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OPINION

Human embryonic stem cells: research, ethics and policy

Guido de Wert[1,3] and Christine Mummery[2]

[1]Institute for Bioethics, University of Maastricht, Postbus 616, 6200 MD Maastricht and [2]Hubrecht Laboratory, Netherlands Institute of Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

[3]To whom correspondence should be addressed. E-mail: g.dewert@ige.unimaas.nl

The use of human embryos for research on embryonic stem (ES) cells is currently high on the ethical and political agenda in many countries. Despite the potential benefit of using human ES cells in the treatment of disease, their use remains controversial because of their derivation from early embryos. Here, we address some of the ethical issues surrounding the use of human embryos and human ES cells in the context of state-of-the-art research on the development of stem cell based transplantation therapy.

Key words: cell therapy/cloning/embryos/ethics/stem cells

Introduction

Human embryonic stem cells (hES cells) are currently discussed not only by the biologists by whom they were discovered but also by the medical profession, media, ethicists, governments and politicians. There are several reasons for this. On the one hand, these 'super cells' have a major clinical potential in tissue repair, with their proponents believing that they represent the future relief or cure of a wide range of common disabilities; replacement of defective cells in a patient by transplantation of hES cell-derived equivalents would restore normal function. On the other hand, the use of hES cells is highly controversial because they are derived from human pre-implantation embryos. To date, most embryos used for the establishment of hES cell lines have been spare embryos from IVF, but the creation of embryos specifically for deriving hES cells is also under discussion. The most controversial variant of this is the transfer of a somatic cell-nucleus from a patient to an enucleated oocyte (unfertilized egg) in order to produce hES cells genetically identical to that patient for 'autologous' transplantation (so-called 'therapeutic' cloning); this may prevent tissue rejection.

The question 'Can these cells be isolated and used and, if so, under what conditions and restrictions' is presently high on the political and ethical agenda, with policies and legislation being formulated in many countries to regulate their derivation. The UK has been the first to pass a law governing the use of human embryos for stem cell research. The European Science Foundation has established a committee to make an inventory of the positions taken by governments of countries within Europe on this issue (European Science Foundation, 2001).

In order to discuss the moral aspects of the isolation and use of hES cells, which is the aim of the present article, it is first essential to understand exactly what these cells are, where they come from, their intended applications and to define the ethical questions to be addressed.

What are (embryonic) stem cells?

'Stem cells' are primitive cells with the capacity to divide and give rise to more identical stem cells or to specialize and form specific cells of somatic tissues. Broadly speaking, two types of stem cell can be distinguished: embryonic stem (ES) cells which can only be derived from pre-implantation embryos and have a proven ability to form cells of all tissues of the adult organism (termed 'pluripotent'), and 'adult' stem cells, which are found in a variety of tissues in the fetus and after birth and are, under normal conditions, more specialized ('multipotent') with an important function in tissue replacement and repair.

hES cells are derived from the so-called 'inner cell mass' of blastocyst stage embryos that develop in culture within 5 days of fertilization of the oocyte (Thomson *et al*., 1998; Reubinoff *et al*., 2000). Although hES cells can form all somatic tissues, they cannot form all of the other 'extraembryonic' tissues necessary for complete development, such as the placenta and membranes, so that they cannot give rise to a complete new individual. They are therefore distinct from the 'totipotent' fertilized oocyte and blastomere cells deriving from the first cleavage divisions. hES cells are also immortal, expressing high levels of a gene called telomerase, the protein product of which ensures that the telomere ends of the chromosomes are retained at each cell division and the cells do not undergo senescence. The only other cells with proven pluripotency similar to that of ES cells are embryonic germ (EG) cells, which as their name implies, have been derived from 'primordial germ cells' that would ultimately form the gametes if the fetus had not been aborted. In humans, hEG cells were first established in culture in 1998, shortly after the first hES cells,

from tissue derived from an aborted fetus (Shamblott *et al*., 1998). Biologically, hEG cells have many properties in common with hES cells (Shamblott *et al*., 2001).

In the adult individual, a variety of tissues have also been found to harbour stem cell populations. Examples include the brain, skeletal muscle, bone marrow and umbilical cord blood, although the heart, by contrast, contains no stem cells after birth (reviewed in McKay 1997; Fuchs and Segre, 2000; Watt and Hogan, 2000; Weissman *et al*., 2000; Blau *et al*., 2001; Spradling *et al*., 2001). These adult stem cells have generally been regarded as having the capacity to form only the cell types of the organ in which they are found, but recently they have been shown to exhibit an unexpected versatility (Ferrari *et al*., 1998; Bjornson *et al*., 1999; Petersen *et al*., 1999; Pittenger *et al*., 1999; Brazelton *et al*., 2000; Clarke *et al*., 2000; Galli *et al*., 2000; Lagasse *et al*., 2000; Mezey *et al*., 2000; Sanchez-Ramos *et al*., 2000; Anderson *et al*., 2001; Jackson *et al*., 2001; Orlic *et al*., 2001). Evidence is strongest in animal experiments, but is increasing in humans, that adult stem cells originating in one germ layer can form a variety of other derivatives of the same germ layer (e.g. bone marrow-to-muscle within the mesodermal lineage), as well as transdifferentiate to derivatives of other germ layers (e.g. bone marrow-to-brain between the mesodermal and ectodermal lineages). To what extent transdifferentiated cells are immortal or acquire appropriate function in host tissue remains largely to be established but advances in this area are rapid, particularly for multipotent adult progenitor cells (MAPCs) of bone marrow (Reyes and Verfaillie, 2001). Answers to these questions with respect to MAPCs, in particular whether they represent biological equivalents to hES and can likewise be expanded indefinitely whilst retaining their differentiation potential, are currently being addressed (Jiang *et al*. 2002; Schwartz *et al*., 2002; Verfaillie, 2002; Zhao *et al*., 2002). For other adult stem cell types, such as those from brain, skin or intestine (Fuchs and Segre, 2000), this may remain unclear for the immediate future. Although the discussion here concerns hES cells and the use of embryos, the scientific state-of-the-art on other types of stem cell is important in the context of the 'subsidiarity principle' (see below).

