led to the model that males and females each contribute unique and irreplaceable factors (the sperm from the male and the egg and uterus from the female) in generating a new human being. Many philosophers consequently view fertilization as the substantial change that marks the transition from nonhuman to human in development, although they disagree whether the substance change is the fusion of the sperm and egg or the fusion of the nuclei (when the paternal and maternal genes are present together for the first time) or the first cell division (when the first totipotent cells arise, each containing a nucleus with complete maternal and paternal genomes).

Some philosophers find substance changes in the cell divisions that occur between the 2-cell stage and the 16-cell stage (Smith and Brogaard, 2003; Brown, 2007), although they also find a final substance change later, at gastrulation, so that substance changes before gastrulation would not be determinative for moral status. At the four- and eight-cell stages, all cells are totipotent, although they differ in their biases (i.e., some of the cells contribute to embryonic tissues more efficiently, whereas others are more likely to contribute to extraembryonic tissues; Zernicka-Goetz, Morris, and Bruce, 2009, 471–474). At the eight-cell stage, some of the cells divide symmetrically while others divide asymmetrically so that one daughter cell is entirely surrounded by other cells while the other is on the outer surface of the embryo. These outer and inner cells become more constrained in their developmental potential (see Sections II.C and II.D).

Since the 1970s, researchers have greatly reduced the list of factors required for generating a new human being. In vitro fertilization (IVF) demonstrates that embryos can be generated and maintained in the laboratory through the blastocyst stage, which consists of approximately 60–150 cells (Hardy, Handyside, and Winston, 1989). This is the stage at which embryos normally implant in the uterus. Because implantation in a uterus is sufficient for an embryo generated in the laboratory to produce a human being, when ethicists apply the rules of strict ontology, they generally accord IVF embryos the same moral status as embryos conceived without intervention. For these ethicists, human moral status does not depend on precisely how the embryo is generated.

Cloning, or somatic cell nuclear transfer (SCNT), proves that sperm are not necessary for embryo formation. In this procedure, the nucleus of a cell that had been committed to a particular lineage (e.g., a skin cell) can generate a functional embryo upon introduction into an egg lacking a nucleus. For ethical reasons, SCNT has not been performed on human beings, although it has been successful for many mammals. SCNT works because the egg cytoplasm reprograms the skin cell nucleus to totipotency, giving it a developmental potential it never would have had otherwise. Because SCNT generates new

organisms and could presumably generate a mature human being, some philosophers maintain that the joining of the nucleus and the enucleated egg is a substance change analogous to fertilization: "this is not merely the placing of the somatic cell into an environment hospitable to its continuing maturation and development. Rather . . . [the entity] brought into being by this process is radically different from the constituents that entered into its generation (George and Lee, 2009a, 301–302). In fact, the nucleus of the somatic cell and the cytoplasm of the oocyte are still present; what has changed is that the oocyte cytoplasm has reprogrammed the somatic cell's nucleus. According to these philosophers, then, the nuclear reprogramming must itself constitute a substance change of enormous ethical and ontological significance. Clearly, the programming of the nucleus warrants further attention.

II.B. Nuclear Programs, Cell Types, and Moral Status

All animals are made up of multiple specialized cell types that act together to promote the survival and reproductive success of the organism. All cells carry out an impressive array of coordinated functions. For example, cells alter their metabolism in response to nutrient abundance and energy stores, and they alter their patterns of division and behavior in response to internal and external signals. In addition to these highly general functions, each cell type has specific functions it carries out. Nerve cells specialize in a particular type of cell–cell communication, muscle cells specialize in force-generating contractions of their cytoskeletons, and so on. Several types of white blood cells move within the body quite independently of other cells, and one cell type, the sperm, develops in the male but carries out its primary function in the female's reproductive system.

The differences we observe in the behavior of cells in an organism, whether we are comparing a single cell responding to an environmental change or two cells of different types, are due to differences in nuclear programming. The cells in a human being differ not in the genetic information (genes) they contain but rather in the subset of the genes they use or "express."[1] Each expressed gene is used as a template for producing a particular protein that carries out a specific function.[2] Genes include instructions not only for how to make a protein but also for how much of the protein to produce and under what circumstances to produce it. A skin cell and an adjacent nerve cell share identical genes and very similar locations in the body; they differ enormously in their appearance and the tasks they carry out because skin cells express the genes that encode skin cell proteins and nerve cells express the genes that encode nerve cell proteins. In other words, a human being's cell types differ because their nuclei access different subsets of the same genetic information: they differ in their "expression profiles," that is, in how much protein is produced from each gene. A cell of a given type

can respond to different environmental stimuli or different internal states by altering its expression profile as well.

