HUMAN EMBRYONIC STEM CELLS: SCIENCE AND ETHICS

INTRODUCTION

1. Every day thousands of people of all ages are admitted to hospitals because of disease of some vital organ. Some of these diseases do not have permanent cures as yet and because of a dearth of transplantable organs, many of these people eventually die. A dramatic example reported by the American Heart Association is that only 2,300 of the 40,000 Americans who needed a new heart got one (Scientific American, 1999). Even in Singapore there is a long line of patients waiting for heart transplants. Cancer, HIV, diabetes and neuro-degenerative diseases are other life-threatening ailments that add to this list.

2. An exciting new strategy is poised to revolutionize treatment for such diseases. The ultimate cell, the human embryonic stem (ES) cell that can be engineered to produce replacement cells of any type, help to create new tissues and eventually new organs for transplantation has been developed. The ES cell commonly referred to as the 'mother of all cells' promises to open a new era in regenerative medicine with tremendous hope for the cure of a variety of incurable diseases. However this new science has been recently surrounded by ethical sensitivities because the source for derivation of such cells are human embryos and this has impeded the progress of this science. This paper will address the science of ES cell biology, critically evaluate the ethical sensitivities and recommend to the Human Stem Cell Research sub-committee policies, with the hope of protecting the rights and welfare of individuals while allowing this science to develop and realize its full potential for the benefit of mankind.

THE SCIENCE OF ES CELL BIOLOGY

What are stem cells?

3. Stem cells are unspecialized cells in the human body that are capable of renewing themselves and also being able to specialize into other new cell types, each with specialized functions.

Sources of human stem cells

4. Several sources of human stem cells have been recognized today. These have been isolated from the preimplantation embryo, fetus and adult. Embryonic stem cells have been confirmed to be widely pluripotent compared to fetal and adult stem cells. Stem cell sources, acronyms and pluripotentiality are shown in Table 1.

Sources for derivation of ES cells

5. Embryonic stem cells can be derived by a number of methods:

(a) Human embryos created by in vitro in fertilization as a method for treatment of infertility. These embryos are excess of fertility need and are voluntarily donated by subfertile couples who no longer plan to use the embryos and do not wish they be donated to other couples or be disposed.

(b) Human fetal tissue following elective abortion.

(c) Human embryos created by in vitro fertilization with sperm and eggs donated for the sole purpose of providing research material.

(d) Human or hybrid embryos generated asexually by somatic cell nuclear transfer of the adult human cell nucleus into an enucleated human or animal egg (therapeutic cloning).

6. Of the above four types, only types (a) and (b) have been utilized.

Human embryonic development

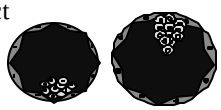
7. Through the technology of in vitro fertilization it has been possible for the first time to observe and accurately describe the stages and timing of early human embryonic development (Bongso et al 1998; Table 2).

Table 1: Sources of human stem cells

Name	Acronym	Source	Pluripotentiality*
Embryonic stem cell	ES	5 day old embryos (Blastocysts)	Widely pluripotent
Embryonic germ cell	EG	First trimester abortuses	Pluripotency not confirmed
Adult stem cell	AS	Adult tissues (Blood, bone-marrow, umbilical cord, liver, brain)	Not widely pluripotent (multipotent)

*Pluripotentiality: ability to specialize into other cell types.

Table 2: Human embryonic stages observed each morning from day 1 to 6

Day (hr)	Embryonic stage	Description	
1 (18-20)	Two Pronuclear stage	Male and female pronuclei present	
2 (48)	Cleavage stage	4 cells	
3 (72)	Cleavage stage Compacting	8 cells discrete 8 cells fusing	
4 (96)	Compacting Compacted Early cavitating	8 cells fusing All cells fused (morula) First signs of blastocoele	
5 (120)	Late cavitating Early blastocyst Expanding blastocyst	Distinct blastocoele, ICM, TE not distinct Distinct ICM, TE Distinct ICM, TE; embryo diameter increased	

ICM: Inner cell mass (future ES cells); TE: Trophectoderm (future placental cells)

