Non-Destructive Sources for Embryonic Stem Cells: A Moral Analysis

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Human embryonic stem cells (hES cells) have the potential promise to cure a variety of human ailments. Such cells are pluripotent (from the Latin for "many powers"), and may act as "starter" cells to grow new nerve tissue, heart muscle tissue, or glandular tissue, which has many scientists excited about new treatments for heart disease, strokes, Parkinson's disease, diabetes, and many other disabling conditions.

Current U.S. policy permits private companies to engage in hES cell research, but prohibits federal funding for such research through the National Institutes of Health, except research using a limited number of existing stem cell lines.[1] The ethical concern for the sanctity of human life causes many to object to the destruction of embryos to obtain hES cells, because they believe that embryos are human persons, and thus have a right to life. This view equates destroying human embryos to abortion.[2-8]

Despite a divisive public debate, including objections from a wide range of religious and conservative secular writers, there appears to be no end to the desire among scientific researchers for unfettered access to human embryos for experimentation. Currently, there are two possible sources of such embryos: excess frozen embryos from *in-vitro* fertilization procedures and embryos derived from human cloning.

Harvesting of stem cells necessitates the death of the embryos from which they are derived. Is it possible to produce pluripotent stem cells in other ways? This paper will examine the technical aspects of three proposed alternatives, providing an ethical analysis of each. The newest development in the debate was announced in November of 2007: the derivation of pluripotent stem cells directly from human skin cells.[9] A moral analysis of this new development will contrast it with the three extant proposals.

Background Definitions

Human fertilization begins with fertilization of an ovum in the Fallopian tube by a single sperm. This creates a diploid single-celled cell with the full complement of DNA necessary for later development, called a zygote. At approximately 24 hours, the first cell division takes place, forming a two-cell embryo. The four-cell stage occurs at 48 hours, and the eight-cell stage is reached after three days. Subsequent cell divisions lead to a solid ball of cells called a morula at four days. These cells continue to divide, and the center of the embryo forms a cavity, with a surrounding trophoblast made up of blastomere cells at the periphery. One pole of the embryo thickens to form the inner cell mass (ICM), which will eventually form the definitive embryo proper (the trophoblast develops into cells of the placenta). This hollowing out and division into trophoblast and ICM is complete at five days, and the embryo is now called a blastocyst. The blastocyst has enzymes that allow it to implant into the inner wall of the uterus (endometrium), a process that begins at about 6.5 days after fertilization.[10]

In vitro fertilization (IVF) is a technique to assist in the reproductive process by combining sperm in a Petri dish with multiple ova (produced by hyperstimulation of the ovaries). The resulting embryos are then allowed to develop, with attempts at implantation made at the blastocyst stage. If the embryo is to be used for stem cell research, the cells are taken from the ICM at this point as well, which causes destruction of the embryo.

The PGD Proposal

In preimplantation genetic diagnosis (PGD), a single cell (blastomere) is removed from a three day-old (eight-cell) embryo. After such a blastomere biopsy, a genetic analysis is performed, and the embryo can be discarded if defects are found. However, if there are no genetic defects, the remaining seven-cell embryo is

implanted. Removing a single blastomere does not seem to affect the viability of an embryo for immediate implantation,[11,12] although cryopreservation is less successful.[13]

PGD has been used to screen for a variety of monogenic conditions, such as cystic fibrosis, Duchenne muscular dystrophy, hemophilia A, fragile X syndrome, retinitis pigmentosa, and Tay Sach's disease.[14] Ethical concerns revolve around the destruction of the affected embryos. Nonetheless, PGD has been a well-accepted part of IVF.

The same PGD technique may be used to remove a single cell from a three day-old embryo, and use this as a starter cell for a stem cell line, leaving the embryo intact to be implanted later. Would this be a feasible and ethically acceptable way to produce hES cells, since the embryo is not destroyed in the process?