In different cell types, there are different regulatory proteins, called transcription factors, that determine which genes are expressed and at what levels. Each type of transcription factor recognizes and binds tightly to specific portions of certain genes, and it increases or decreases expression of those genes. A cell's expression profile therefore depends to a great extent on the transcription factors that are active in its nucleus.

Scientists have known for decades that introducing a single transcription factor into a cell can, in some cases, alter the cell's fate. For example, expressing the transcription factor MyoD in fibroblast cells reprograms them to develop as muscle cells (Enver et al., 2009, 387). However, most changes in gene expression do not alter cell types; each cell type includes a range of expression profiles. Changes within this range would never be deemed substance changes.

Transcription factors positively and negatively regulate each other's expression in addition to regulating the genes that cells need to carry out their specialized tasks. Consequently, some combinations of transcription factors reinforce themselves and result in stable cell types, whereas others are internally inconsistent and are not characteristic of stable cell types (Graf and Enver, 2009, 590). Cells are induced to express different sets of transcription factors and take on different cell fates based on signals from other cells or from their environment, based on levels of transcription factors they inherit from their mother cells when they are born, or based on factors scientists introduce into them during genetic engineering experiments. Transitioning from one cell type to another can be accomplished by multiple routes; there is no one sequence of gene expression changes that must be followed for a cell to differentiate into a particular cell type or to be reprogrammed to a less differentiated state (Enver et al., 2009, 389).

In general, although cell types differ only by virtue of their expression profiles, and although all cells carry out an impressive array of coordinated functions, we do not ascribe moral status to any single human cell except the one-celled embryo.[3] Those who would assign moral status to the fertilized egg based on biological arguments must then explain what distinguishes this cell from every other human cell type. One argument put forward by some philosophers is that in addition to possessing the genetic information required for developing into an adult (which almost all the cells of the body have), the fertilized egg is unique because it has the potential to develop into an adult. Some prefer to state that the embryo "possesses an active disposition to develop itself using [its genetic] information" (George and Tollefsen, 2008, 53). Before discussing how recent biological advances undermine these assertions (see Sections II.D–II.F), we must first examine the divide between the totipotent cells of the fertilized egg and very early embryo and the developmentally limited cells of the later embryo.

II.C. From the 16-cell Stage to Blastocysts and Implantation

Several transcription factors carry out important nuclear programming events between the 16-cell stage and the implantation. The transcription factor CDX2 is found disproportionately in the outer cells of the embryo beginning at the 16-cell stage. This protein is necessary for the development of the cells that will give rise to the placenta. The inner cells express the transcription factors OCT4, SOX2, and NANOG, which all reinforce each other's activity. These factors repress differentiation and promote pluripotency, the capacity to give rise to all the tissues of the embryo-proper and to many extraembryonic tissues but not to the placenta. OCT4 and CDX2 repress each other but activate themselves. Yet at the 16-cell stage, the embryo's cell fates are still not fully determined. Repositioning cells between the inner and outer portion of the embryo can alter which transcription factors predominate and whether the cells will contribute to placenta or embryonic tissues (Zernicka-Goetz, Morris, and Bruce, 2009, 469–475).

All of the cells of the embryo and most extraembryonic tissues derive from the population of OCT4-expressing inner cells. The outer cells contribute only to the placenta. Some of these outer cells secrete a fluid such that a large border of outer cells surrounds a smaller ball of pluripotent inner cells resting on top of a fluid-filled cavity. At this developmental stage the embryo is called the blastocyst, and it is at this stage that an IVF or SCNT embryo must be implanted if it is to develop into a fetus. At implantation, the outer cells differentiate and invade the uterine lining to form the placenta so that oxygen and nutrients can diffuse from the mother's circulatory system to the fetal circulatory system while waste moves in the opposite direction. The placenta also releases hormones to maintain pregnancy. In other words, the outer cells that express CDX2 eventually connect to and mediate the functions of the uterus: they maintain pregnancy and transport nutrients and waste. Despite the many changes in cell fate, expression profiles, and physiology that accompany these changes at implantation, most ethicists and philosophers who apply strict ontology criteria do not count implantation as a substance change. If they did so, they would not accord human moral status to preimplantation IVF embryos or to early embryos in general.