8. We now know that optimum numbers of motile sperm have to encounter an egg with its enclosed cell vestments so as to produce optimum fertilization (10,000 sperm per egg per 100 µl of nutrients) (Bongso et al 2000). Interestingly, optimum fertilization is completed in one hour (Gianorrali 2000). Visual evidence of fertilization (the two pronuclear stage) is noticed only at 18-20 hours after sperm-egg interaction. Each pronucleus containing maternal and paternal genetic make-up (23 chromosomes each) have not as yet fused or joined to establish the 46 chromosome state. Fusion of both pronuclei (syngamy) occurs at approximately 20 to 23 hours and the first cleavage division (2-cell stage) occurs at 24-25 hours. This would be the completion of Day 1 of embryonic development. At 48 hours (Day 2) the embryo is a ball of 4 cells, at 72 hours (Day 3) a ball of 8 cells with the cells fusing in some embryos and on Day 4 is the cavitating stage when the first signs of a cavity (blastocoele) is formed. Migration of cells within the embryo takes place between days 4 and 5 to produce on days 5 and 6 blastocysts at various stages of development. The blastocyst is still a ball of cells, but the cells have migrated within the embryo to form two distinct cell layers, the outer cell mass (trophectoderm) and inner cell mass (ICM) (Table 2). The trophectoderm has about 200 cells, which form the future placenta and the ICM about 30 cells that differentiate to form the future foetus. The blastocyst is enclosed in a shell called the zona pellucida. ES cells are isolated at this blastocyst stage from the 30 ICM cells. This 5 day old stage is the best and the most optimal stage for ES cell derivation because the ICM can be visually recognized (Table 2).

How are ES cells isolated and propagated

9. In vitro fertilized frozen surplus preimplantation stage 2 to 5 days old embryos that are not used for clinical treatment are donated voluntarily with informed consent by patients undergoing in vitro fertilization procedures. Frozen instead of fresh embryos are used for ES cell derivation so as to give both husband and wife ample time to think and agree whether such embryos should be donated for this specific research. If the frozen embryos are 2 days old, they are thawed and grown to the 5^{th} day (blastocyst stage) for isolation of the ICM. The ICM is separated by immunosurgery and grown on mouse embryonic fibroblast feeder cell layers. The mouse feeder cells are previously treated with mitomycin-C to arrest their growth and act only as a supplier of nutrients and specific undifferentiating factors by cell to cell contact. After about 2 weeks, the expanded ICM lump is separated from the feeder layer with enzymes, dissociated into small pieces and re-grown on fresh mouse fibroblast feeder layers. The ICM cells, now called ES cells are continuously grown in this way to expand cell

numbers. At alternate generations, an aliquot of ES cells are injected under the testicular or kidney capsule of severely combined immunodeficient (SCID) mice to allow the growth of these cells in 4 weeks into differentiated human cells and tissues including gut (endoderm); cartilage, bone and muscle (mesoderm) and nervous tissue, skin (ectoderm). These three cellular layers (endoderm, mesoderm and ectoderm) have the potential to form all the 210 cell types of the body and the ES cells are then confirmed truly and widely pluripotent. Four such cell lines have already been developed in Singapore with informed patient consent and according to NIH, USA and NUH ethical committee guidelines.

Benefits of ES cells to mankind

10. ES cells hold promise to mankind in three major areas: (1) Transplantation therapy (2) Pharmaceutical development (3) Human developmental biology.

1. Transplantation therapy

The potential therapeutic impact of ES cells in transplantation therapy is enormous because of their capability to produce virtually unlimited quantities of any cell in the body. Additionally, they have the potential to be genetically engineered to prevent their immune rejection by the transplant recipient thereby bypassing the need to provide each recipient with his/her own ES cells via therapeutic cloning. Examples of medically relevant cells that could be developed from ES cells for human transplantation therapy are cardiomyocytes for the treatment of myocardial infarction and congestive heart failure; neuronal cells for the treatment of stroke, Parkinson's and Alzheimer's diseases; blood cells for the treatment of blood related cancers and HIV (after genetically engineering these cells to resist infection by the HIV virus); pancreatic islet cells for the treatment of diabetes; skin cells for the treatment of wounds, burns and for the cosmetic industry; and cartilage cells for the treatment of osteoarthritis. These clinical applications will involve direct injection of ES cell-derived differentiated cells into the diseased sites. Further research could lead to development of complex multi-cellular solid tissues and organs by encouraging these cells to interact with scaffolds made of degradable polymers.

2. Pharmaceutical development

Permanent stable sources of normal differentiated human cells can be developed for drug screening and testing, drug toxicology as well as new drug target identification

and the screening of teratogens (drugs causing birth defects) extending the capability of current screening using animal cell lines, bacterial and laboratory animal systems.

