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of haploid, round spermatid(-like) cells from iPSCs and/or ESCs^{3–7}.

Three major approaches have been used to induce fertile germ cells from somatic cells using stem cell technology (Fig. 1). The first makes use of the current primary methodology of stepwise differentiation of human iPSCs (generated from an individual's somatic cells) to germ cells *in vitro* (Fig. 1a). To date, directed differentiation of human iPSCs under the existence of bone morphogenetic proteins, retinoic acid and subsequent treatment (Forskolin, human leukemia inhibitory factor, basic fibroblast growth factor and R15866), or mouse spermatogonial stem cell (SSC) culture conditions has resulted in generation of primordial germ cells (PGCs)^{4,8}, SSCs⁹, spermatocytes^{5,9} and haploid, round spermatid(-like) cells expressing ACROSIN^{5,9}, despite a low induction efficiency. However, fertilizing oocytes with such immature male germ cells would result in poor embryonic development¹⁰. Moreover, although a combined differentiation protocol using both *in vitro* and *in vivo* systems induced fertile oocytes from mouse iPSCs², *in vitro* generation of human female germ cells has not been manifestly demonstrated.

With the advent of genome-editing technology, we can expect research of human germ-cell induction to advance rapidly. For example, genome editing has been used to induce PGCs from human pluripotent stem cells *in vitro*^{11,12}. Using gene editing in germ-line-competent human ESC lines, SOX17 has been demonstrated to be a key regulator of PGC-like cell fate¹¹. In addition, PGC-like cells can be differentiated from genetically edited human iPSCs, which display primed pluripotency, not naive potency, via incipient mesoderm-like cells¹². Of particular note is the recent finding that human iPSCs transplanted directly into mouse seminiferous tubules not only differentiate extensively to germ-cell-like cells with morphology indistinguishable from that of fetal germ cells, but also express PGC-specific proteins¹³. This xenotransplantation experiment suggests that culture conditions mimicking the stem cell niche in the fetal testis or ovary can effectively direct human iPSCs to germ cell differentiation *in vitro*. Park *et al.*⁸ have also improved PGC generation using a co-culture system with human fetal gonadal cells. Moreover, Bucay *et al.*¹⁴ have reported that PGC generation from human ESCs was accompanied by the development of Sertoli-like support cells, suggesting the potential utility of support

Creating human germ cells for unmet reproductive needs

To the Editor:

Current assisted reproductive technology (ART), such as *in vitro* fertilization and intracytoplasmic sperm injection, depends on the premise that both partners produce fertile eggs or sperm (gametes). As yet, there is no ART treatment for individuals with few or no gametes, unless donor gametes are used. Ongoing research suggests that autologous female or male germ cells may be regenerated from somatic cells by controlling cell fate; if so, there is a possibility that ART might aid infertile couples and even same-sex couples in the future. Before such ART treatments using induced germ cells can be considered in the clinical context, however, a great many questions concerning the safety and efficacy of such procedures must be answered. Moreover, profound ethical and social concerns will arise if such

induced germ cells are needlessly generated, cryopreserved and used for reproductive purposes in clinics without a definition of their appropriate roles in ART. As a response to the increasing feasibility of inducing germ cells from human pluripotent stem cells, we discuss below the current technical challenges to creating induced human germ cells and explore some of the ethical, legal and social issues associated with their use in clinical practice.

Recent work has shown that mouse oocytes as well as spermatozoa can be differentiated from induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) via primordial germ-cell-like cells (PGCLCs), resulting in the birth of healthy offspring^{1,2}. Furthermore, rapid advances have also been made on human germ-cell induction research, including the generation

cells in germ-cell induction¹⁴. These findings indicate that the creation of increasingly elaborate co-culture systems, including gonadal tissue cells, has the potential to enhance the efficiency of human germ cell induction *in vitro*.

Although there is compelling evidence for spermatogonial stem cells, controversy remains about the existence of oogonial stem cells^{15,16}. If oogonial stem cells can be isolated in a reproducible manner and are sufficiently characterized, the possibility of carrying out genome editing for research use may also lead to insights that ultimately could enhance female germ-cell induction *in vitro* for clinical applications.

A second approach for generation of germ cells (Fig. 1b) involves the overexpression of key genes associated with the development of germ cells. Overexpression of genes, including *DAZL*, *DAZ*, *BOULE* and *VASA* has facilitated entrance into meiosis, resulting in the generation of haploid, round spermatid(-like) cells from human iPSCs and/or ESCs^{3,4,6}. Moreover, a recent mouse experiment demonstrated that simultaneous overexpression of three transcription factors, *Blimp1*, *Prdm14* and *Tfp2c*, efficiently differentiate epiblast-like cells to PGCs, without cytokines¹⁷. These findings suggest the overexpression of genes potentially induces more advanced germ cells or bona fide spermatozoa from human iPSCs if an

optimized combination of key factors is identified.