II.D. Embryonic Stem Cell Technology

The description of preimplantation development might leave the misleading impression that these early events are inevitable or determined. In fact, however, development is much more tractable than would first appear, and the early embryo is quite plastic. Most embryos fail to develop beyond the first few weeks. Although embryos that develop successfully inside the female do follow a predictable course, this is not proof of determinism. Some illustrate this point by comparing the embryo's course of development to a journey downstream on a river. The most energetically favorable developmental events are comparable to steep cascades, where the course is most

predictable (Hemberger, Dean, and Reik, 2009), but changing the shape of the river (by exposing the embryo to different signals or by otherwise reprogramming nuclei) can change the destination of the journey considerably.

Observations in the laboratory show that embryonic development can proceed along many different paths. If the blastocyst-stage embryo is dissociated, cells isolated from the pluripotent inner cells can be cultured as embryonic stem (ES) cells.[4] ES cells remain pluripotent: they can be used to generate any of the tissues in the embryo, although they do not generate placenta. If ES cells are added to cells that generate only placenta (because those cells have been manipulated to have a condition fatal to other cell types), then upon implantation the ES cells will develop into a complete, viable, mature organism. However, if the transcription factors OCT4 or SOX2 are silenced in ES cells, they develop as "outer" (placenta-generating) cells rather than as embryonic tissues. Moreover, even more subtle, quantitative changes in gene expression can be responsible for the differences between cell types of the greatest developmental significance. For example, increasing OCT4 levels in ES cells by 50% induces loss of pluripotency and differentiation into specific embryonic cell types that can no longer generate entire organisms (Chambers and Tomlinson, 2009). Altering levels of transcription factors such as CDX2 also affects the ES cell's predisposition toward pluripotency or toward generating specific cell fates.

We see, then, that what may have appeared to be a significant ontological divide between two strictly defined categories of substances (one that could generate a complete adult and one that can generate only a specific tissue type) is instead an easily manipulated transition between states. Where strict ontology would lead us to expect abrupt events leading to stark, qualitative changes, we see instead that reversible, modest, quantitative changes in gene expression alter the predispositions of cells and tissues to develop along different developmental paths. A recent paper shows how reversible these transitions often are: ES cells and iPS cells "cycle in and out" of a totipotent state (MacFarlan et al., 2012). Strict ontology does not, therefore, account for the developmental plasticity of the embryo.

II.E. Strict Ontology and ES Cells

Because ES cells are capable of generating entire embryos (including all the tissues present in adults), one might have expected that ethicists who attribute human moral status to fertilized eggs and to IVF embryos would also attribute this same status to ES cells. Surprisingly, these ethicists treat ES cells like any other cell (except for strong compunctions about how they are generated). George, Lee, Tollefsen, and other ethicists instead determine that introducing the ES cells to cells capable of generating placenta alters their moral status. Their argument depends on two propositions. First, that ES cells do not "by their own internal self-direction" develop into an adult, whereas the embryo does. Second, as strict ontology requires, "the aggregation of

the stem cells with the cells [capable only of producing placenta] generates a new organism" (George and Tollefsen, 2008, 165–166). In other words, bringing the two types of cells together causes a substance change. Biology supports neither of these propositions.

The first proposition, that embryos but not ES cells develop into adults "by their own internal self-direction," does not mean that the early embryo is conscious but rather that its developmental trajectory is entirely determined by an internal program. Strictly speaking, this would appear to be a capabilities-based argument in which the (asserted) developmental autonomy of the embryo earns it a high moral status. As such, the analysis of the argument would be beyond the scope of this paper. However, George and Tollefsen attempt to use this capabilities-based argument to buttress the ontological claim of their second proposition: "[the capacity to direct its own development] is central to the embryo's nature, as it is not to the nature of an embryonic stem cell" (George and Tollefsen, 2008, 165). We are therefore compelled to examine whether embryonic development is internally determined.