3. Human developmental biology

Since ES cells can be made to differentiate into a variety of functional cell types in a laboratory dish, they offer a unique platform to understand and harness nature's mechanisms of embryonic development, tissue differentiation and repair. Such understanding will contribute to the treatment of fertility disorders, the prevention of premature pregnancy loss and diagnosis and prevention of birth defects. The availability of ES cells may facilitate research in these areas without the need to use human embryos or fetuses.

Unique characteristics of ES cells

i. Wide pluripotency

HES cells can form virtually any cell in the body. They have been shown to form derivatives of all three primary cell layers (ectoderm, mesoderm and endoderm) in immunodeficient SCID mice (Reubinoff et al 2000) and hence have the potential to be directed into gut, cartilage, bone, muscle (heart and other muscle), nerve, skin, pancreas etc. Already differentiated adult stem cells have limited pluripotency to form certain cell types for eg. bone-marrow to heart in the mouse (Orlic et al 2001), bone-marrow to neurons in the mouse (Brazelton et al 2000), bone-marrow to liver in the mouse (Petersen et al 1999).

ii. Self-renewing capacity

Under specific culture conditions ES cells can repopulate themselves while remaining in an undifferentiated state. Their growth in vitro is also prolific and as such once isolated from a few embryos they will be a continuous source of normal pluripotent stem cells. The major benefit of the already developed 4 cells lines from 4 donated embryos is that they not only can be scaled up in large numbers but also can be provided for research worldwide without the need to isolate more cells from embryos or fetal tissue. It has not been possible to maintain long-term self-renewing capacity of adult human stem cells in culture. The ability of ES cells to propagate indefinitely in the undifferentiated state without losing pluripotency is a feature that distinguishes these cells from all other 'multipotent stem cells' discovered todate in the human.

iii. Telomerase expression and immortalization

Telomerase is an RNA-dependent DNA polymerase which when reactivated in normal cells allows their continual proliferation. ES cells express abundant amounts of telomerase. The continuous steady release of telomerase activity in ES cells conveys replicative immortality. Adult stem cells express telomerase at low levels or only periodically and may therefore age and stop dividing with time.

iv. Normal genetic make-up with continuous growth

ES cells maintain a normal genetic make-up even after prolonged growth in vitro. They do not undergo chromosomal changes, as is characteristic of most adult cells grown in vitro. It is not known whether adult stem cells will show such genetic changes with prolonged growth in vitro because as of now no adult stem cell has been serially sub-cultured as a cell-line for many generations.

v. Isolation and availability

ES cells have been isolated from frozen embryos with very high efficiency (eg. 4-cell lines from 7 embryos, ESI). The cells propagated from these existing 4 cell lines are enough for research for all centers worldwide and can be scaled up even further for application because of their prolific growth. (Biocentury, May 2001). Adult stem cells in the human body except for bone-marrow and umbilical cord cells are very few in number and not easily accessible in the human body. The extent of growth in vitro is yet unknown for all adult stem cells including bone marrow and umbilical cord

cells. In some situations like the brain, isolating the stem cells would be difficult and a dangerous procedure itself. For some acute disorders there may not be long enough time to scale up enough cells for treatment.

Current state of ES cell research

11. Undifferentiated human embryonic stem cell lines from embryos have been developed by two groups in the world (Bongso et al 1994; Thomson et al 1998; Reubinoff et al 2000). One group (Thomson et al 1998) has 5 cell lines (with patient consent for research only) with no compliance to NIH ethical guidelines. The other group (Bongso et al 1994; Reubinoff et al 2000) have 2 cell-lines for research (non-NIH compliant) and 4 cell lines for research and application compliant with NIH, MOH, Singapore and NUH ethical committee guidelines. All these cell lines have been serially propagated thus far at least 200 times and have been confirmed pluripotent at all generations by demonstration of human tissues in immunodeficient SCID mice. Whilst it is true that ES cells have the potential to become every cell type in the body, they require certain triggers to persuade them to develop along specific cell lineages. In the embryo for example what cell type a cell will eventually become is determined by a combination of factors including physical forces, electrical charges, hormones and growth factors. All these forces combine to determine the cells future by switching certain genes on and other genes off. Some of these triggers have already been worked out for ES cells and pure nerve and heart cell lines have already been developed that are undergoing characterization in animal models (Reubinoff et al and Mummery et al, unpublished data). These tasks become even more difficult to direct adult stem cells since nothing is presently known as to what factors can de-differentiate already differentiated adult cells.

