Forum

Ethical and Legal Issues Arising in Research on Inducing Human Germ Cells from Pluripotent Stem Cells

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Abstract: Derivation of eggs or sperm from pluripotent stem cells or direct reprogramming from somatic cells would have huge effects on assisted reproductive technology. Here we discuss important ethical, legal, and social issues that would be raised by the development of such female or male gametes for clinical use.

Recent advances in stem cell biology have now made it conceivable that human eggs or sperm could potentially be derived from pluripotent stem cells or direct reprogramming from somatic cells. Most notably, Saitou and colleagues recently demonstrated the successful induction of sperm and oocytes from mouse embryonic stem cell (ESC)s and induced pluripotent stem cell (iPSC)s (Hayashi et al., 2011, 2012). Although there is room for improvement in the protocols, the work abolished several technical barriers in mouse cells with the demonstration that viable progeny could be derived from pluripotent stem cells. Similarly, over the last decade, much progress has been made in the differentiation of human germ cells from both human embryonic stem cells (hESCs) and hiPSCs. A recent report indicated that hiPSCs can be differentiated into primordial germ cells (PGC)s via use of bone morphogenetic proteins (Panula et al., 2011). In addition, hESCs and hiPSCs differentiated directly into male germ cell lineages, including postmeiotic, spermatid-like cells, without genetic manipulation (Easley 4th et al., 2012). Although production of human oocytes from female pluripotent stem cells has not yet been described, the data from the mouse experiments may help overcome some of the critical problems and lead to the rapid derivation of, human oocytes.

Five years have passed since an international consortium published a

consensus paper onvarious issues concerning stem cell-derived gametes (Hinxton Group, 2008). The time is right to reconsider the research response to the production of human germ cells *in vitro* from PSCs or somatic cells, as well as what steps might ensure the ability to move research to clinical applications. This research should improve our understanding of human developmental biology and should contribute to advancing knowledge of the pathology, diagnosis, and uses of assisted reproductive technology (ART). As we move forward, however, many ethical, legal, and social issues lie ahead.

One key issue is the need to assess the function of induced germ cells; this implies the need for research that tests human induced sperm and eggs to determine if they can successfully participate in fertilization and can produce normal embryos. For example, generation of sperm and eggs positive for specific male and female germ cell markers and negative for those specific to pluripotent stem cells should be ensured. Additionally, appropriate epigenetic programming, properly imprinted sperm and egg chromatin, and appropriate organization of the sperm and egg nucleus and mitochondrial structure should be evaluated. Thereafter, the creation of human embryos may, as a final biological assay, be exceptionally necessary in a preclinical stage to ensure safety of the induced cells. Currently, many countries allow derivation of hESCs from surplus *in vitro* fertilization (IVF) embryos, subject to some conditions, but many jurisdictions limit or ban production of human embryos for research purposes. This raises the question of where and under what circumstances the research necessary to explore the medical potential of human induced germ cells can be legally and ethically performed. Should such cells be brought to the clinic, a different set of controversial issues will appear. In this article, we scrutinize the questions concerning embryo research and point out some of the issues that eventual clinical use will raise.

Creating Human Embryos for Research

In fertility clinics, ART generally begins with ovulation via hormonal stimulation followed by oocyte retrieval for IVF. In cases of male infertility, the use of intracytoplasmic sperm injection (ICSI) is often required to produce viable embryos with one or a few sperm. Following fertilization, embryos are cultured, generally for 3 – 5 days and one or more is selected for transfer to the uterus (Niakan et al., 2012). Remaining pre-implantation embryos are then either stored under cryopreservation for future embryo transfer to a patient or discarded. Following successful pregnancies, cryopreserved embryos may subsequently be discarded, given to other prospective parents, or donated to research. Research that makes use of surplus embryos must

meet guidelines of institutional review boards (IRB) or equivalent bodies, especially in terms of prior informed consent of parental donors. Notably, the derivation of hESC lines is generally conducted using the existing and surplus embryos that were originally created for ART and are no longer required for reproductive purposes. For many people, and governments, the fact that the embryos were initially created for reproductive purposes is crucial to their ethical use for research once intended reproductive uses are no longer contemplated.

International Regulatory Landscape

Regarding national policy of human embryo creation for research purposes, we investigated 17 countries that permit hESC derivation from the surplus embryos (Supplemental Table 1). Fifteen of these countries permit creation of embryos for research purposes in at least some circumstances: Australia, Belgium, Canada, China, Denmark, India, Israel, Japan, Singapore, South Africa, South Korea, Spain, Sweden, UK, and US (in some but not all states). All of these countries, by statutes or by guidelines, limit the culture period of the created embryo to either the 14th day of embryo development or the formation of primitive streak, which begins roughly at that time. This restriction has been justified on the ground that the formation of the primitive streak signifies the start of a unique, human being (President's Council on Bioethics, 2002). In some of the permissive countries, researchers are required to provide specific justification for why they need to create embryos for research. In Australia, Belgium, Canada, India, Israel, Japan, Singapore, South Africa, South Korea, Spain and the UK, the laws or guidelines require a public license or authorization to create human embryos for research. In addition to the need to account for the creation of embryos for research purposes, some explanation for the decision not to use surplus IVF embryos is also required in review process.