Stem cell cultures have usually been derived from a large number of cells taken from the ICM in a five-day embryo. Using just one blastomere from a three-day embryo might be inadequate to establish a stem cell line. Scientists in Belgium were able to derive hES cell lines from embryos that had undergone PGD (destroying the embryos),[15] but could a single cell biopsy start a stem cell culture *and* preserve the embryo?

A research group at Advanced Cell Technology, led by Robert Lanza, claimed to have accomplished this challenge, in a report that appeared in the journal *Nature*.[16] When the study was published online, Lanza stated this in the *Nature* podcast: "What we have done, for the first time, is to actually create human embryonic stem cells without destroying the embryo itself."[17]

Criticism soon appeared in a variety of news sources, including the Washington Post and the New York Times.[18] In fact, none of the embryos Lanza's team biopsied survived the procedure. All the embryos were broken up, and 91 cells were used from 16 embryos. Only two stem-cell lines were created, a net yield of only 2%.[17] In defense of the research, the authors described their own findings as a "proof of principle" only.[16] Despite the controversy, it appears that using PGD methods to non-destructively create stem cell lines is technically feasible.

Though PGD can be performed without necessarily destroying embryos, it does increase the overall likelihood of embryo loss. For those who affirm the personhood of embryos, any procedure that puts embryos at greater risk is morally impermissible,[19] because human beings are used as means and not valued as ends in themselves. Using embryos for such utilitarian purposes violates the principle that it is unjust to abuse a vulnerable minority despite a potential benefit to others. The 18th Century philosopher Immanuel Kant stated as one of his Categorical Imperatives: "[H]uman beings, and in general every rational being, exist as ends in themselves, not as mere means for arbitrary use by another will."[20]

Despite the Lanza team's apparent success, the actual yield of a viable hES cell line from a single blastomere may be very low. The normal time to remove pluripotent cells for research is five days, when such cells are obtained from the ICM. Using a single cell from a three-day embryo may simply be an inadequate starting point for cell culture.[21]

It seems unlikely that couples who have gone to all the expense, difficulty, and sacrifice to engage in IVF would be willing to implant embryos that have been manipulated in this way, especially if there are no pre-existing genetic conditions that must be ruled out through PGD. Adding one more obstacle to successful embryo implantation is unjustified, as success rates of IVF remain fairly low.

The label "designer babies" has been used as a criticism of PGD: selecting embryos based on desirable traits. Even though the new use of PGD as a source of stem cells does not directly destroy embryos, it can increase the overall use of PGD as a screening technique. In addition, some have suggested expanding the indications for PGD to include using it for gender selection or to select out an HLA-matched embryo for tissue donation for an existing sibling.[22,23] For some, this raises the specter of eugenics.

The goal of the eugenics movement of the early 20th Century was to "perfect" the human species, by encouraging those with "better" genetic traits to have babies, and by sterilizing the mentally handicapped (and even some minorities) against their will. Condemned as pseudoscience, the movement died out during World

War II. Nonetheless, the idea of manipulating or selecting embryos using PGD strikes some as a variant of eugenics thinking.[24-26] Using blastomere biopsy as a source of hES cells is an unacceptable ethical compromise for conservatives.

The Non-Viable Embryo Proposal

Another recent proposal suggests that "non-viable" embryos, those embryos from IVF procedures that have stopped dividing in culture, may be a source of hES cells. Such embryos are "already dead" in some sense, so they could be ethically disaggregated.[27] The resulting stem cells may be stimulated to divide in cell culture:

> [This method] seeks to identify a subset of cryopreserved embryos that might reasonably be regarded as non-viable in advance. That is, although the embryos are not dead, and could presumably be thawed and would still exhibit some cellular function, they would be unlikely to survive even if transfer were available.[28]

In theory, no ethical violation occurs if the embryos have already stopped dividing. Some have suggested that the ethical considerations mirror those of donating organs from "brain dead" patients.