12. In the mouse, ES cells have been genetically stabilized into heart cells (Klug et al 1996) and modified into nerve cells with retinoic acid (Deacon et al 1998). Recently, beating ventricular-like heart cells from murine ES cells were separated in vitro (Muller et al 2000) and murine ES cells were successfully directed into insulin-producing cells in vitro (McKay et al 2001). These cells were able to release insulin in the presence of blood sugar. When ES-derived murine nerve cells were transplanted into mice with spinal cord injuries and brain disorders, engraftment of the injected cells into the diseased sites occurred with improvement of nerve function (Deacon et al 1998). Similarly, the transplantation of ES-derived heart cells into the scar tissue of ischaemic adult mouse hearts showed engraftment, improvement of

new blood vessel formation (angiogenesis) and improvement of heart function (Klug et al 1996).

13. Getting human ES cells to turn into many cell types targeted against specific diseases is an ongoing area of research. A lack of government funding for this promising area of research has slowed down its progress because of the debate on the ethical issues involved in deriving ES cells from human embryos. Once these issues are cleared, the potential benefits are expected to be reaped at least within the next 10 years.

THE ETHICS OF ES CELL BIOLOGY

Status of the human embryo

14. Just about anything with the label 'embryo' or 'fetus' arouses the concerns of many people about the dignity of human life or human potential. It is important to note that a 5 day old blastocyst is not yet a so called 'embryo'. Any particular cell in a blastocyst is as likely to become part of the placenta, which will be discarded at, birth, as it is to become part of a 'potential person'. Ethics commissions in several countries including the United Kingdom (Warnock report), the USA (NIH Human Embryo Research Panel, 1994), Australia and Denmark have approved research on the human embryo up to 14 days. Up to 14 days it is more correctly called a 'pre-embryo' because the embryo has not differentiated into tissues. At 14 days, a structure called the 'primitive streak' appears which later becomes the brain and spinal cord and which then differentiates embryo from placenta. Before 14 days there is no possibility of pain or sentience and no cells that will definitely become part of an individual.

15. It would not be right to readily dismiss the objections that using embryos for ES cell research is an insult to human dignity. The frozen embryos used for ES cell derivation are in excess of fertility need and already abandoned by their parents as by-products of other conception attempts. Currently these embryos have a zero chance of ever maturing to human beings. Stem cell research offers the cells more opportunity for life than they would otherwise see (Scientific American, May 2001).

Do the potential benefits justify embryonic stem cell research?

16. Interestingly, knowledge gained thus far from the 4 existing ES cell-lines confirm that ES cells are not only versatile but prolific in their growth. There is virtually no limit to the quantity of stem cells that can be generated from these few cell-lines (BioCentury, May 21,

2001). Interestingly, the efficiency was over 60% to generate these 4 cell lines because they originated from 7 frozen pre-embryos (Bongso and Fong 2001). These cell-lines are now virtually immortal because they have been serially subcultured over 200 times and the available cells can be supplied for researchers around the whole world forever without destroying any additional embryos. Thus future research does not depend upon continued use of pre-embryos.

17. Given the fact therefore that ethical sensitivities are now no more an issue for ES cell research, the potential benefits in the final usage of these cells is tremendous. Because of the versatility of these cells (widely pluripotent) almost any disease has a potential cure by transplantation therapy once target cells or tissues are derived by differentiating these ES cells. ES cell-derived nerve, heart, blood and pancreatic cells will have cures for stroke, Parkinson's disease, Alzheimer's disease, heart diseases, cancers and diabetes. Because ES cells are widely pluripotent, prolific in their growth and 'younger' cells, they would be the gold standard over adult stem cells for replacing bad tissue with good. Even though they are donor cells unlike the patient's own adult stem cells, their histocompatibility genetic make-up can be engineered to prevent rejection after transplantation. However, it is important to note that when attempting to seek clinical benefits as fast as possible the use of both ES and adult stem cells for research should be encouraged because we do not know at this point in time which stem cell will be best suited for a particular disease.

What source of stem cells should be used for research?

18. Both ES and adult stem cells should be used as sources of cells for stem cell research. Even though adult stem cells may be more convenient to use, the scientific fact is that we do not yet know whether the adult stem cells necessarily retain the full plasticity of ES cells. Research should and will continue on adult stem cells and if they ultimately prove as capable as or better than ES cells, it might then be wise to forsake ES cells in deference to the moral debate over whether an embryo is really a human being. Until then, adult stem cell research can only be an adjunct to ES cell work. Polls taken in the USA have suggested that most of the American public think that ES cell research should continue. This means that the American Congress must decide how to balance ethical objections with the potential benefits of ES cell research. Should we ignore research that offers the best hope for treating or curing many illnesses? (Scientific American, May 2001). The overwhelming consensus among the real scientists involved in both ES and adult stem cell research is that no avenue of

stem cell research can be safely ignored (The Scientist, May 28, 2001). We simply do not know what types of cells would work best for particular diseases. In January 2001, after contentious debate lasting more than 8 hours the British House of Lords voted overwhelmingly to allow research on ES cells (The Scientist, May 28, 2001).