A final approach for germ-cell induction involves the use of somatic cell nuclear transfer to human ESCs (Fig. 1c)¹⁸. A major challenge associated with germ cells induced from cloned ESCs is the high likelihood of heteroplasmy due to carried-over mitochondria in the karyoplast. Such heteroplasmy may unbalance evolutionarily-optimized interactions between nuclear DNA and mitochondrial DNA¹⁸ with potentially deleterious biological and pathological consequences for the offspring's health. But as in the ART of mitochondrial donation—where the lack of abnormalities observed in adult nonhuman primates born after mitochondrial donation was cited, in part, as evidence for the technique's safety in the scientific review reported to the UK's Human Fertilisation and Embryology Authority last year²⁰—experiments to study the offspring of larger animals could similarly be carried out to establish the safety of induced germ cells. But even if these concerns can be addressed, this third approach appears the least feasible of the induced germ-cell approaches.

Whatever method is used, germ-cell induction involves reversal of the Weismann barrier, wherein hereditary information moves only from germ

cells to somatic cells²¹. The implications of reversing this process not only for ensuring the developmental potential of induced germ cells but also for assuring the health of resulting offspring over an entire life time should be carefully considered. Notably, some studies on the genetic integrity of human iPSCs suggest a tendency to develop changes in nuclear DNA and/or mitochondrial DNA in addition to chromosomal aberrations during reprogramming and/or subsequent culture²². Moreover, although human iPSCs derived from dermal fibroblasts have already been used in a clinical trial for retinal regeneration²³, the somatic cell type of origin must be contemplated in terms of its clinical availability and cumulative burden of *de novo* mutations²⁴.

With this in mind, it will be necessary to take precautions against the genetic instability of iPSCs used to induce germ cells, regardless of the establishment method used. Clearly, although intergenerational monitoring of mouse offspring derived from induced germ cells will provide valuable insight into the safety of this technology over an offspring's lifetime, additional studies using other animal species, particularly nonhuman primates, will be needed before clinical application is contemplated.

Quite apart from the technological hurdles that face the application of human germ-cell technology to clinical practice, these procedures raise several ethical concerns²⁵. First, human fetal ovarian or testicular tissue will likely be required for effectively inducing oocytes or spermatozoa *in vitro*⁸. When the fetal tissues are derived from elective abortions, some might feel that the germ-cell generation comes at the expense of other human lives. However, fetal tissues can be ethically procured if informed consent is obtained from patients who undergo the surgery for a clear medical reason (for example, ectopic pregnancy). In such cases, the disclosure of information regarding the materials and methods is important.

Compared with *in vitro* fertilization using donor gametes, one advantage of *in vitro* gametogenesis procedures is that genetic parentage of the child is the same as in normal reproduction.

In addition to these concerns, ART using germ cells derived from cloned human ESCs poses two other serious ethical challenges (Fig. 1c). First and foremost is the concern that the creation of human ESCs would involve the destruction of human embryos. In addition, the establishment of a cloned ESC would require oocyte retrieval with

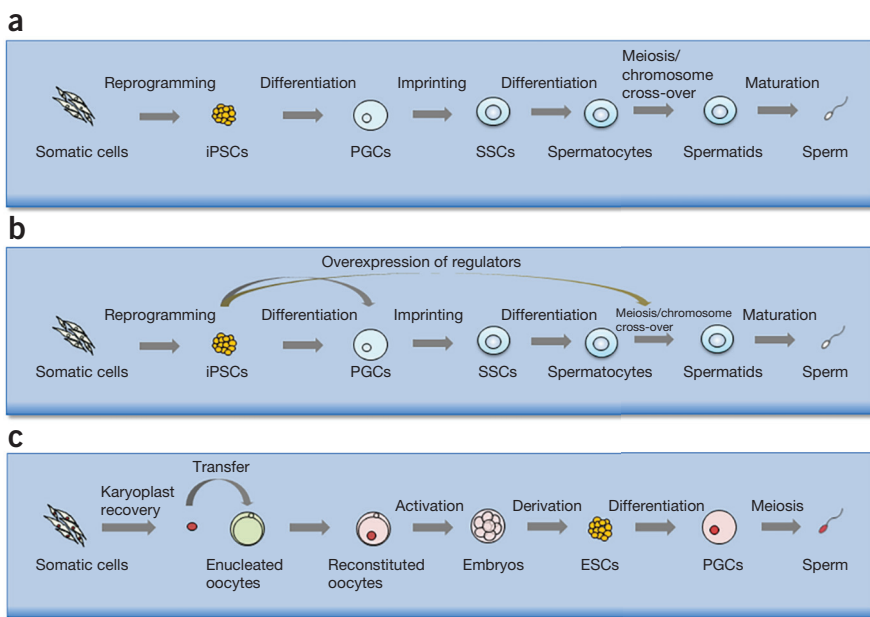


Figure 1 Three potential approaches for inducing fertile germ cells by stem cell technology. (a) Differentiation of human iPSCs to germ cells; (b) overexpression of genes associated with germ cell development; and (c) somatic cell nuclear transfer to oocytes, derivation of human ESCs and differentiation to germ cells. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; PGCs, primordial germ cells; SSCs, spermatogonial stem cells.