Potential applications of hES cells and state-of-the-art

In theory, hES cells could be used for many different purposes (Keller and Snodgrass, 1999). Examples in fundamental research on early human development are the causes of early pregnancy loss, aspects of embryonic ageing and the failure of pregnancy in older women (where genetic defects in the oocyte appear to be important). A second category might be toxicology, more specifically research on possible toxic effects of new drugs on early embryonic cells which are often more sensitive than adult cells (drug screening). The most important potential use of hES cells is, however, clinically in transplantation medicine, where they could be used to develop cell replacement therapies. This, according to most researchers in the field represents the real 'home run' and it is the ethics of using embryos in this aspect of medicine that will be discussed here. Examples of diseases caused by the loss, or loss of function, of only one or a limited number of cell types and which could benefit from hES cell-based therapies include diabetes, Parkinson's disease, stroke, arthritis, multiple sclerosis, heart failure and spinal cord lesions. Although it is known that hES cells are capable of generating neural, cardiac, skeletal muscle, pancreas and liver cells in teratocarcinomas *in vivo* in immunodeficient mice as well as in tissue culture, it would be an illusion to consider that cell-therapies will have widespread application in the short term (i.e. within a couple of years). It is unfortunate that sensational treatment in the media, which implied the generation of whole organs from hES cells, initially left this impression so that the more realistic view emerging is already a disappointment to some patient groups. Nonetheless, a proper scientific evaluation of the therapeutic potential is being carried out in countries that allow the isolation and/or use of existing hES cells. The ethical questions here then also include whether the establishment of new hES cell lines can be justified, in the realisation that eventual therapies, based on either hES or adult stem cells are long-term perspectives.

There are, at least in theory, various sources of hES cells. In most cases to date, these have been spare IVF embryos, although IVF embryos have been specifically created for the purpose of stem cell isolation (Lanzendorf *et al*., 2001). In one variant of 'embryo creation', it has even been reported that normally organized blastocysts develop from chimeras of two morphologically non-viable embryos (Alikani and Willadsen, 2002). The most revolutionary option would be the creation of embryos specifically for the purpose of isolating stem cells via 'nuclear transfer' ('therapeutic cloning'). This option is purported to be the optimal medical use of hES technology since the nuclear DNA of the cells is derived from a somatic cell of a patient to receive the transplant, reducing the chances of tissue rejection (see Barrientos *et al*., 1998; 2000). It is of note that the oocyte in this case is not fertilized, but receives maternal and paternal genomes from the donor cell nucleus. Since by some definitions an embryo is the result of fertilization of an oocyte by sperm, there is no absolute consensus that nuclear transfer gives rise to an embryo (see below).

The establishment of embryonic cell lines is becoming increasingly efficient, with up to 50% of spare IVF embryos that develop into blastocysts after thawing at the 8-cell stage reported to yield cell lines. There are reports of efficiencies much lower than 50%, however, the quality of the donated embryos being an important determinant of success. Growth of the cell lines over extended periods and in some cases under defined conditions (Xu *et al*., 2001) has also been reported, but the controlled expansion and differentiation to specific cell types is an area where considerable research will be required before cell transplantation becomes clinical practice (for review, see Passier and Mummery, 2003). In addition, research will be required on how to deliver cells to the appropriate site in the patient to ensure that they survive, integrate in the host tissue and adopt appropriate function. These are the current scientific challenges that will have to be overcome before cell therapy becomes clinical practice; the problems are common to both hES and adult stem cells. The efficiency of establishing

embryonic stem cell lines from nuclear transfer embryos is currently unknown, but expected to be lower than from IVF embryos.

Ethical exploration

In the following section, the status of hES cells is first considered. The questions of whether it is acceptable to use pre-implantation embryos as a source of ES cells for research on cell transplantation therapy and if so, whether embryo use should be limited to spare embryos or may also include the creation of embryos via nuclear transfer ('therapeutic cloning'), are then addressed.

The status of hES cells

What is the ontological status of hES cells? Should they be considered equivalent to embryos or not? Let us first consider the status of the 'naked', isolated inner cell mass (ICM; the source for deriving hES cell lines). The ICM is as it were the 'essence' of the pre-implantation embryo, the precursor of the 'embryo proper'. The isolated ICM, however, no longer has the potential to develop into a fetus and child, as trophoblast cells, necessary for implantation and nourishment of the embryo, and extra-embryonic endoderm, are absent. It does not necessarily follow, though, that the isolated ICM is no longer an embryo—we suggest that the whole, isolated ICM could best be qualified as a disabled, 'non-viable' embryo (even though it might, at least in theory, be 'rescued' by enveloping the ICM with sufficient trophoblast cells).