In countries that allow creation of embryos for research, therapeutic cloning using somatic cell nuclear transfer is frequently indicated as a permitted purpose. Others include hESC derivation, parthenogenesis, and special embryos including "hybrid embryos" in Australia and "cytoplasmic hybrids" in Singapore and UK. Belgium, Canada, Denmark, Japan and UK permit research that creates human embryos for improving or providing instruction in ART. The Australian act indicated the possibility of creating human embryos using "precursor cells" from human embryos. Notably, the "precursor cells" might be regarded as the germ cells induced from hESCs but not from hiPSCs. Japan has guidelines for inducing germ cells from human iPSCs, ESCs and tissue-specific stem cells, but those guidelines currently prohibit fertilization

using the induced germ cells. Thus, it seems clear that researchers in Australia, Belgium, Canada, Denmark and the UK, might, after prior consultation or permission from a regulatory authority, be allowed to create human embryos and develop them in culture for about 14 days in order to investigate developmental potential of the induced germ cells.

In the US, current federal laws and NIH guidelines only prohibit federal funding of research that results in destruction or risk of damage to human embryos. Research that does not receive federal funding is not subject to that restriction. In contrast, several states enacted statutes that directly impact human embryo research. These statutes vary widely (National Conference of State Legislatures, 2008). The statutes in California, Connecticut, Illinois, Iowa, Maryland, Massachusetts, New Jersey, and New York generally encourage embryonic stem cell research, but with varying restrictions (Supplemental Table 1). On the other hand, some states, such as Michigan and Louisiana, discourage or ban human embryo research including hESC research. Yet, except in some states with restrictive policies, US researchers with non-federal funding may be allowed to create human embryos using induced germ cells for research, though perhaps after approval by both a local stem cell research oversight committee as well as an IRB.

The Regulatory Process in Japan

To our knowledge, Japan is the only jurisdiction with a special guideline on human germ cell induction from stem cells. In Japan, hESC guidelines were established by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in 2001. The guidelines banned germ cell induction research after considering ethical issues raised by creation of an individual with the induced germ cells. In 2004, the Council for Science and Technology Policy adopted a basic policy, holding that in order to preserve human dignity, human embryo creation for research purposes is in principle not permitted. But, the policy contained two exceptions: research for improving or providing instruction in ART, and hESC derivation from surplus embryos. For the purpose of ART, only gametes donated by infertility or gynecologic patients may currently be used.

On November 30, 2007, Yamanaka and colleagues reported the generation of hiPSCs (Takahashi et al., 2007). A week later, on December 7, 2007, Yamanaka suggested that research on human germ cell induction should be temporarily restricted until MEXT established proper guidelines. The Ministry issued a notification informing research institutions of the temporary prohibition on germ cell induction

from hiPSCs on February 21, 2008, just 3 months after the derivation of hiPSCs was reported. After this temporary prohibition, the MEXT Bioethics and Biosafety Committee continued to investigate the requirements that would permit the research. Following a year of discussion, the committee concluded that the ban on germ cell induction could be conditionally lifted considering major scientific and medical benefits, such as elucidation of gametogenesis, the pathologies of infertility, and congenital diseases. However, the ban on creating human embryos using germ cells induced from iPSCs continued, both because of the 2004 national basic policy banning human embryo creation for research purposes and the perception of technical barriers for obtaining mature and fertile germ cells from iPSCs or tissue stem cells at that time.

In 2010, the Ministry formulated "Guidelines on Research into Producing Germ Cells from Human Induced Pluripotent Stem Cells or Human Tissue Stem Cells". At about the same time, the Ministry revised the hESC utilization guidelines to allow germ cell induction from hESCs. The Japanese authorities thus acted quickly to respond to ethical concerns triggered by a scientific breakthrough, imposing a temporary ban and then lifting it, in part, within two years due to constructive discussions by researchers, policy makers, and the committee.

The Path Forward

The use of germ cells derived from pluripotent hESCs or iPSCs to create human embryos for research purposes is legal in some countries and not in others. Except for Japan, no country seems to have considered this issue directly. If human germ cells induced from pluripotent stem cells are to have an opportunity to be used in clinical applications, the creation of human embryos will be essential as a final functional assay. However, this functional assay should be limited in use as an exceptional assay to evaluate the developmental potential of induced germ cells in a preclinical stage. Countries will have to consider, or reconsider, their positions on creation of human embryos for research in light of this possible application. In doing so, some countries may want to consider whether, in terms of moral status, embryos created for research purposes from pluripotent stem cell-derived germ cells are similar to, or are different from other embryos.