NIH guidelines for research using human embryonic stem cells

19. On August 25, 2000, the NIH, USA brought into effect its guidelines allowing research on human embryonic stem cells. This was after receiving 50,000 comments from members of Congress, patient advocacy groups, scientific societies, religious organizations and private citizens. (NIH Website, Aug, 2000). In its guidelines the NIH concluded that it was possible that no single source of stem cells is best or even suitable/usable for all therapies. Different types of sources of stem cells may be optimal for the treatment of specific conditions. In order to determine the very best source of many of the specialized cells and tissues of the body for new treatments or cures, it was concluded that it was vitally important to compare the potential of adult stem cells with that of ES cells. Unless all stem cell types were studied the differences between adult stem and ES cells will not be known.

20. The conditions for the derivation and utilization of ES cells from human **embryos** set out by the NIH are described below.

i. The ES cells must be derived from human embryos that were created for the purpose of fertility treatment and were in excess of the clinical need of the individuals seeking such treatment. It must be ensured that the donation of human embryos in excess of the clinical need is voluntary and, no inducements, monetary or otherwise, should have been offered for the donation of human embryos for research purposes. Fertility clinics and/or their affiliated laboratories should have implemented specific written policies and practices to ensure that no such inducements are made available.

ii. There should have been a clear separation between the decision to create embryos for fertility treatment and the decision to donate human embryos in excess of clinical need for research purposes to derive pluripotent stem cells. Decisions related to the creation of embryos for fertility treatment should have been made free from the influence of researchers or investigators proposing to derive or utilize human pluripotent stem cells in research. To this end, the attending physician responsible for

the fertility treatment and the researcher or investigator deriving and/or proposing to utilize human pluripotent stem cells should not have been one and the same person.

iii. To ensure that human embryos donated for research were in excess of the clinical need of the individuals seeking fertility treatment and to allow potential donors time between the creation of the embryos for fertility treatment and the decision to donate for research purposes, only frozen human embryos should have been used to derive human embryonic stem cells. In addition, individuals undergoing fertility treatment should have been approached about consent for donation of human embryos to derive pluripotent stem cells only at the time of deciding the disposition of embryos in excess of the clinical need.

iv. Donation of human embryos should have been made without any restriction or direction regarding the individual(s) who may be the recipients of transplantation of the cells derived from the human pluripotent stem cells.

v. Informed consent should have been obtained from individuals who have sought fertility treatment and who elect to donate human embryos in excess of clinical need for human embryonic stem cell research purposes. The informed consent process should have included discussion of the following information with potential donors, pertinent to making the decision whether or not to donate their embryos for research purposes.

21. Informed consent should have included:

i. A statement that the embryos will be used to derive human pluripotent stem cells for research that may include human transplantation research;

ii. A statement that the donation is made without any restriction or direction regarding the individual(s) who may be the recipient(s) of transplantation of the cells derived from the embryo;

iii. A statement as to whether or not information that could identify the donors of the embryos, directly or through identifiers linked to the donors, will be removed prior to the derivation or the use of human pluripotent stem cells;

iv. A statement that derived cells and/or cell lines may be kept for many years;

v. Disclosure of the possibility that the results of research on the human pluripotent stem cells may have commercial potential, and a statement that the donor will not receive financial or any other benefits from any such future commercial development;

vi. A statement that the research is not intended to provide direct medical benefit to the donor; and

vii. A statement that embryos donated will not be transferred to a woman's uterus and will not survive the human pluripotent stem cell derivation process.

22. Derivation protocols should have been approved by an Institutional Review Board (IRB) established in accordance with NIH or FDA regulations. The conditions for the derivation and utilization of ES cells from human **fetuses** set out by NIH are described below.

23. As a policy matter, deriving or utilizing human pluripotent stem cells from fetal tissue should comply with the informed consent law applicable to fetal tissue transplantation research together with the following conditions. The informed consent process should have included discussion of the following information with potential donors, pertinent to making the decision whether to donate fetal tissue for research purposes.