In practice, however, assessing which embryos have stopped dividing has been fraught with uncertainty. One recent study has sought to address this question by defining the criteria whereby embryos could be declared "dead:"

> Many nonviable embryos (n = 142 out of 444) were hypocellular and lacked compaction on embryonic day 5 (ED5). All of these hypocellular embryos did not progress to compacted morula or normal blastocyst when observed further. No criteria could be discerned for the diagnosis of death on ED3.[29]

The authors go on to recommend that such non-viable embryos be considered a source for hES cell cultures for research, relying on the ethical parallel of organ donation after brain death in adults. If the diagnosis of embryo death is as reliable as this study suggests, then a *prima facie* case can well be made for this idea as an ethical source of embryonic stem cells.

There are, however, two issues that remain. First, it is not yet proven that such cells can actually be used in place of blastomeres derived from viable embryos. Perhaps the very reason that the embryos are non-viable is an inability of the blastomeres to undergo mitosis. Or there may be a more subtle biological problem with such cells that would make them unsuitable for stem cell cultures. This is a pragmatic concern and a matter for additional research rather than an ethical objection.

More troubling for some ethicists is the parallel with brain death criteria in adult organ transplantation. The analogy may not be convincing when one recognizes that the most rudimentary neural structure, the neural plate, does not appear in a normal embryo until day 18 of development.[10] On the other hand, research scientist Maureen Condic has described "integrated organismal function" as the core meaning of brain death in adults, and as the basis of a parallel idea in embryos. In this, she was defending the ethical idea that embryos should be considered persons from conception, despite not yet having a functioning brain.[30]

Borrowing from Condic, then, it seems reasonable to conclude that non-viable embryos are those that have lost integrated organismal function, which is the equivalent of brain death in adults. Thus, such embryos may be regarded as truly "dead," therefore available for hES cell culture.

Altered Nuclear Transfer

Altered nuclear transfer (ANT) was first proposed in 2004 by William Hurlbut, a Stanford University physician and ethicist, and a member of the President's Council on Bioethics. He suggests using somatic cell nuclear transfer (SCNT - commonly called cloning). SCNT inserts a diploid somatic cell nucleus into an ooplast (enucleated oocyte), which then is stimulated to form a zygote capable of cell division and embryogenesis. The Hurlbut proposal would use gene modification techniques to create a non-implantable entity:

> Using the techniques of somatic cell 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.[31]

Specifically, Hurlbut suggests using RNA interference to block expression of cdx2, a gene responsible for formation of the trophectoderm, a precursor to the development of the placenta. Blocking this produces an embryo without the proper structural integrity to implant.[32,33] Hurlbut does not even refer to the resulting entities as embryos; he calls them 'biological artifacts.'[34] Such entities never have the moral status of human embryos, and therefore their destruction should be morally unobjectionable, Hurlbut claims. A 'proof of concept' study has already appeared in a scientific journal, using an animal model:

> Cloned [mouse] blastocysts [from donor fibroblasts] were morphologically abnormal, lacked functional trophoblast and failed to implant into the uterus. However, they efficiently generated pluripotent embryonic stem cells when explanted into culture.[35]

Though this idea may be possible in humans, some in the ethical debate are not convinced that it would be morally justifiable. The problem is that a cdx2-silenced embryo develops normally and appears indistinguishable from a normal embryo until the morula stage (at three days).[36] Richard Doerflinger of the National Council of Catholic Bishops has said, "A short-lived embryo is still an embryo." Even Robert Lanza, cited earlier as a proponent of the PGD idea, objects to the Hurlbut proposal as creating "crippled embryos to please the Church."[37] Those that hold to the conception view of personhood would never consent to creating and destroying such 'biological artifacts.'

Amore recent proposal would epigenetically alter the state of the transferred diploid nucleus in SCNT so that the resulting entity would never have the self-organizing capability of a zygote. This idea suggests using the overexpression of a transcription factor called Nanog, which has been shown in mouse embryos to maintain blastomeres in a pluripotent state. The procedure, called "Altered Nuclear Transfer-Oocyte Assisted Reprogramming (ANT-OAR)," would use SCNT methods to produce "embryonic" stem cells without preexisting embryos. In other words, by genetically manipulating both the ooplast and the inserted diploid DNA, these materials could be combined in such a way as to *directly* produce pluripotent cells. A major proponent of this idea is biologist and theologian Nicanor Austriaco.[38] Because of its ethical appeal, it has

received the cautious endorsement of a number of prominent pro-life scientists, theologians, and ethicists, including Hadley Arkes, Nigel Cameron, Maureen Condic, and Robert George.[39]