Embryonic development is anything but determinate. IVF embryos can be implanted or they can be dissociated to generate ES cells. ES cells can then be used to produce any number of tissue types. In utero, the cells of the embryo are generally exposed to predictable signals that often impel them toward fetal development. In other environments, however, or given exposure to different signals, their trajectories differ considerably.

The issue of signals and how they impact nuclear programs (and thus, developmental trajectories) is key. George and Tollefsen, for example, claim that the embryo is independent from its environment from fertilization onward. They distinguish environmental from internal factors in this way: "something that qualifies as 'merely environmental' does not enter into an organism and modify its internal parts." In contrast, they claim that in SCNT the enucleated egg and the donor nucleus are both internal rather than environmental because "the cytoplasm, or factors in the cytoplasm, reprogramme the nucleus fused with it" (George and Tollefsen, 2008, 302). The distinction George and Tollefsen attempt to draw here is that between what developmental biologists call permissive and instructive environmental factors. Classic permissive factors include oxygen, nutrients, and other necessary external factors that have not generally been thought to direct development. Instructive factors, such as the signals by which cells communicate with each other, direct development by inducing cells to modulate their behavior and their gene expression profiles. We have come to realize, however, that there are no purely permissive factors. Cells, tissues, and organisms are more sophisticated than that. Cells routinely alter their gene expression profiles in response to different oxygen levels, different levels of nutrients, and so on (Ma and Blenis, 2009). As Sagan and Singer point out, "at least from implantation onwards there is a constant acquisition of substances from the outside that modify the embryo's internal parts, including its gene expression" (Sagan and Singer, 2009). In support of Sagan and Singer's

claim, recent work has shown that nutritional states in utero result in altered nuclear programs (expression profiles) that continue to impact predisposition to chronic diseases even late in life (McMillen et al., 2004; Wu et al., 2004). If such changes in genetic programming alter moral status, then the fetus is not internally determinate because the uterus instructs such changes. If such changes are not significant, then philosophers are not justified in classifying the introduction of embryonic precursor cells to placental precursor cells as a substance change. A further note: one of the main functions of the placenta is to release hormones that reprogram the expression profiles of cells in the mother. By the standard of George and Tollefsen, neither mother nor embryo is independent of the other. Clearly, there is no clear-cut distinction, no binary ontological divide, between independence and dependence. Arguments concerning internal determination do not, therefore, help distinguish the moral status of different developmental stages.

The second proposition is that the introduction of ES cells to cells capable only of producing placenta is a substance change responsible for generating an embryo with full human moral status. George and Lee explain this stance by analogy with their view of what takes place when scientists generate SCNT embryos. When a skin cell nucleus is placed in an egg cytoplasm, they say, this "generates a wholly distinct, self-integrating and entirely new organism—it generates an embryo" (George and Lee, 2009a, 302). The cytoplasm is not merely a suitable environment for the nucleus because "something that is 'merely environmental' does not enter into an organism and modify its internal parts resulting in an entity with a new developmental trajectory" (George and Lee, 2009a, 302). "Just as in SCNT cloning, so here: the manipulation . . . generates a new organism" (George and Tollefsen, 2008, 166–167).

In fact, bringing ES cells into association with cells that generate placenta is much more closely analogous to implantation of IVF embryos than it is to SCNT cloning. When IVF embryos are implanted in the uterus or when ES cells are brought together with cells that generate placenta, cells of different tissue types establish close contacts and influence each other's development so that one cell type can provide life support for the other. In both cases, cells signal each other to alter each other's gene expression profile and developmental trajectory. In both cases, the completion of development requires both tissue types, although the mature human is composed of only one of the tissue types. Either both events are substance changes or neither is. If both events are substance changes, then the rules of strict ontology indicate that the last substance change is the relevant event, and preimplantation embryos would not have human moral status. If neither event is a substance change, then ES cells, iPS cells, and single-celled embryos should all have the same moral status.