Box 1 Induced human germ cells in the clinic

One use case for induced human germ cells would involve a same-sex couple. Self-use of induced oocytes derived from XY cells or induced spermatozoa from XX cells by a same-sex couple is conceivable³¹; however, it remains only a remote scientific possibility²⁵. Apart from the technical challenges, one ethical issue for such an application is that children resulting from such a procedure might suffer distress and stigma associated with being born as a result of an unnatural biological process.

A second use case of the clinical availability of induced oocytes would be couples looking to create a 'savior sibling' who could provide a compatible transplant for an existing sick child. Such a savior sibling would be identified by human leukocyte antigen typing via preimplantation genetic diagnosis; such an approach is sometimes attempted with traditional ART but is relatively inefficient³². That said, human oocyte induction currently remains a work in progress as discussed above, and creating a savior sibling may be ethically controversial because it implies a moral decision by parents to favor the welfare of an existing original sick child over that of the new savior offspring³³.

A third use case is the adoption of induced oocytes to assist females who have experienced recurrent ART failures due to aneuploidies associated with age-related oocyte senescence, but do not wish to use donor gametes. Although preimplantation

genetic diagnosis to test for aneuploidy is already in practice to improve the ART success rate, its clinical effectiveness is currently unclear³⁴. However, as mentioned above, human oocyte induction requires further research before such procedures could be contemplated. What is more, induced oocytes would also be unlikely to benefit all females of advanced age. As women get older, implantation and ability to carry a viable pregnancy may be compromised³⁵.

A final application involves infertile survivors of malignancies. Recent studies have reported that 46% of male survivors and 16% of female survivors of childhood cancer manifest infertility³⁶. One of the major reasons is germ-cell loss due to chemotherapy and/or radiation therapy³⁷. Although for male cancer survivors sperm cryopreservation is an effective method to safeguard spermatozoa, not all male patients who are offered this method bank sperm because of stress, severe illness or sexual inexperience³⁷; for such individuals, induced spermatozoa may provide a means of ART. For prepubertal boys, cryopreservation and subsequent transplantation of testicular tissue could restore the survivor's fertility in the future³⁷. But ART using induced spermatozoa may be a potential reproductive option for them after understanding other options (uses of donor sperm and adoption) in addition to the risks associated with *in vitro* gametogenesis.

medication and hormone injections that might impose distress or potentially could lead to ovarian hyperstimulation syndrome²⁶ in healthy oocyte donors. Clearly, approaches using iPSCs obtained under informed consent from an adult as a starting point (Fig. 1a,b) pose fewer ethical concerns than somatic cell nuclear transfer to human ES cells (Fig. 1c).

Then, there is the question of medical need. The potential benefits of induced germ cells are likely found in self-use by a couple because it is their germ cells that would be regenerated to have a genetically related child. To illustrate some of the scenarios^{25–31}, we discuss four relevant cases in **Box 1**.

The first reproductive use of induced spermatozoa would occur in a clinical trial. In ART treatments, informed consent is provided by prospective parent(s). For informed consent, the potential risks associated with ART using induced germ cells include embryonic arrest, implantation failure, miscarriage and childbirth with congenital anomalies. Parental consent for the reproductive use of such germ cells is justifiable if the safety can be considered to be equivalent to that of conventional ART treatments according to the results of preclinical research²⁷.

Beyond the above technical, ethical and social issues, the clinical use of induced germ cells must take place in an appropriate legal environment. According to the European Society of Human Reproduction and

Embryology, the most ART-active countries in the world are Japan and the United States²⁸. Twenty-three European countries have legislation on ART²⁹, whereas the United States and Japan have few federal or national laws or guidelines directly pertaining to ART³⁰. In general, ART is, without strict regulations, conducted as a type of advanced medicine in the United States and in Japan. The US Food and Drug Administration (FDA) regulates gamete donation with a view of preventing the transmission of infectious diseases, and this is a minimum requirement for all ART procedures. But given that the reproductive use of induced germ cells appears to be legal both in the United States and in Japan, the elaboration of clear regulations that guide socially acceptable uses of induced germ cells should be a priority. It is a paradox that although oversight of clinical practice remains lax in the United States, the Dickey-Wicker amendment (H.R. 2880, Sec. 128) prohibits the use of appropriated funds for the creation of human embryos for research purposes or for research in which human embryos are destroyed.