What, then, is the status of the individual cells from the ICM once isolated, and the embryonic stem cell lines derived from them? Should we consider these cells/cell lines to be non-viable embryos too? We would argue that when the cells of the ICM begin to spread and grow in culture, the ICM disintegrates and the non-viable embryo perishes. Some might argue that hES cells are embryos, because, although hES cells in themselves cannot develop into a human being, they might if they were 'built into' a cellular background able to make extra-embryonic tissues necessary for implantation and nutrition of the embryo. At present this is only possible by 'embryo reconstruction' in which the ICM of an existing embryo is replaced by ES cells (Nagy *et al*., 1993). Commentators who, against this background, regard hES cells as equivalent to embryos, apparently take recourse to the opinion that any cell from which a human being could in principle be created, even when high technology (micromanipulation) would be required to achieve this, should be regarded as an embryo. An absurd implication of this 'inclusive' definition of an embryo is that one should then also regard all somatic cells as equivalent to embryos—after all, a somatic nucleus may become an embryo after nuclear transplantation in an enucleated oocyte. It is therefore unreasonable to regard hES cells as equivalent to embryos.

Instrumental use of embryos

Research into the development of cell-replacement therapy requires the instrumental use of pre-implantation embryos from which hES cells are derived since current technology requires lysis of the trophectoderm and culture of the ICM; the embryo disintegrates and is thus destroyed. As has already been discussed extensively in the embryo-research debate, considerable differences of opinion exist with regard to the ontological and moral status of the pre-implantation embryo (Hursthouse, 1987). On one side of the spectrum are the 'conceptionalist' view ('the embryo is a person') and the 'strong' version of the potentiality-argument ('because of the potential of the embryo to develop into a person, it ought to be considered as a person'). On the other side of the spectrum we find the view that the embryo (and even the fetus) as a 'non-person' ought not to be attributed any moral status at all. Between these extremes are various intermediates. Here, there is a kind of 'overlapping consensus': the embryo has a real, but relatively low moral value. The most important arguments are the moderate version of the potentiality argument ('the embryo deserves some protection because of its potential to become a person') and the argument concerning the symbolic value of the embryo (the embryo deserves to be treated with respect because it represents the beginning of human life). Differences of opinion exist on the weight of these arguments (how much protection does the embryo deserve?) and their extent (do they apply to pre-implantation embryos?). In view of the fact that up to 14 days of development, before the primitive streak develops and three germ layers appear, embryos can split and give rise to twins or two embryos may fuse into one, it may reasonably be argued that at these early stages there is in principle no ontological individuality; this limits the moral value of an embryo.

Pre-implantation embryos are generally regarded from the ethical point of view as representing a single class, whereas in fact ~50–60% of these embryos are aneuploid and mostly non-viable. For non-viable embryos, the argument of potentiality does not of course apply. Their moral status is thus only based on their symbolic value, which is already low in 'pre-individualized' pre-implantation embryos. The precise implications of this moral difference for the regulation of the instrumental use of embryos is, however, beyond the scope of the present article.

The view that research with pre-implantation embryos should be categorically forbidden is based on shaky premises and would be difficult to reconcile with the wide social acceptance of contraceptive intrauterine devices. The dominant view in ethics is that the instrumental use of pre-implantation embryos, in the light of their relative moral value, can be justified under certain conditions. The international debate focuses on defining these conditions.

Ethics of using surplus IVF embryos as a source of hES cells

Possible objections are connected to the principle of proportionality, the slippery slope argument, and the principle of subsidiarity.

Proportionality

It is generally agreed that research involving embryos should be related to an important goal, sometimes formulated as 'an

important health interest' (the principle of proportionality). Opinions differ on how this should be interpreted and made operational. In a number of countries, research on pre-implantation embryos is permitted provided it is related to human reproduction. Internationally, however, such a limitation is being increasingly regarded as too restrictive (De Wert *et al*., 2002). The isolation of hES cells for research into cell-replacement therapies operates as a catalyst for this discussion. It is difficult to argue that research into hES cells is disproportional. If embryos may be used for research into the causes or treatment of infertility, then it is inconsistent to reject research into the possible treatment of serious invalidating diseases as being not sufficiently important. The British Nuffield Council on Bioethics (Nuffield Council on Bioethics, 2000) also saw no reason for making a moral distinction between research into diagnostic methods or reproduction and research into potential cell therapies.

Even if one argued that there is a difference between the two types of research, research on cell therapy would, if anything, be more defensible than research on reproduction. One (in our opinion somewhat dubious) argument is to be found in McGee and Caplan (1999); here the suggestion is made that in using embryos for cell therapy, no embryos are actually sacrificed: 'In the case of embryos already slated to be discarded after IVF, the use of stem cells may actually lend permanence to the embryo. Our point here is that the sacrifice of an early human embryo, whether it involves a human person or not, is not the same as the sacrifice of an adult because life of a 100-cell embryo is contained in its cells nuclear DNA.' In other words, the unique characteristic of an embryo is its DNA; by transplanting cells containing this DNA to a new individual, the DNA is preserved and the embryo therefore not sacrificed—a 'win–win' situation for both the embryo and cell transplant recipient. The implication is thus that the use of embryos for cell transplantation purposes is ethically preferable to disposing of them or using them in other ('truly destructive') types of research. This extreme genetic 'reductionism' is highly disputable and not convincing: the fact that embryos are actually sacrificed in research into cell therapy is masked. A second, more convincing, argument, that the instrumental use of embryos is in principle easier to justify for isolation of hES cells than, for example, research directed towards improving IVF, is that it has potentially far wider clinical implications. It therefore, unquestionably meets the proportionality requirement.