II.F. iPS Cell Technology

SCNT cloning and ES cell production demonstrate how we can produce new embryos in the laboratory, but each of these technologies requires either an

egg cell or an early embryo. iPS cell technology reprograms differentiated cells (e.g., adult skin cells or neurons) into pluripotent cells by transiently expressing several transcription factors. iPS cells, like ES cells, can differentiate into all embryonic tissues but not into placenta. Either cell type will develop into viable embryos when combined with cells that generate placenta (Jaenisch and Young, 2008, 569–572). Because iPS cells can generate embryos but originate from cells of very limited developmental potential, strict ontology demands that iPS cell production should entail a substance change. An examination of how scientists produce iPS cells should allow us to test this prediction.

iPS cell technology is based on the fact that different cell types, including pluripotent cell types, are characterized by different sets of expressed transcription factors, which themselves interact to increase or decrease expression of other genes to induce particular expression profiles (Graf and Enver, 2009, 590). Cells are induced to express different sets of transcription factors and thereby to take on different fates based on signals from other cells or from their environment, based on levels of transcription factors they inherit from their mother cells when they are born, or based on factors scientists introduce into them during genetic engineering experiments. Work on iPS cells has confirmed that many different cell types can be induced to adopt a pluripotent fate, but some cell types are more easily induced to pluripotency than others. For example, neurons require only the transient introduction of the transcription factor OCT4 to be reprogrammed into iPS cells. Pluripotency results because the introduced OCT4 helps to activate expression of the cell's endogenous OCT4 along with other transcription factors before it disappears. Fibroblasts require several transiently introduced transcription factors for reprogramming into iPS cells, although different combinations suffice. For example, fibroblasts were first shown to take on iPS fate upon transient introduction of the transcription factors OCT4, SOX2, KLF4, and MYC. More recent work has shown that other transcription factors can substitute for KLF4 and MYC or even for OCT4. None of these factors are therefore absolutely required, nor is any given factor sufficient in all circumstances for pluripotency: germ cells express OCT4 but are not pluripotent (Rossant, 2009; Heng et al., 2009). Even the introduction of exogenous genes or proteins may be dispensable in generating iPS cells. A recent protocol reprograms fibroblasts to pluripotency using a cocktail that includes a drug in place of SOX2 (Ichida et al., 2009). It is possible that all the factors required for reprogramming could be replaced by temporary exposure to drugs in the cells' growth medium.

With iPS technology, potentially any cell in the body with two sets of genetic information could be used to generate a human being without recourse to sperm or egg. iPS technology removed any final doubts: cells differ from each other solely by virtue of their expression profiles. Adopting a pluripotent fate is biologically similar to adopting any other fate. Although

scientists have not attempted to reprogram cells to be totipotent (like the cells of the two- or four-cell embryo), iPS cell technology demonstrates that in principle, it should be possible to do so. Pluripotent cells like differentiated cells and totipotent cells differ from each other because of the different levels of the different proteins they contain. Human development, awe-inspiring as it is, is not a mystical, irreducible event but a process that we can manipulate handily and predictably by altering the concentrations of a handful of transcription factors. Changes in protein levels that lead to pluripotency are achieved with no more effort, drama, or mystery than any other changes in gene regulation. The biology of iPS technology and of cell reprogramming in general does not distinguish some of these changes as pertaining to accidents and others as pertaining to substances. Rather, all the techniques for generating iPS cells are quantitative, transient, and reversible. Strict ontology does not adequately account for the power and simplicity of iPS cell technology.

II.G. Individuals, Twins, and Coordinated Development

Strict ontology places great emphasis on individuation, or the separation of the embryo from other entities and from its environment because, according to its rules, substances must be independent in the sense that they do not require any other specific entity to exist (Smith and Brogaard, 2003, 48). Despite its importance, different philosophers approach individuation in very different ways. Smith and Brogaard (2003) take an anatomical approach to determine at what stage the embryo is topologically separate from the mother or from other embryos. Alternatively, many philosophers claim that so long as an embryo retains the capacity to twin, the embryo is not a human being because strict ontology requires that a single entity must undergo a substance change if it is to be replaced by two entities. Early embryos, which can twin, are therefore not the same individuals we observe at later developmental stages because they have different persistence conditions (Olson, 1999; Wiggins, 2001, 72–73; Brown, 2007; Smith and Brogaard, 2003). Other philosophers assert that the embryo's individuality is evident because it acts as a "unified, integrated whole" during early development (George and Tollefsen, 2008, 151–156).