However, molecular biologist Malcolm Byrnes asserts, "[T]he scientific evidence is overwhelming that Nanog cannot single-handedly establish pluripotency in stem cells, and its ability to do so in the foreign milieu of a newly cloned embryo would be even less likely."[38] Byrnes' concern is that the ANT-OAR proposal is overly speculative and may even not be scientifically feasible. He adds, however, this positive statement: "These results nevertheless provide hope that one of the holy grails of stem cell biology – the reprogramming of adult cells directly into a pluripotent cell so that embryos are not used at all – may be achievable."[38] This statement, made in *Stem Cell Reviews* in January, 2007, may well have been prophetic, given the developments that occurred later the same year.

Using Human Skin Cells

Whether it will be the "holy grail" of stem cell biology remains to be seen, but a startling announcement was made online by two independent researchers on November 20, 2007. Junying Yu and colleagues from Madison, Wisconsin (senior researcher Thomson, reporting in the journal *Science*) and Kazutoshi Takahashi and associates from Kyoto, Japan (senior researcher Yamanaka, reporting in the journal *Cell*) simultaneously revealed a truly innovative approach to pluripotency, using a non-controversial source: human skin cells. To achieve this, both teams added four transcription factors to human skin fibroblasts, which created "induced pluripotent stem cells" (IPSCs). In both cases, the reprogrammed cells resembled hES cells in every measurable way, and demonstrated the ability to develop into all three primary germ layers. Ironically, the Yamanaka group accomplished this without even using the Nanog transcription factor, and the Thomson group showed that it was not absolutely essential.[40,41]

The intense international furor over this development would seem to imply that stem cell cures to human diseases have already been found, which of course is far from the case. The next research steps are critical. For example, it remains to be seen if these genetically-altered cells can safely be grown into the various human tissues required to treat Parkinson's, diabetes, spinal cord injuries, heart failure, and the huge list of other chronic diseases that might benefit from replacement cells. Control of cell division and the prevention of a variety of aggressive tumors remain vexing problems to be solved.

At first glance, this new technology seems to be the best outcome imaginable. Charles Krauthammer can perhaps be excused for some hyperbole when he writes:

> [It is] one of the great scientific breakthroughs since the discovery of DNA: an embryo-free way to produce genetically matched stem cells. Even a scientist who cares not a whit about the morality of embryo destruction will adopt this technique because it is so simple and powerful. The embryonic stem cell debate is over . . .
>
> [The] Holy Grail has now been achieved. Largely because of the genius of Thomson and Yamanaka. And also because of the astonishing good fortune that nature requires only four injected genes to turn an ordinary adult skin cell into a magical stem cell that can become bone or brain or heart or liver . . . Providence . . . saw to it that the technique be so elegant and beautiful that scientific reasons alone will now incline even the most willful researchers to leave the human embryo alone.[42]

Not all participants in the debate are happy at the news. One ethicist has said, "It's going to fuel those who call for preferential federal funding only for non-embryonic stem cell research and it will certainly complicate any efforts to expand funding for embryonic stem cell research at the federal level"[43] Even James Thomson, senior scientist of the Wisconsin stem cell group, joins Alan Learner, publisher of *Science*, to write:

> A new way to trick skin cells into acting like embryos changes both everything and nothing at all . . . Further delays in pursuing the clearly viable option of embryonic stem cells will result in an irretrievable loss of time, especially if the new approach fails to prove itself.[44]

Aside from funding and political considerations, the ethical controversy is far from over. If the technical problems associated with IPSCs cannot be overcome, then there will be a resurgence of interest in the PGD proposal, the non-viable embryo proposal, and the ANT or ANT-OAR ideas.