Slippery slope

The slippery slope argument can be considered as having two variants, one empirical and the other logical. The empirical version involves a prediction of the future: 'Acceptance of practice X will inevitably lead to acceptance of (undesirable) practice Y. To prevent Y, X must be banned'. The logical version concerns the presumed logical implications resulting from the moral justification of X: 'Justification of X automatically implies acceptance of (undesirable) practice Y'. In this context the problem often lies in the lack of precise definition of X: 'The difficulty in making a conceptual distinction between X and Y that is sharp enough to justify X without at the same time justifying Y, is a reason to disallow X.' Both versions of the argument play a role in the debate about the isolation of hES cells for research into cell replacement therapy. An example of the logical version is that acceptance of hES cells for the development of stem cell therapy for the treatment of serious disease automatically means there is no argument against acceptance of use, for example, for cosmetic rejuvenation (Nuffield Council on Bioethics, 2000). The main difficulty is, according to these critics, the 'grey area' between these two extremes. One answer to this objection is to consider each case individually rather than reject all cases out of hand. One could use the same objection for example against surgery, which can equally be used for serious as well as trivial treatments.

An example of the empirical version of the slippery slope argument is that the use of hES cells for the development of cell therapy would inevitably lead to applications in germ-line gene therapy and in therapeutic cloning, then ultimately reproductive cloning. This version of the argument is unconvincing too; even if germ line gene therapy and therapeutic cloning would be categorically unacceptable, which is not self-evident, it does not necessarily follow from this that the use of hES cells for cell-therapy is unacceptable. The presumed automatism in the empirical version of the slippery slope argument is disputable.

Subsidiarity

A further condition for the instrumental use of embryos is that no suitable alternatives exist that may serve the same goals of the research. This is termed 'the principle of subsidiarity'. Critics of the use of hES cells claim that at least three such alternatives exist, which have in common that they do not require the instrumental use of embryos: (i) xenotransplantation; (ii) human embryonic germ cells (hEG cells), and (iii) adult stem cells.

The question is not whether these possible alternatives require further research (this is, at least for the latter two, largely undisputed), but whether only these alternatives should be the subject of research. Is a moratorium for isolating hES cells required, or is it preferable to carry out research on the different options, including the use of hES cells, in parallel?

The answer to this question depends on how the principle of subsidiarity ought to be applied. Although the principle of subsidiarity is meant to express concern for the (albeit limited) moral value of the embryo, it is a sign of ethical one-dimensionality to present every alternative, which does not use embryos, as *a priori* superior. For the comparative ethical analysis of hES cells from pre-implantation embryos on the one hand, and the possible alternatives mentioned on the other, a number of relevant aspects should be taken into account. These include: the burdens and/or risks of the different options for the patient and his or her environment; the chance that the alternative options have the same (probably broad) applicability as hES cells from pre-implantation embryos; and the time-scale in which clinically useful applications are to be expected.

A basis for initiating a comparative ethical analysis is set out below:

(i) Xenotransplantation is viewed at present as carrying a risk, albeit limited, of cross-species infections and an accom-

panying threat to public health. This risk is, at least for the time being, an ethical and safety threshold for clinical trials. Apart from that, the question may be raised from a perspective of animal ethics whether it is reasonable to breed and kill animals in order to produce transplants, when at the same time spare human embryos are available which would otherwise be discarded;

(ii) In principle, the use of hEG cells from primordial germ cells of dead fetuses seems from a moral perspective to be more acceptable than the instrumental use of living pre-implantation embryos, provided that the decision to abort was not motivated by the use of fetal material for transplantation purposes. To date, however, hEG cells have been difficult to isolate and culture, with only one research group reporting success (Shamblott *et al*., 1998; 2001). In addition, research in mice suggests abnormal reprogramming of these cells in culture: chimeric mice generated between mouse (m)EG cells and pre-implantation embryos develop abnormally while chimeras using mouse (m)ES cells develop as normally as non-chimeric mice (Steghaus-Kovac, 1999; Surani, 2001). This makes the outcome of eventual clinical application of these cells difficult to predict in terms of health risks for the recipient.

(iii) Analysis of the developmental potential of adult stem cells is a rapidly evolving field of research, particularly in animal model systems. Experiments carried out within the last two years have demonstrated, for example, that bone marrow cells can give rise to nerve cells in mouse brain (Mezey *et al*., 2000), neural cells from mouse brain can turn into blood and muscle (Bjornson *et al*., 1999; Galli *et al*., 2000), and even participate in the development of chimeric mouse embryos up to mid-gestation (Clarke *et al*., 2000). Although apparently spectacular in demonstrating that neural stem cells from mice can form most cell types under the appropriate conditions, it is still unclear whether true plasticity in terms of function has been demonstrated or whether the cells simply 'piggy-back' with normal cells during development. Published evidence of 'plasticity' in adult human stem cells is more limited, but recent evidence suggests that the MAPCs from bone marrow may represent a breakthrough (Jiang *et al*., 2002; Schwartz *et al*., 2002;). They are accessible. Collection is relatively non-destructive for surrounding tissue compared, for example, with the collection of neural stem cells from adult brain, although their numbers are low: 1 in 10^8 of these cells exhibit the ability to form populations of nerve, muscle and a number of other cell types and they only become evident after several months of careful culture. Clonal analysis has provided rigorous proof of plasticity: a single haematopoietic stem cell can populate a variety of tissues when injected into lethally irradiated mice (Krause *et al*., 2001) or into blastocyst stage embryos to generate chimeric embryos (Jiang *et al*., 2002). Nonetheless, there are potential hazards to using cells that have been cultured for long periods for transplantation and although MAPCs seem to have normal chromosomes, it is important to establish that the pathways governing cell proliferation are unperturbed. This is also true for hES cells. However, the powerful performance of mES cells in restoring function in a rat model for Parkinson's disease (Kim *et al*., 2002), has not yet been matched by MAPCs. Bone marrow stem cells have been shown very recently to restore function to some extent in a mouse heart damaged by coronary ligation, an experiment that mimics the conditions of the human heart soon after infarction (Orlic *et al*., 2001). Although clinical restoration of function in a damaged organ is usually sought rather longer after the original injury than in these experiments, which were performed before scar tissue had formed, this approach will certainly be worth pursuing. An alternative, non-invasive, haematopoietic stem cell source is umbilical cord blood. This is used clinically for transplantation as an alternative to bone marrow in patients for whom no bone marrow match is available. Cord blood contains precursors of a number of lineages but its pluripotency, or even multipotency, is far from proven. Nevertheless, the prospect of autologous transplantation of haematopoietic stem cells of bone marrow in the long term makes this an important research area in terms of alternatives to therapeutic cloning (see below).

