

Stem cell research – new challenges for the ban on cloning and treatment of artificially created germ cells?

AD HOC RECOMMENDATION

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Introduction

New developments in research on human embryonic stem cells produced via cell nuclear transfer and induced pluripotent stem cells are enabling the artificial creation of germ cells and embryos. Controversy has ensued over the extent to which these entities are covered by the relevant legislation in Germany. On a purely technical level, these new developments have increased the likelihood that human beings will be cloned for reproductive purposes in the future.

In view of this situation, the Conference of Health Ministers (*Gesundheitsministerkonferenz*) asked the German Ethics Council to evaluate current developments in stem cell research. The central concern is that the cloning of humans for reproductive purposes via these new methods might not be subject to a clear ban by German legislation, particularly the Embryo Protection Act (*Embryonenschutzgesetz*) and the Stem Cell Act (*Stammzellgesetz*). On 8 May 2014, the German Ethics Council held a public hearing¹ to consider the scientific, legal, and ethical questions that have arisen in this context and subsequently developed the present recommendation.

Current situation

There are currently two methods in the field of stem cell research that would make it possible to develop patient-specific pluripotent stem cell lines capable of differentiation. Such cell lines could be used to harvest a variety of different cell types compatible with a patient's immune system for therapeutic purposes. The first method is the creation of embryonic stem cells via somatic cell nuclear transfer (SCNT), and the second is the generation of induced pluripotent stem cells (iPS cells).

In somatic cell nuclear transfer, the nucleus of a somatic cell is removed and transferred into an egg cell that has had its own nuclear DNA removed. Under the proper conditions, the egg cell can develop into an embryo with cells that are genetically identical to the original donor. Such an embryo is, effectively, a clone of the donor. It may even continue to develop up to the point of birth, as was proven in the case of Dolly the sheep, when the technique was first successfully used on a mammal. Miscarriages, defects, and illnesses quite frequently emerge during this process, however.

Alternatively, scientists can isolate the inner cell mass of such a clone embryo at the blastocyst stage – approximately five days into its development and continue to grow the cells in culture as pluripotent embryonic stem cells. Their ability to differentiate and the fact that they are genetically identical to the original donor theoretically makes it possible to generate a variety of cell types from such a stem cell line to treat the donor with little risk of immune rejection. The creation of pluripotent stem cells for research purposes with therapeutic aims is often referred to as “therapeutic cloning”. The clone embryo is destroyed during the removal of the stem cells.

In 2013, scientists succeeded for the first time in growing an SCNT human embryo to the point that its embryoblast cells could be cultivated as embryonic stem cells.² At least two other groups of researchers have since been able to repeat the experiment using different donor cells.³ Given these results, the probability is growing that the cloning of humans for reproductive purposes will become at least technically possible.

A second method of producing the body's own pluripotent stem cells consists of using certain biomolecules to reprogram adult cells to an embryonic stem cell-like state. Induced pluripotent stem cells were first successfully cultivated in 2006.⁴ A variety of different cell types have since been developed from them.

1 The complete documentation of the hearing can be found under <http://www.ethikrat.org/veranstaltungen/anhoerungen/forschung-an-ips-zellen-und-an-hes-zellen> [2014-09-01].

2 Cf. Tachibana, M. et al. (2013): Human embryonic stem cells derived by somatic cell nuclear transfer. In: *Cell*, 153 (6), 1228–1238.

3 Cf. Chung, Y. G. et al. (2014): Human somatic cell nuclear transfer using adult cells. In: *Cell Stem Cell*, 14 (6), 777–780; Noggle, S. et al. (2011): Human oocytes reprogram somatic cells to a pluripotent state. In: *Nature*, 478 (7367), 70–75.

4 Cf. Takahashi, K.; Yamanaka, S. (2006): Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. In: *Cell*, 126 (4), 663–676.

Questions about reproductive cloning also arise in connection with iPS cells. In 2009, researchers were able to cultivate a viable mouse embryo from iPS cells using the tetraploid complementation assay.⁵ With this method, iPS cells are injected into a tetraploid embryo in which every chromosome exists fourfold. The tetraploid embryo is created by fusing the blastomeres of an embryo in the two-cell stage. The iPS cells can, in combination with the tetraploid embryo, develop into an embryoblast with the potential to further develop into a viable individual which would be a clone of the iPS donor. The cells of the tetraploid embryo form only extra-embryonic tissue such as the placenta. In principle, applying this technology to reproductive cloning using human iPS cells is conceivable.