It is important to point out that it will be necessary for ART practitioners using induced germ cells to provide evidence that no genetic alternation has occurred during the process because in many countries, genetic modification of the germ line is banned; indeed, a recent survey of regulations suggests at least 29 countries

(including Japan but not the United States) ban germ-line gene modification for reproductive purposes³⁰. In the United States, the FDA regulates germ-line gene modification for safety reasons³⁰, and the US National Institutes of Health (NIH) Recombinant DNA Advisory Committee states in its Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, that it will not, at present, entertain proposals for germ-line gene alterations³⁰. Similarly, Japan bans clinical research that intentionally conducts, or may result in, the genetic modification of human germ cells or embryos under the Guidelines of Clinical Research Regarding Gene Therapy³⁰.

Overall, autologous induced germ cells from autologous iPSCs could benefit many couples who at present do not benefit from ART. Although a caring, nurturing and loving environment is important for family building, in reality most parents would prefer to have their own genetically related child rather than one created from an unrelated gamete donor²⁷. Given that a mere single perfectly induced spermatozoon would be sufficient for starting intracytoplasmic sperm injection if a fertile oocyte were available, we believe that a likely first clinical trial using induced germ cells would evaluate induced spermatozoa in ART for male cancer survivors. Nevertheless, before such a trial can take place, many ethical, legal and social issues remain to be addressed.

We contend that now is the time to start discussions about how induced germ cells will be adopted into clinical ART practice.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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German Federal Office of Consumer Protection and Food Safety (BVL; Berlin) to assess the herbicide-resistant canola variety under the German regulation for GMOs (GenTG), with a view to carrying out a field test. The following January, on the basis of recommendations from the German Central Committee of Biological Safety (ZKBS; Berlin)⁴, BVL decided that this canola variety should not be considered as a GMO, according to the German GenTG⁵. BVL's statement is in line with other decisions on products of new breeding technologies provided by regulatory authorities in Finland, Sweden and the United Kingdom (UK)⁶.

In the case of the German ruling, however, there was another development. Although the national Finnish, Swedish and UK assessments went unremarked by nongovernmental organizations (NGOs), BVL's decision prompted an outcry from Greenpeace Germany (Hamburg, Germany), Testbiotech (Munich, Germany) and others, with these organizations publishing a counterstatement. The intention of these NGOs was to delay any final decision assigning Cibus' canola as a non-GMO, as this would set a precedent for other products of modern breeding technologies. Last June, the BVL rejected the NGOs' objection against its decision⁷. In response, three parties, including the German branch of Friends of the Earth (Berlin), went to the Administrative Court in Braunschweig, Germany, to protest the decision. As a result, the BVL decision is in limbo until the legality of indict has been proven⁸.

At the end of June 2015, Testbiotech announced that the European Commission (EC; Brussels) has expressed its own different opinion on this matter and “prohibited the field release” of the Cibus RTDS canola. Counter to this claim, the BVL indicated that the European authority had merely sent ‘a request’ to the EU Member States either to wait with a decision about concrete plans of field-testing plants produced by novel breeding technologies or to handle already given decisions carefully (for example, by specific confinement or monitoring activities) until the EC has delivered an opinion on the legal status of these plants.

Of course, there are many other novel breeding technologies in addition to Cibus' RTDS technology being applied to generate new plant products. Besides ODM, these include gene editing with DNA endonucleases, agroinfiltration,

Cibus' herbicide-resistant canola in European limbo

To the Editor:

A single herbicide-resistant canola variety developed by the company Cibus¹ (San Diego) continues to languish, caught while European regulators deliberate whether to torture their creaking genetically modified organism (GMO) rules to encompass new breeding technologies. Given the low popularity of genetically modified (GM) food in the European Union (EU; Brussels), the question is whether Cibus' canola and other products of new breeding technologies will be subject to scientific regulation or sacrificed on the altar of European political expediency as was the case with their older transgenic counterparts.

Cibus is a US biotech company with a focus of developing alternative ways of genetically improving crops². Its

herbicide-resistant canola variety was not modified by classic genetic engineering via *Agrobacterium tumefaciens* or particle gun, but through the company's rapid trait development system (RTDS), developed and patented a few years ago³. RTDS exploits oligonucleotide-directed mutagenesis (ODM; Fig. 1), whereby chemically synthesized oligos are introduced into plant cells, where they are then used as repair templates to generate mismatches in DNA of the target region, which are then corrected by the cell's DNA-repair machinery³. Because during the repair process the introduced oligo DNA is broken down, the herbicide-resistant Cibus canola variety is genetically edited but not genetically modified (transgenic).

In July 2014, Cibus approached the