Although studies with adult stem cells so far have been encouraging, Galli (2000), author of the first adult neural stem studies and much cited by advocates of the view that adult stem cells have a proven developmental potency equal to that of ES cells, himself disagrees entirely with this viewpoint (see Editorial, 2000). It has even been suggested that the results from adult stem cell research are being misinterpreted for political motives and 'hints of the versatility of the adult cells have been over interpreted, overplayed and over hyped' (Vastag, 2001). Opponents of ES cell research are now heralding Verfaillie's adult stem cells as proof that work on hES cells is no longer needed. However the stem cell research community and Verfaillie herself (Vastag, 2002) have called for more research on both adult and embryonic stem cells. ES cells that can perform as powerfully as those described by Kim *et al*. (2002) in the rat Parkinson model make it far too early in the game for them to be discounted (Editorial, 2002).

The question remains, however, should a moratorium be imposed on isolating hES cells for research in cell therapy in the light of the indisputably promising results from adult stem cell research? The lack of consensus arises largely from disagreement on interpretation of the subsidiarity principle. Against the restrictive viewpoint that research on hES cells may only take place if there is proof that adult stem cells are not optimally useful, there is the more permissive viewpoint that hES cell research may, and indeed should, take place so long it is unclear whether adult stem cells are complete or even partial alternatives.

On the basis of the following arguments, a less restrictive interpretation of the subsidiarity principle is morally justified. (Stem Cell Research, 2000) To begin with, the most optimistic expectation is that only in the long run will adult stem cells prove to have equal plasticity and developmental potential as hES cells (and be as broadly applicable in the clinic), and there is a reasonable chance that this will never turn out to be the case. If hES cells from pre-implantation embryos have more potential clinical applications in the short term, then the risk of a moratorium is that patients will be deprived of benefit. This in itself is a reason to forgo a moratorium—assuming that the health interests of patients overrule the relative moral value of pre-implantation embryos. Secondly, the simultaneous devel-

opment of different research strategies is preferable, considering that research on hES cells will probably contribute to speeding up and optimising clinical applications of adult stem cells. In particular, the stimuli to drive cells in particular directions of differentiation may be common to both cell types, while methods of delivery to damaged tissue are as likely to be common as complementary. A moratorium on hES cell research would remove the driving force behind adult stem cell research.

A final variant on adult stem cell sources concerns the use of embryonal carcinoma (EC) cells, a stem cell population found in tumours (teratocarcinomas) of young adult patients. These cells have properties very similar to hES cells. The results of a phase I (safety) trial using these cells in 11 stroke victims in the USA have recently been published and permission granted by the Food and Drug Administration (FDA) for a phase II trial (effectivity) (Kondziolka *et al*., 2000). The patients received neural cells derived from retinoic acid (vitamin A) treatment of teratocarcinoma stem cells. Although the scientific and ethical consensus is that these trials were premature in terms of potential risk of teratocarcinoma development at the transplant site, all patients survived with no obvious detrimental effects, no tumour formation and in two cases a small improvement in symptoms. After two years, the transplanted cells were still detectable by scanning (Kondziolka *et al*., 2000). Despite its controversial nature, this trial has nevertheless probably set a precedent for similar trials using neural derivatives of hES, the best controlled differentiation pathway of hES cells at the present time (Reubinoff *et al*., 2001; Zhang *et al*., 2001). Proponents believe that such trials would be feasible even in the short term (McKay, 1997). Neural differentiation of hEC cells is fairly easy to induce reproducibly but most other forms of differentiation are not; even if ultimately regarded as 'safe', hEC cells will not replace hES cells in terms of developmental potential and are therefore not regarded as an alternative.

In view of both the only relative moral value of pre-implantation embryos and the uncertainties and risks of the potential alternative sources for the development of cell therapy, a moratorium for isolating human embryonic stem cells is unjustified.

Therapeutic cloning

Before discussing the ethical issues around 'therapeutic cloning', the term itself requires consideration. To avoid confusion, it has been proposed that the term 'cloning' be reserved for reproductive cloning and that 'Nuclear transplantation to produce stem cells' would be better terminology for therapeutic cloning (NAS report, 2002; Vogelstein *et al*., 2002). Others have pointed out the disadvantage of this alternative term, namely that it masks the fact that an embryo is created for instrumental use. More important in our opinion however, is that the use of the adverb 'therapeutic' suggests that hES cell therapy is already a reality: *strictu sensu* there can only be a question of therapeutic applications once clinical trials have started. In the phase before clinical trials, it is only reasonable to refer to research on nuclear transfer as 'research cloning' or 'nuclear transplantation for fundamental scientific research', aimed at future applications of therapeutic cloning.

Some consider this technology to be ethically neutral; they claim that the 'construct' produced is not a (pre-implantation) embryo. Qualifications suggested for these constructs include: activated oocyte, ovasome, transnuclear oocyte cell, etc. (Kiessling, 2001; Hansen, 2002) However, to restrict the definition of 'embryo' to the product of fertilization in the post-Dolly era is a misleading anachronism. Although the purpose of therapeutic cloning is not the creation of a new individual and it is unlikely that the viability of the constructed product is equivalent to that of an embryo derived from sexual reproduction, it is not correct to say that an embryo has not been created.