1. Anatomical boundaries. Smith and Brogaard focus on determining when the embryo is physically isolated from its environment to discover when it is an Aristotelian substance. They cite anatomical and topological criteria to buttress their claim that from gastrulation onward the embryo is a "unified causal system that is relatively isolated from its surroundings" (Smith and Brogaard, 2003, 49). They dismiss signs of coherence from the two-cell stage through the blastocyst stage, and they conclude that at gastrulation the "boundaries of a discrete, coherent entity have been formed" for the first time (Smith and Brogaard,

2003, 63). Specific problems with Smith and Brogaard's biological analysis have been addressed elsewhere (Damschen, Gomez-Lobo, and Schonecker, 2006), but a greater problem is that one cannot determine, as they try to, whether one is looking at an individual substance by examining its boundaries to see if they are shared or not. For example, Smith and Brogaard are forced to consider two conjoined twins to be a single substance because they share a boundary (Smith and Brogaard, 2003, 53), yet these twins are undoubtedly two people sharing a body. They have different interests, talents, passions, loves. Although they are often impossible to separate without killing one or both of the twins, legally, socially, and psychologically, they are two individuals (Dreger, 2005, 17–50). For human beings, at least, topology does not determine individuality.

2. Twinning. Identical twins form when early embryos divide. According to strict ontology, one substance cannot be continuous with two substances. A given embryo in the process of twinning might split very unevenly; in these cases, strict ontological rules permit us to view one of the twins as continuous with the original embryo and the other to be a newly generated being. When a twinning embryo splits more equally, strict ontology would require us to conclude that the original embryo died and was replaced by two new embryos (Smith and Brogaard, 2003, 66–69; Damschen, Gomez-Lobo, and Schonecker, 2006, 173–174). Although strict ontology should predict that we would observe some recognizable evidence of death in this process, metabolism does not pause when an embryo twins. There is no cessation of biological function whatever (Olsen, 1999, 114–119). Further, although anything that might have marked all or part of the embryo before twinning (e.g., mutation, an epigenetic change, or physical damage) will be present in at least one of the twins, we cannot, according to the rules of strict ontology, take this as evidence of continuity. If shortly after twinning, the twins rejoined, strict ontology would describe the series of events this way: one entity died as two new entities came into being for a brief time before these beings also died, leaving us with a fourth, completely new entity. The fourth entity would likely be indistinguishable from the first entity, but we would nevertheless consider it a different substance. There is no scientific justification for this bizarre account of events.

Philosophers applying the rules of strict ontology do not all agree on the significance of twinning to the embryo. Some argue that in most cases when twinning does not take place, the individual's existence begins at fertilization and generalize from those cases that embryos are human substances from that point (George and Tollefsen, 2008, 55). Others point out that twinning is common enough to "erase any doubt that embryos are divisible, even if most

embryos do not as a matter of fact divide" and conclude that the embryo can only be a human substance after it has lost the capacity to twin (Brown, 2007, 611). The main weakness of all these philosophers' arguments lies not in their disagreements but in the premises they all accept. Death and twinning are both undoubtedly biological processes, and there is no biological evidence of death or cessation of any sort when an embryo twins. None of the strict ontology arguments ethicists apply based on cases of twinning can be convincing because the strict ontology account flies in the face of biological evidence.

3. Coordination and unity of action. Some philosophers perceive the coordinated action of the early embryo to be proof that even at the earliest stages the embryo is an individual rather than a collection of cells. These philosophers put forward three lines of evidence: (i) the embryo has complex goals that it accomplishes in its first week, which demonstrates that it acts as a "unified, integrated whole"; (ii) the cells of the embryo exhibit differences that are quite pronounced by the stage at which the embryo attaches to the uterine lining of the mother; these differences reflect the complementary functions one would expect from different parts of a single organism; and (iii) the cells of the early embryo function in concert, as witnessed by the fact that they generate one individual rather than each developing on its own and by the fact that cells in the embryo take on different fates depending on their position (George and Tollefsen, 2008, 151–156). Each of these points requires discussion.
   (a) George and Tollefsen attribute three "goals" to the embryo in the week after fertilization. The first goal claimed for the embryo to is "get itself to the uterus." In fact, the embryo is passively moved toward the uterus by the cilia lining the walls of the oviduct. The second goal is to develop the tissues required for implantation. This goal is inseparable from forming different tissue types, which will be discussed in item b. The embryo's third goal is to "preserve its structural unity." The authors cite the presence of a protective protein sheath around the embryo as evidence for the third goal. This sheath, however, is not a product of the embryo and cannot be interpreted as a sign of unity of a new individual. Maternal cells provide the unfertilized egg with a protein sheath. The unfertilized egg contains granules that, upon exposure to calcium ions, modify the protein coat to make it impermeable (Gilbert, 2010, 152–153). Fertilization induces calcium release, but even an unfertilized egg will undergo the same reaction if exposed to calcium. In fact, such treatment is sufficient to induce unfertilized eggs to develop as far as the blastocyst stage (Toth et al., 2006). If one feels compelled to attribute a goal or design to this process, any credit would have