The question of whether iPS cells may – at least in a *cell cluster* – be deemed totipotent since they can, in effect, be used to produce all cell types, remains a matter of some controversy. For one thing, they have only realised this potential thus far in combination with tetraploid embryos. Additionally, experts doubt whether the cell plasma of iPS cells (unlike the totipotent cells of an early embryo) has the factors necessary for embryonic development, as these are contributed by the egg cell during fertilization.

Scientists have already been successful in developing germ line cells from iPS cells and cultivating them into fully functional germ cells following implantation in the gonads of animals. In animal experiments, sperm⁶ and egg cells⁷ artificially produced from such iPS cells have, through fertilization, lead to the creation of viable mice. It cannot be ruled out that, in future, this technology could be applied to human reproduction in constellations where procreation by natural means is impossible. Same-sex couples could, for example, produce children that are genetically related to both parents. The method could even be used to combine artificially created male and female germ cells generated from the same individual, resulting in an embryo. This raises an issue that is at least related to concerns regarding cloning: although the embryo is not a clone, it has only one genetic parent.

As is the case with cell nuclear transfer, reproductive technologies involving iPS cells lead significantly more frequently to miscarriages and diseases. Among other factors, this could also be due to the fact that changes in DNA caused by mutations will amass over the course of a lifetime and continue to be present in a reprogrammed cell. The reprogramming process also does not always unfold in a predictable manner.

In summary, the current results of stem cell research are moving the cloning of humans for reproductive purposes closer to the realm of the technically possible. Moreover, the use of reprogrammed somatic cells and artificially produced embryos, also makes it technically possible to cross the boundary between somatic cells and germ line cells, out of which a new life can develop.

5 Cf. Zhao, X.-Y. et al. (2009): iPS cells produce viable mice through tetraploid complementation. In: *Nature*, 461 (7260), 86–90; Kang, L. et al. (2009): iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. In: *Cell Stem Cell*, 5 (2), 135–138.

6 Cf. Hayashi, K. et al. (2011): Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. In: *Cell*, 146 (4), 519–532.

7 Cf. Hayashi, K. et al. (2012): Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. In: *Science*, 338 (6109), 971–975.

Legal Questions

In Germany, the legislative acts most relevant to stem cell research are the Stem Cell Act and the Embryo Protection Act.

Stem Cell Act

The Stem Cell Act regulates the importation and use of (human) embryonic stem cells already in the country. According to Section 3 of the Stem Cell Act

- » stem cells are all human cells that have the ability to multiply through cell division in the appropriate environment, and which by themselves or through their daughter cells are capable, under favourable conditions, of developing into specialized cells, without, however, the potential to develop into an individual (pluripotent stem cells),
- » embryonic stem cells are all the pluripotent stem cells derived from embryos created outside of the body and not intended to result in a pregnancy or extracted from a woman's body prior to implantation in the womb,
- » embryo means already any human totipotent cell which, exposed to the necessary conditions, has the potential to divide and develop into an individual.

The conclusion is that the law does not cover iPS cells as such. They are pluripotent, but in order to be subject to the Stem Cell Act, they not only have to be derived from an embryo, they have to have already been pluripotent at the time that they were derived from an embryo. The latter is not the case, since they only become pluripotent through the reprogramming process. In other words, in their form as pluripotent cells, they are not directly extracted from a totipotent entity (= embryo).

However, unlike the case with iPS cells, as soon as pluripotent stem cells are extracted from a human totipotent entity, the regulations of the Stem Cell Act apply to their importation and their use within Germany regardless of what method was used to generate the totipotent entity. According to the Stem Cell Act, an embryo created through cell nuclear transfer or tetraploid complementation is indisputably a totipotent entity. The totipotent cell that is deemed an embryo need not itself have been extracted from an embryo. The Stem Cell Act's definition of an embryo is thus much broader than that of the Embryo Protection Act.