The core of the problem is that here human embryos are created solely for instrumental use. Whether or not this can be morally justified—and if so, under what conditions—has already been an issue of debate for years in the context of the development of 'assisted reproductive technologies' (ART). Is it acceptable to create embryos for research, and if so, is therapeutic cloning morally acceptable too?

A preliminary question: is it justified to create embryos for research?

Article 18 of the European Convention on Human Rights and Biomedicine forbids the creation of embryos for all research purposes (Council of Europe, 1996). However, this does not close the ethical and political debates in individual EU member states.

In the 'classical' normative debate on embryo research, two perspectives can be distinguished: a 'fetalist' perspective (focusing on the moral value of the embryo), and a 'feminist' perspective (with the interests of women, particularly candidate oocyte donors, playing a central role) (Raymond, 1987). Both perspectives have a different outlook on the question of whether or not there is a decisive moral distinction between research with spare IVF embryos on the one hand, and creating embryos for research on the other. In other words: is the difference between these practices such that the former can be acceptable under specific conditions, and the latter absolutely not?

Fetalist perspective

Instrumentalization of the embryo is sometimes regarded as far greater and fundamentally different when it involves the creation of embryos for research purposes rather than the use of spare embryos. This difference, however, is just gradual. Not only is the embryo used completely instrumentally in both cases, the moral status is also identical. The difference is in the intention at fertilization, which, although a real difference, is relative. It is a misconception to think that in the context of regular IVF treatment every embryo is created as a 'goal in itself': the goal is the solution of involuntary childlessness and the loss of some embryos is a calculated risk beforehand.

Feminist perspective

From a feminist perspective, the creation of embryos for research should be evaluated critically in as far as it may

require hormone treatment of a woman to obtain oocytes for research purposes: can this be morally justified when it requires unpleasant treatment of the donor with no benefit at all, or even a detrimental outcome, for her own state of health? A first objection is that women themselves become objects of instrumental use. Here, however, an analogy can be made with recruiting healthy research subjects. Relevant considerations concern whether or not the research serves an important goal, whether the burdens and risks to the subjects are proportional, and whether valid informed consent of the research subject/donor is given. The second objection is that the health risks to the women themselves are too high and the degree of discomfort disproportional. Difference of opinion exists, however, also among women, about the disproportionality of hormone treatment. There are, furthermore, several potential alternatives that do not require hormone treatment of healthy women. One involves the in-vitro maturation (IVM) of immature oocytes after their isolation from dead donors or donors having ovaries removed for other reasons. IVM is successful in cattle and sheep (efficiency ~40%), although it is, for the moment, much lower in humans.

In conclusion, from both a fetalist and a feminist perspective there is no overriding categorical objection against bringing pre-implantation embryos into existence for instrumental use. If the research cannot be conducted using spare embryos and its importance for human health is beyond doubt, we believe the creation of embryos specifically for research is morally justified subject to the required oocytes being obtained in a morally sound way.

Ethics of therapeutic cloning

Can therapeutic cloning be morally acceptable? The principle of proportionality, the slippery slope, and the principle of subsidiarity enter the debate again, but in a slightly different way.

Proportionality

It is doubtful whether the principle of proportionality provides a convincing a-priori objection against therapeutic cloning. If it is considered acceptable to create embryos for research aimed at improving ART (freezing of oocytes; IVM of oocytes, etc…), then it is inconsistent to reject therapeutic cloning beforehand as being disproportional. Maybe even some opponents of creating embryos for the improvement of ART can conditionally accept therapeutic cloning because of the important health interests of patients.

Slippery-slope

A consequentialist objection (fashioned as a 'slippery-slope' argument) is that therapeutic cloning will inevitably lead to reproductive cloning. This objection is not convincing; if reproductive cloning is categorically unacceptable (the debate on this issue is still ongoing), it is reasonable to prohibit this specific technology, and not to ban other, non-reproductive, applications of cloning. A second objection that could be raised in this context is that the creation of embryos through cloning for the isolation of stem cells could in the long term be used to justify the initiation of pregnancy from these embryos and their use simply as a vehicle for generating sufficient cells of the required type for transplantation; the pregnancy would be interrupted the moment the appropriate developmental stage was reached (Lanza *et al*., 2002). Relevant questions here are: is this a realistic scenario in the human (or just science fiction), would it be unacceptable, and is it unavoidable?

In terms of being a realistic means of generating genetically identical (fetal) tissue for transplantation, it could theoretically be an option, but whether it would actually be useful would depend on the alternatives available at the time transplantation techniques themselves have been perfected to clinical applicability (see below).

In terms of moral acceptability, most people would consider pregnancy-and-abortion-for-transplantation to be far more difficult to justify than the creation of pre-implantation embryos for instrumental use *in vitro*, firstly because of the higher moral status/symbolic value of the fetus, and secondly because of the significantly greater burden of pregnancy-and-abortion-for-transplantation for women. (De Wert *et al*., 2002) Even though many countries do forbid pregnancy-for-transplantation, it has been argued that it could be morally justified as a last resort, on the basis that sacrificing a fetus (a potential person) may be justified in order to rescue the life of a person.

Finally, in scrutinising the slippery slope argument, it is important to assess whether instrumental use of pre-implantation embryos makes pregnancy-for-abortion unavoidable. Again, the apparent automatism is disputable: if we reject pregnancy-for-abortion as being unacceptable, we can continue its prohibition.