The main difference that we see of possible ethical significance is that embryos created from induced germ cells from hiPSCs would not require the process of gamete donation, which, for women, is unpleasant and risky. This difference, however, seems to be minor compared to the overall similarities given that all embryos have the potential to become human life. As a result, all the embryos created either for research

or for reproductive purposes deserve equivalent moral respect.

The Social Effects of Successful Research on Human Induced Germ Cells

When we are able to generate germ cells from human pluripotent stem cells or somatic cells, people may benefit, both from making ART possible in cases where few or no eggs or sperm are produced and from improved understanding of and treatment for reproductive failure. Autologous production of germ cells from pluripotent stem cells might provide hope for patients who were never fertile or who lost their fertility from chemotherapy, radiation therapy, or other events but who wish to have a child that carries half of their genome. The creation of mature metaphase II oocytes from the patient's own iPSCs might also provide a pool of oocytes for use in age-related oocyte senescence or to support a type of germline gene therapy that replaces mitochondrial DNA in human oocytes by spindle transfer for inherited mitochondrial diseases (reviewed in Trounson and Dewitt, 2013). Induced germ cells could also be useful in basic research and contribute further to unveiling pathways of human pre-implantation development. This could lead to findings that improve IVF success rates by providing new culture techniques and improved cryopreservation methods.

Conversely, adoption of this kind of technology without rigorous study could have adverse consequences. The most worrisome possibility is that children conceived from germ cells differentiated from pluripotent stem cells might suffer serious health impairments, at birth, later in childhood, or even in adulthood. Thalidomide and Diethylstilbestrol were powerful warnings about the possible dangers of insufficiently tested interventions early in embryonic development.

In addition to issues of physical safety, the possible uses of human germ cells differentiated from pluripotent stem cells raise the possibility of perplexing new ethical, legal, and social issues. For example, posthumous conception using germ cells that are infinitely generated from iPSCs or somatic cells might produce unprecedented social concerns, as well as novel pedigree diagrams. Or if germ cells were frequently induced from a particular iPSC line, many siblings might be born in a region without knowing about their genetic relationships, potentially expanding an issue that may already be a problem with sperm donation. The technology could also be used to produce many embryos, allowing prospective parents to select their "best" embryo from scores of options, where their idea of "best" might be driven by many different ideas of the "best" genetic traits for a child – or even to create a "savior sibling" primarily to provide transplantation therapy for a relative. The clinical uses of induced germ cells would greatly expand the current issues that ART already confronts. Broad and open

discussions with the general public as well as medical, scientific, and ethical experts will be essential.

Furthermore, a different set of controversies might be raised if one could induce oocytes from XY (chromosomally male) cells or inducing sperm from XX (chromosomally female) cells (Hinxton Group, 2008), however remote this possibility may be. Researchers will have to justify such a research to the public in terms of both scientific plausibility and medical benefits.

Conclusion: Reconsidering Regulations

Research on differentiation of germ cells from human iPSCs raises many ethical, legal and social implications. Herein, we reconsider the regulations. A retrospective look at ART reveals a complicated history. Some technologies that were once considered controversial, such as intrauterine insemination and in vitro fertilization, are now considered and accepted as mainstream, with an estimated 5 million births aided by IVF to date (Lomax and Trounson 2013). Human induced germ cells could aid even more people.

However, if we are to fully harness the potential of human induced germ cells generated from hiPSCs for medical applications, research that involves the in vitro creation of human embryos and subsequent culture for a short period is likely to be necessary. *In vitro* human embryo culture until the 14th day is currently viewed as the ethically permitted maximum period to assess developmental potential of the induced germ cells. If this time window was insufficient to provide scientific grounding for clinical uses of the induced germ cells additional measures might be necessary to evaluate details of differentiation potential and whether imprinted genes are expressed exclusively from either the paternal or the maternal alleles.

The countries, including Japan, that are currently restrictive to human embryo research would benefit from increasing their flexibility when formulating their cautious regulations regarding embryo creation using induced germ cells if those countries would like to fulfill the potential medical value of the induced germ cells. Those countries will need to consider research justifications and decide whether they should permit the research or not. In contrast, countries that currently permit embryo research, including the US, would benefit from added clarity and caution to their more flexible regulations in light of the moral respect owed to human embryos. Researchers will have to act openly and justify the research to the public based on both scientific rationality and medical benefit. All countries where such research is undertaken will need continuous discussion of the requirements for ethical research and proper clinical

applications of induced germ cells.

Supplemental information

Some of the national and state policies regarding creation of human embryo for research purposes are shown in supplemental table 1 at http://dx.doi.org/10.1016/j.stem.2013.07.005.

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Web Resources

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