In addition, the new developments are not without ethical controversy. Buried in the experimental procedures reported by the two groups is the surprising detail that cell cultures used to extract the DNA to accomplish the reprogramming were from aborted fetuses:

> For example, while Dr Yamanaka reports using PLAT-E, PLAT-A and 293FT cells in his paper, the proper name for these cell lines is HEK (human embryonic kidney) 293. The cells were obtained from an electively aborted baby by Dr. Alex Van der Eb, of Crucell NV, who also produced aborted fetal cell line PER C6 from the retinal tissue of an 18-week gestation baby.[45]

The Thomson group also used 293 FT cells as well. Note that the concern here is moral complicity with elective abortion, though "after the fact." In other words, the ethical objection is not the ongoing destruction of nascent human life, but association with a morally tainted procedure that took place in the past. Such was the case with the rubella vaccine, a live (though attenuated) virus developed in tissue culture from aborted fetuses.[46] The vaccine is still in use today.

Though "after the fact" complicity concerns do not seem to rise to the same level as ongoing embryo destruction, these hidden research details will make many pro-life forces uncomfortable. As research scientist Theresa Deisher points out: "The genes and virus material used to transform the adult cells could have readily been produced by any number of other cell lines available for this purpose, such as HeLa, COS or CHO cells, none of which come from electively aborted fetuses."[47] If Deisher is right, then perhaps future developments on IPSCs could take place without utilizing questionable techniques that lead to divisive debates.

There is also the potential for abuse. Denker has pointed out that "embryonic stem cell lines can form an entire, normal organism when combined with helper cells, either blastomeres from cleavage stages or blastocysts, that have been made tetraploid." This procedure is called tetraploid complementation.[48] While it remains to be seen whether IPSCs are capable of such transformation, the lure of research cloning of human embryos will always be tempting to scientists.

Conclusion

Major attempts to produce pluripotent stem cells without the destruction of human embryos remain ethically controversial. The PGD proposal uses an established procedure from *in vitro* fertilization techniques to extract a single blastomere from a three-day old embryo, developing a stem cell line while allowing the possibility of embryo implantation later. This idea is ethically objectionable to many because it violates time-honored traditions of human experimentation and informed consent. Furthermore, it may not be scientifically possible or pragmatically feasible.

The non-viable embryo proposal suggests disaggregating embryos that have already stopped dividing in culture, on the assumption that such embryos are "dead" in some sense, borrowing from the concept of "brain death" in adult patients. Since there is not yet a nervous system in an early embryo, there may be little ethical warrant for such a comparison. Furthermore, the biological defect that caused such embryos to cease developing may at the same time make their component cells inappropriate for stem cell lines.

Altered Nuclear Transfer, as proposed by William Hurlbut, would use SCNT techniques to produce genetically modified 'biological artifacts,' incapable of implantation. Critics of ANT claim that such procedures would merely create 'crippled embryos,' and that their creation would violate sanctity of life concerns. An alternative idea, ANT-OAR, might obviate such concerns, but may not be scientifically possible.

Finally, the news that induced pluripotent stem cells can be made from adult human fibroblasts seems like a unifying idea that may allow the development of pluripotent stem cells without ethical compromise, though some potentially troubling questions remain. Maureen Condic, a stem cell research herself, recently wrote that IPSCs are superior for a number of key reasons. First of all, they can produce patient-specific (genetically-matched) stem cell lines. They are available right now, and can easily produce multiple stem cell lines for the study of possible treatments for a wide range of human disorders. She goes on to add:

> IPSCs are simpler to produce than stem cells from human embryos, and they are ethically uncompromised and therefore fully eligible for federal funding. These features make the cells attractive

to scientists who have avoided embryo-destructive research from technical, ethical, or financial concerns.[49]

The stem cell wars have been bitter and divisive. Despite some residual methodological concerns that involve fetal cell lines, IPSCs seem to "pass the test" ethically on most counts. This new development promises a future where all parties can work together without ethical compromise. Scientists can compete for federal grants based on the merits of their proposals, and stem cell research may the move forward to accomplish its ultimate goal: the actual cure of human disease.

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