Taking these points for and against together, the slippery slope argument does not provide a convincing basis for banning therapeutic cloning.

Subsidiarity

Therapeutic cloning can only be morally acceptable if there are no good alternatives. It is important to note that therapeutic cloning *strictu sensu* is not likely to be short-term prospect. Apart from unsolved technical difficulties with nuclear transfer itself in human oocytes (Cibelli *et al*., 2002), much basic research is still needed to determine whether the differentiation of hES cells can be controlled and sufficient cell numbers generated to be a useful therapy. This research can be done with spare IVF embryos. In this light, creation of embryos for therapeutic cloning is, in our opinion, premature. Although critics of this point of view could use our own argument that delay in the development of research cloning could, just as a moratorium on hES cell isolation and research, have negative consequences for patients, the evidence suggests that further optimization of the technology as such could take place in animals. We believe that the duration of any 'delay' in offering therapy to patients would not then be of real significance.

At the same time, research on potential alternatives for therapeutic cloning, which likewise avoid (or at least reduce) the problem of rejection but which do not involve the creation of human embryos for instrumental use, should be stimulated. For the comparative ethical analysis, it is again important to avoid the pitfall of one-dimensionality. Possible alternative options include: (i) the use of adult cells, both stem cells and

differentiated cells; (ii) making optimal use of spare embryos: embryo-banks and immuno-tolerance and (iii) the use of entities with an undetermined status: 'hybrids' and 'parthenotes'.

Adult cells

Adult tissue is a potential source of two alternatives: stem cells, which may be induced to transdifferentiate by extracellular signals, and somatic cells (nuclei) which require direct reprogramming signals, for example from an oocyte after nuclear transfer, to adopt a new fate. Both sources will, however, require substantial research to become realistic alternatives. Until it has been shown that adult stem cells at some point re-express ES cell markers we will never know if transdifferentiation or direct reprogramming are the same or not.

For direct reprogramming of somatic nuclei, new methods may be developed which do not require nuclear transfer to oocyte cytoplasm. Examples of current work in this area include the study of cellular hybrids derived from the fusion of (embryonic) stem cells with somatic or adult stem cells (Surani, 2001; Terada *et al*., 2002; Ying *et al*., 2002). An understanding of the basic mechanisms underlying reprogramming is already being undertaken in mice, cattle and sheep and indeed, the creation of 'Dolly' re-initiated a wave of research in nuclear reprogramming in mammals. The ultimate aim of this research in the context of cell transplantation therapy would be chemically-induced nuclear re-programming in the test-tube to derive the required cell type, obviating the necessity for therapeutic cloning altogether. First evidence that this might be feasible demonstrated direct reprogramming of fibroblasts to neural cells and T-cells in culture by temporary permeabilization of the fibroblasts to allow them to take up extracts of neural and T-cells, respectively (Hakelien *et al*., 2002). In this sense, therapeutic cloning may be regarded, perhaps, as a temporary option; in the long term it will be replaced by a direct reprogramming alternative.

Research on direct reprogramming of adult somatic nuclei may ultimately require the creation of human embryos for instrumental use. In view of the importance of this research, both in terms of the contribution to the development of cell therapy and the potential ultimately to reduce the instrumental use of human embryos by developing an alternative for therapeutic cloning, this research would no doubt also meet the principle of proportionality.

Optimal use of spare embryos

Various strategies should be considered. Firstly, the generation of a bank of hES cell lines from a wide spectrum of genotypes is required to be able to offer a reasonable tissue match for every patient requiring a cellular transplant. Estimates of the number of independent cell lines that would actually be required for this vary greatly, from a few hundred to several thousand. Such a bank is already being discussed in the UK but could ultimately be established as a European resource. However, even very good tissue matches between donor and recipient require some degree of immunosuppressive therapy, which has long term negative side-effects for patients, including increased risk of tumorigenesis

Secondly, there should be further development and application of 'immunotolerance' methodology. This may be particularly useful in combination with matching from an hES cell bank. The observation that patients receiving bone marrow transplants are more immunotolerant to other tissue transplantation from the same donor have led to the suggestion that immunotolerance may also be induced by initial injection of hES-derived haematopoietic cells followed by the cell type of interest derived from the same hES cell line (Kaufman *et al*., 2001). The transplant may then be tolerated without being genetically identical, and lower doses or no immunosuppressives required. The combination of 'near match' with immunotolerance is probably a promising option.

For certain genetically based diseases, autologous transplantation may not always be appropriate since the transplanted tissue will bear the same genetic defect. Immunotolerance hES cell strategies may then be a particularly attractive or the only option. Should the success rates be very high, then attempts to create genetically identical transplantable tissue may become superfluous, not only for these, but for all patients. If, however, it works imperfectly or only for some patients, then therapeutic cloning may well remain an important option for the majority of all other patients.

Creating entities with an undefined status

Various alternative options raise classification problems, as the entities created to obtain cells have an undefined status. Firstly, transplanting the somatic nucleus of a patient into an enucleated animal oocyte. The logic behind this variant of therapeutic cloning is twofold: one, assuming that the 'units' thus created are not human embryos because only their nuclear but not mitochondrial DNA is human, advocates of this strategy argue that it circumvents the controversial issue of the instrumental use of human embryos. Two, a technical advantage of this approach would be that plenty of animal oocytes would be available; the feminist objection to creating human embryos for research would, of course, not apply.