to go to the mother, not the embryo. Attributing "goals" to a cell to buttress an argument for its moral status is tendentious at best, in any case. One could attribute goals and concerted action to a white blood cell at least as compellingly as for a zygote. A white blood cell will migrate to an infection site and kill the pathogens it finds there. Nevertheless, philosophers who apply the rules of strict ontology do not attribute individuality or high moral status to the white blood cell.

(b) George and Tollefsen point to the distinction between embryo precursor cells and placental precursor cells by the time of implantation as evidence of coordination because different cell types carry out different functions. They further suggest that this coordination begins at fertilization because the sperm entry point in the oocyte influences which parts of the oocyte cytoplasm will contribute to which type of precursor cells later (George and Tollefsen, 2008, 154–155). This analysis is not convincing. First, every organ is made up of different cell types with different functions. No moral status accrues from this division of labor, nor are organs considered to be individuals. Further, to the extent that sperm entry organizes the oocyte cytoplasm (which is currently a subject of intense research and debate), differentiation would be attributed to the parents rather than to any new individual.

(c) Several authors claim that because cells in the early embryo develop differently if they are isolated or if their position in the embryo changes, it implies that they "shift their development in response to the needs of the whole and the tasks of its surrounding cells" and that they are therefore parts of an individual (Damschen, Gomez-Lobo, and Schoenekecker, 2006, 170; George and Tollefsen, 2008, 155–156). This analysis ignores the many examples of cells and organisms that coordinate their developmental and cell cycle programs with their neighbors even when their neighbors are clearly different organisms. For example, when exposed to pheromone released by its neighbors, the roundworm, Caenorhabditis elegans, arrests its growth and dramatically alters its developmental program, morphology, and behavior, although it continues to respond to its environment (Rottiers et al., 2006). Many types of tissue culture cells that do not otherwise appear to cooperate or coordinate will also cease dividing when surrounded by neighbors. The cells resume dividing if their neighbors are removed. Cells and organisms coordinately regulate their nuclear and developmental programs regardless of whether they are individuals. Cells and organisms respond to signals from their environment whether those signals derive from neighboring cells in a tissue or from other organisms or from abiotic factors. This fact has no bearing on moral status.

To sum up, strict ontology holds individuation to be highly significant for determining moral status. Unfortunately, none of the strict ontology–based claims for the embryo's individuality withstand close examination. There is no stage at which the embryo's developmental program is entirely internally directed, nor is early development fully determined (see Section II.D). Anatomical criteria cannot be used to determine if or when an embryo is an individual. The strict ontology–based view of twinning is unscientific and implausible. Arguments based on goals, different tissue types, or coordination of development all fail to distinguish individuals either from collections or from parts of individuals (e.g., organs). Individuation, then, like internal determination, is an important component of strict ontology that cannot be used to determine the status of embryos.

II.H. Identifying Substance Changes Later in Development

According to the logic of persistence conditions, only the final substance change in development marks the moment at which the fully realized human being comes into existence. It is therefore essential to examine the developmental events that have been suggested as possible candidates for final substance change. George, Lee, and Tollefsen simply deny that there are any substance changes after fertilization. These authors view twins as exceptional cases that should not inform their sense that embryos develop along an internally determined, invariant trajectory (George and Tollefsen, 2008, 55). Smith and Brogaard assert that a substance change must mark the distinction between an early embryo, which can twin, and an embryo after day 16, which cannot. These authors dismiss the ontological significance of postgastrulation milestones, although their justifications for these decisions frequently appear arbitrary. For example, the onset of neurulation (in which the cells that will eventually give rise to the central nervous system are fated) coincides with gastrulation, their "preferred threshold," so it does not merit separate consideration (Smith and Brogaard, 2003, 63). Brain stem formation, they state, is merely part of neurulation (ironically, given the continuity of all development) because they conclude that this aspect of development is a continuous process (Smith and Brogaard, 2003, 63–64). Sentience, they claim, is a change in one part of the organism and so could not cause a substantial change in the whole organism (Smith and Brogaard, 2003, 65–67). Why the physical extent of a feature should be relevant to its ontological importance is unclear; in any case, their premise is false because the peripheral nervous system extends throughout the organism. The transition to viability and birth itself are not substantial changes, they say, because changes in technology affect viability and because birth is merely entry into a new environment. They assert that such externally determined qualities cannot reflect substance changes (ibid, 64, 73). This catalog of rationalizations helps to illustrate that if substance changes occur in the generation