Embryo Protection Act

a) Entities covered by the law, with particular respect to the ban on cloning

Section 8 (1) of the Embryo Protection Act contains a definition of an embryo. It states that "for the purpose of this Act, an embryo already means the human egg cell, fertilised and capable of developing, from the time of fusion of the nuclei, and further, each totipotent cell removed from an embryo that is assumed to be able to divide and to develop into an individual under the appropriate conditions". The entity that exists from the moment of the first cell division following fertilization of the egg cell is not specifically mentioned here. However, the cell cluster (organism) that results from the fertilization process of course also constitutes an embryo according to the Embryo Protection Act.

The question of whether the definition in Section 8 (1) of the Embryo Protection Act as well as the act as a whole (including the cloning ban in Section 6 of the act, see below) applies to entities created neither through gamete fusion after fertilization (Section 8 (1) No. 1) nor through embryo splitting (Section 8 (1) No. 2) remains a matter of debate. This affects in particular entities created via cell nuclear transfer, as well as those created through tetraploid complementation.

The predominant interpretation, considering both Section 8 (1) and Section 6 of the Embryo Protection Act, is that Section 8 of the act on its own is not intended to be a complete definition of an embryo. The word “already” in the first version of Section 8 (1) should rather be understood to mean “also”, so that viable entities created via other methods would also be considered embryos. The use of the term “means” is also an indication that the law should remain adaptable to new scientific facts. It follows, then, that fertilization is an example of *one* possible way to create a human egg cell that is capable of development; it does not exclude from the law’s definition of an embryo entities with the capacity for development created via other methods. It is not the method that is important, rather, in addition to the presence of human⁸ source material, the result of the process, meaning the creation of *functionally equivalent entities* that are comparable to a fertilized embryo. In accordance with this view, the Embryo Protection Act equally applies to viable entities created via tetraploid complementation and cell nuclear transfer. The same would then also be true for a cell cluster capable of development that has not descended in a cell line from a totipotent cell.

Proponents of the contrasting interpretation, however, argue that there is a legal loophole. For them, the word “already” is merely of temporal significance, meaning that methods of creating a cell structure other than fertilization are not covered by the legal definition of an embryo, putting Section 8 of the Embryo Protection Act in conflict with Section 6. The federal government’s 1998 report on cloning⁹ already refers to this ambiguity in the definition of an embryo in Section 8.

b) The need for the “same” genetic information with reference to the cloning ban

Section 6 (1) of the Embryo Protection Act states: “Anyone who causes artificially a human embryo to develop with the same genetic information as another embryo, fetus, human being or deceased person will be punished with imprisonment up to five years or a fine.” Legal experts argue about whether the use of the word “same” indicates a quantitative or qualitative view, and about where the relevant lines should be drawn. What is clear is that the law does not demand the presence of “identical” genetic information.

The fact that there is no unanimity regarding the term “same genetic information” can be demonstrated by the evaluation of mitochondrial genes in connection with somatic cell nuclear transfer. Many authors assume that, due to quantitative reasons, the existence of foreign mitochondrial DNA is insignificant, since it makes up only from 0.01 to 0.02 percent of the total genome. Hence for them, the creation of a cell nuclear transfer embryo constitutes a form of banned cloning. In contrast, those taking a qualitative view warn that there is a strong interaction between mitochondrial genes and the genome of the nucleus and that certain mutations of mitochondrial genes could result in severe diseases, for example.

The consequence of the ambiguous use of the term “same genetic information” is a lack of legal clarity about if or when an infraction of the cloning ban is committed during the creation of cell nuclear transfer embryos or embryos created by tetraploid complementation. The federal government’s 1998 report on cloning also referred to this ambiguity.¹⁰ The same applies to the question of *what change* in the genetic information before cell nuclear transfer, for example, could result in no longer being able to speak of the “same” genetic information.¹¹ This is particularly relevant with regard to mutations, which usually amass over the course of time in the somatic cell from which the nucleus is extracted for transfer.

c) Production and use of germ cells from iPS cells

Section 5 (1) of the Embryo Protection Act prohibits artificially altering the genetic information of human germ line cells provided (in short) it hasn’t been ruled out that the cell will be used for fertilization, or that it will be used to produce a germ cell (Section 5 (4)). The definition of germ line cells includes all cells that form a cell line from the fertilized egg cell to the egg or sperm cells of the human being that developed from it.

⁸ In the event that human and animal source material is used, Section 7 of the Embryo Protection Act contains a special regulation (“Chimeras and hybrids”).