It is not yet known whether this is a scientifically realistic option (whether hES cells can be effectively obtained following this approach). Animal research has so far been limited and not generally successful (Barrientos *et al*., 1998; 2001); polymorphic interspecies differences in mitochondrial DNA are thought to make such reconstructed zygotes non-viable or prone to major developmental abnormalities. There are however, unvalidated reports of successful applications of the technique in China. The Donaldson Committee advocated a ban on this approach, but without any argumentation (Stem Cell Research, 2000). However, if this were a realistic option scientifically, then we believe that the issues involved deserve further ethical discussion. The major questions that should be addressed include: is the risk acceptable? As for xenotransplantation, there is also here the risk of cross-species infection, although this may be extremely small, because the nuclear DNA of the animal, which may harbour viruses, is removed from the oocyte. Is it reasonable to argue that this 'artificial

combination' should not be considered equivalent to a human embryo? Since the entire nuclear DNA is human, the reconstructed combination should, we think, be regarded as a human embryo. The procedure should thus not be presented as an 'embryo saving' variant of therapeutic cloning. However, only further in-utero research with reconstructed animal embryos, for example embryos created by transplanting the somatic nucleus of a rat into an enucleated mouse oocyte, will provide a more definitive answer. Finally: in-vitro research may well show that embryos obtained by transplanting a human somatic nucleus into an enucleated animal oocyte are non-viable (like parthenotes, see below). The moral status of non-viable pre-implantation embryos, and more particularly, the question as to whether the conditions for research using non-viable embryos may be more permissive than the conditions for using viable embryos, needs further debate (see earlier).

A second option may be the generation of parthenogenetic embryos for the isolation of hES cell lines. Here, an unfertilized (haploid) oocyte is treated chemically such that it becomes diploid, with two identical sets of the maternal chromosomes. These uniparental embryos are by definition gynogenetic and never result in viable offspring, because they fail to generate extra-embryonic tissues. Nevertheless, in mice (see Boediono *et al*., 1999) and in apes (Cibelli *et al*., 2002), parthenotes have been shown to develop to the blastocyst stage and yield cell lines with properties not distinguishable from ES cells derived from fertilized oocytes. However, in view of the fact that some genes are genomically imprinted, such that they are expressed only if inherited via the male germ line, ES cells derived from parthenotes may well be abnormal. First attempts at parthenogenesis in humans have not yielded hES cell lines (Cibelli *et al*., 2002). It is important to realise that such hES cell lines, if developed in humans, would only provide a tissue match for the oocyte donor, i.e. women of reproductive age. Although it has been speculated that two sets of male chromosomes could also be used in parthenotes, there is no evidence that this is a real option.

Cibelli and colleagues have referred to parthenogenesis as cloning. Whether this is correct depends on the timing of parthenogenesis: if initiated before the first complete meiotic division, then the procedure amounts to cloning (the same genotype as the female); if after the first meiotic division (ie recombination and loss of half) then it is not cloning. In this light, the experiments of Cibelli *et al*. (2002) would not qualify as cloning in the strict sense.

Some will certainly argue that the parthenote is not an embryo; parthenogenesis would then be classified as an 'embryo-saving' strategy. As the parthenote undergoes the first divisions normally and is at these stages not distinguishable from embryos derived by normal fertilization, we would argue that it should be regarded as a non-viable embryo. In the light of its non-viability, the potentiality argument is not applicable. The moral status of parthenotes may therefore be regarded as very low, lower even than that of normal viable embryos at the same stage (see earlier). Thus, although not an 'embryo-saving alternative', all other things being equal, parthenogenesis may be regarded as ethically preferable to the generation of viable embryos by fertilization or nuclear transfer (for instrumental use). In addressing the question of whether this research is premature given the current lack of proof that human ES cells are clinically useful as a source of transplantable cells, the lower moral status of parthenotes should be taken into account.

Conclusions and recommendations

Regarding moral judgements as a 'quasi stable equilibrium' is particularly appropriate when applied to the ethics of isolating hES cells for research into cell replacement therapy. Stem cell research is highly dynamic, with many questions and 'unknowns'. New insights into the effectiveness, risks and usefulness of the various alternatives may have immediate consequences for the ethical evaluation of the isolation of hES cells.

The status of the pre-implantation embryo is the most sensitive and disputed point in the debate on isolation of hES cells for research. The dominant view in ethics, however, is that the moral status of the pre-implantation embryo is relatively low and that the instrumental use of these embryos can be morally justified under some conditions.

The moral status of non-viable pre-implantation embryos is lower than the moral status of viable pre-implantation embryos. The precise implications of this difference in moral status for the regulation of the instrumental use of embryos need further ethical scrutiny.

Both the principle of proportionality and a permissive interpretation of the principle of subsidiarity, make a moratorium on the isolation of hES cells unjustified.

Parallel research on alternatives is important and requires major support. Research on hES cells can provide an important impetus in this context.

The moral difference between research on surplus embryos and the creation of embryos for research is only gradual. A complete ban on creating embryos for instrumental use in research is morally unjustified.

A categorical ban on research on human therapeutic cloning is not justified, although the creation of embryos by cloning for the isolation of hES cells is, at the present time, premature. The necessary research can currently be carried out using animal embryos and surplus human IVF embryos.

Research into potential alternatives for therapeutic cloning, which does not require human embryos or which requires only the use of spare embryos, should be stimulated.

Banning the transplantation of a human somatic nucleus to an animal oocyte (as a variant of therapeutic cloning) is premature and morally unjustified.

The question whether therapeutic cloning should be allowed, becomes acute if research with spare embryos suggests that usable transplants can be obtained *in vitro* from hES cells and if the possible alternatives for therapeutic cloning are less promising or need more time for development than is currently expected. In that case, therapeutic cloning can be morally justified on the basis of both the principle of proportionality and the principle of subsidiarity.

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