of a human being, we cannot look to biology to tell us objectively when they occur. Biology seems to reveal substance changes where philosophers expect to see them and nowhere else.

III. CONCLUSION: FALSE DICHOTOMIES AND SUBSTANCE ABUSE

In attempting to define the point at which an embryo attains human moral status, philosophers applying the rules of strict ontology assume that existence as a human being is sufficient for human moral status. These philosophers then apply the logic of persistence conditions, substance changes, and individuation to determine when in development one becomes human. Unfortunately, although strict ontology offers the comforting appearance of certainty, objectivity, and well-defined criteria for the onset of human moral status, its conclusions are not in accord with the facts of development. Strict ontology fails in its promise to solve problems of assigning moral status because it relies on a false understanding of human development.

Human development is continuous; we define stages and cell types for convenience, but these stages and cell types do not represent natural kinds; rather, they differ quantitatively. We demarcate them for our convenience based on the traits that interest us in a particular discussion. To designate one or another gene expression change or developmental stage transition as substantial or accidental objectively and in a way that reflects nature is not possible. Defining certain stages as independent or internally determined is also subjective and does not reflect biological reality. Recent technological advances show us that inducing ontologically significant changes in cells requires only limited, subtle, reversible interventions that are not otherwise distinguishable from changes we would call "accidental." The moral status of the embryo is therefore not best studied by applying ontological rules for substance sortals, persistence conditions, and individuation.

Ronald Green argued that the continuity of development implies that ethicists have to make subjective decisions in determining the moral status of different developmental stages (Green, 2001, 25–54). In response, George and Tollefsen wrote that there must be "right answers" and that "if truth is merely a matter of what we decide, what is in accord with our 'values' then there is not much point in study after all" (George and Tollefsen, 2008, 126–127). This statement suggests that substance-based arguments can be used to draw clear lines between humans and nonhumans. However, substance-based arguments introduce a false dichotomy between developmental stages on either side of an event denoted as a substantial change and between entities that are independent and internally determined and those that are not. This may explain why those applying the rules of strict ontology cannot agree about which events in human development are determinative. As physicists learned when they tried to determine if light is a wave or a particle, analyses based on false dichotomies yield contradictory or ambiguous answers.

Substance-based arguments are attractive because they offer the promise of establishing universal, objective norms for questions of moral status. However, these arguments do not reflect our understanding of developmental biology. This discussion does not point to any stage of development as having any particular moral status; rather, it points away from arguments that offer a false certainty and toward more realistic, but unavoidably subjective approaches. To rephrase George and Tollefson's statement, if questions of moral status are resolved by our decisions, then the primary work of ethicists, the "point in study" must be to ensure that our decisions reflect our values.

NOTES

1. There are a handful of minor exceptions, none germane to this discussion, to the rule that all cells in a human being are genetically identical. For example, B and T cells undergo rearrangements in several of their genes encoding antigen–recognition proteins. In addition, the DNA in different cells in the body undergoes random chemical damage throughout life. In some cases, these changes can cause cancer.

2. Genes encode not only proteins but also nontranslated RNAs that carry out essential processes, but for the purposes of this paper, nontranslated RNAs and proteins can be discussed together.

3. ES cells are accorded legal protection because their production requires the destruction of embryos, not because of their own properties, although as argued here, that position is not consistent with assigning human moral status to zygotes.

4. On the basis of comparisons with cells isolated from mice, what are generally called human ES cells actually behave more like cells from a slightly later stage in development, the epiblast (Enver et al., 2009, 392), although these cells can still differentiate into any cell type found in the embryo-proper.

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