⁹ Cf. Bundestag printed paper 13/11263, 15; on totipotent tissue structure 22 f.

¹⁰ Cf. *ibid.*, 19.

¹¹ *Ibid.*, 17.

Section 8 (3) of the Embryo Protection Act stretches the definition of a germ line cell to include impregnated egg cells up to the completion of fertilization.

The law does not specify whether the term “germ cell” applies only to naturally created egg and sperm cells or whether it also includes artificially created egg and sperm cells (for example from iPS cells). It is only clear that Section 5 of the Embryo Protection Act only applies to human germ cells, meaning those created or produced exclusively from human material. Given the law’s lack of any statements to the contrary, artificially produced germ cells may be included, as long as they are functionally equivalent to germ cells generated naturally. As long as germ cells are produced from iPS cells, and no germ line cells were used in the production of the iPS cells, there is however no artificial alteration of the genetic information of a human germ line cell according to Section 5 (1) of the Embryo Protection Act.

Section 5 (2) prohibits the use of human germ cells with artificially altered genetic information for fertilization purposes. The language strongly indicates that the genetic information of a given germ cell has to have been changed, and that it is not enough for a germ cell to have been produced (and used in fertilization) that itself was created via a prior manipulation of the genetic information of another cell. In the event that a germ cell, which was created from an iPS cell, is used in fertilization, there is no violation of Section 5 (2) of the Embryo Protection Act. This is also the case should the germ cells come from the same individual.

What is prohibited according to Section 1 (1) and (2) of the Embryo Protection Act, however, is (in short) using a foreign egg cell for reproductive purposes. “Foreign” in this sense is any egg cell that comes from another woman. The use of an egg cell created from iPS cell of another person would thus constitute a foreign cell.

Conclusions

Whilst it is the opinion of the German Ethics Council that, with regard to the ban on cloning, there is no urgent legislative action needed in view of the latest developments in stem cell research, there is however a need for greater clarity with respect to the wide-ranging ethical and legal questions. These questions have resulted mainly in connection with two possible applications of new technologies on human beings for reproductive purposes. The first area of application has to do with cloning resulting from cell nuclear transfer or tetraploid complementation. The second area is in the use of germ cells developed from iPS cells, whereby a differentiation should be made as to whether the iPS cells came from two people or just one individual.

More precise, standardized legal definitions are needed for all areas concerned. The German Ethics Council particularly sees the need for greater clarity of the definition of the word “embryo”. The definition should be the same for both the Embryo Protection Act and the Stem Cell Act. Only the more comprehensive term in the Stem Cell Act does not differentiate according to the method of the embryo’s creation. The definition of totipotency should also be made more precise and its normative significance be clarified within the new context of stem cell research. It should be made clear whether it refers to individual cells or also to cell clusters, and what other further conditions are necessary to enable a cell or cell cluster to develop into a complete organism.

Beyond this, there are further ethical questions arising from the new technical possibilities that should be addressed. In addition to the medical reliability of these applications and their effects on any progeny, the relationship of generations to one another as well as the significance of the natural and the artificial at the beginning of a human life need to be considered and discussed at a level far beyond the past controversies in reproductive medicine. Also in need of clarification is what the significance might be within the framework of reproduction to either relinquish the involvement of two different sexes in sexual reproduction or to dispense entirely with the mode of genetic parentage by two people. On this basis, it should be decided if, and

to what extent, the use of artificially produced germ cells extracted from iPS cells may be used in the creation of an embryo.

The German Ethics Council stresses that the ethical questions raised in this context cannot be thoroughly dealt with in this Ad Hoc Recommendation, nor should they be. On questions of reproductive cloning, the Ethics Council refers to the Opinion of the National Ethics Council “Cloning for reproductive purposes and cloning for the purposes of biomedical research”¹² and to the second interim report by the Bundestag’s Study Commission “Legislation and Ethics in Modern Medicine”¹³. The German Ethics Council affirms the importance of the ban on reproductive cloning. Given the technological advancements that could make the reproductive cloning of humans possible, the Council recommends that Germany work toward an international ban on cloning for reproductive purposes.

¹² National Ethics Council (ed.) (2004): Cloning for reproductive purposes and cloning for the purposes of biomedical research. Berlin.

¹³ Bundestag printed paper 14/7546.

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