24. Informed consent should have included:

i. A statement that fetal tissue will be used to derive human pluripotent stem cells for research that may include human transplantation research;

ii. A statement that the donation is made without any restriction or direction regarding the individual(s) who may be the recipient(s) of transplantation of the cells derived from the fetal tissue;

iii. A statement as to whether or not information that could identify the donors of the fetal tissue, directly or through identifiers linked to the donors,

will be removed prior to the derivation or the use of human pluripotent stem cells;

iv. A statement that derived cells and/or cell lines may be kept for many years;

v. Disclosure of the possibility that the results of research on the human pluripotent stem cells may have commercial potential, and a statement that the donor will not receive financial or any other benefits from any such future commercial development; and

vi. A statement that the research is not intended to provide direct medical benefit to the donor.

Financial issues

25. As suggested in the NIH guidelines this committee agrees that monetary inducement for the donation of human embryos or fetuses for research must be prohibited. The only payment that can be proposed should be one that does not exceed the reasonable costs associated with the transportation, processing, preservation, quality control and storage of ES cells. In order to scale-up and direct the ES cells as fast as possible for therapeutic purposes the results of ES cell research may have commercial potential and must therefore be allowed.

Identifiers

26. If identifiers were to be removed ES cell investigators would not be able to conduct certain genetic studies or develop therapeutic materials. Thus, as recommended in the NIH guidelines, the term 'identifier' should refer to any information from which the donor can be identified, directly or through identifiers linked to the donors. Furthermore since information identifying the donor may be necessary if the derived tissues or cells are to be used in transplantation, it is necessary to state that the informed consent should notify donors whether or not identifiers will be retained. Since ES-derived tissues (heart, nerve, blood etc) will be the best match for the donor supplying the embryos for ES cell derivation, the donor should not be given privilege in the fruits of the successful research either by getting preferential treatment, payments or any other benefits. DNA could also be an identifier and as such all donors of human embryos or fetal tissue should be told that identifiers such as DNA will be retained with the samples. Although DNA can be used to determine an individual from whom a tissue sample was taken, this can be done only when one has a sample from both the

tissue in question and the putative donor. It cannot be used to identify an individual out of a population.

Rights, duties and responsibilities of those handling stem cells for research

27. The following actions may be taken by the awarding agency funding the research. Firstly, compliance to certain guidelines (eg. NIH) can be largely determined prior to the award of funds. Regular progress reports could be requested so as to monitor the research. If necessary the awarding agency can impose special conditions on the award including increased oversight/monitoring/reporting requirements for an institution, project or investigator. If an awardee fails to comply with the terms and conditions of the award, the awarding agency may withhold funds pending correction of the problem or, for more severe enforcement, disallow all or part of the costs of the activity that was not in compliance, withhold further awards for the project, or suspend/terminate all or part of the funding for the project. Individuals or institutions may be debarred from eligibility for all government financial assistance in the future. Harsher punishments than those suggested above will only discourage scientists from getting involved in potential curative stem cell research.

ES Cells and therapeutic cloning

28. Cloning or 'nuclear transfer' in general is of two types viz., reproductive and therapeutic cloning. The process in producing a clone in both cases is the same (generation of an embryo by electric pulse after transplanting any cell with 46 chromosomes into an enucleated human or animal egg). The difference lies in the use put to the generated embryo. In reproductive cloning, the resulting embryo is transferred to the uterus of a woman to deliver a baby. It is important to note that the development of animal reproductive cloning todate has shown a widespread pattern of problems in pregnancy, foetal abnormalities and early deaths of newborn animals. Animal reproductive cloning was strongly criticized in a recent report where it was stated that there is no such thing as a normal clone. Around 75% of cloned cows die in the first two months of pregnancy and miscarriages go on right to the end (Cohen and Concar, 2001). Thus, it has been stated as to why would anyone in their right mind want to clone a human being when animal cloning can go disastrously wrong. This therefore makes it quite clear that for the foreseeable future it would be criminally foolhardy to attempt to clone human beings quite apart from the very strong ethical objections (Bruce, 2001). Thus, this committee recommends very strong objections to producing embryos genetically identical to another human being (reproductive cloning).

29. In therapeutic cloning, ES cells can be derived from the nuclear transferred embryo and these cells can be directed into useful cells and tissues that will benefit mankind. One advantage of deriving differentiated cells from nuclear transferred ES cell lines is that the transplanted cells may not be rejected because the genome of the donor cell used for the nuclear transfer comes from the recipient. However, the major obstacle to therapeutic applications is obtaining stem cells for every given patient. The second limitation is the recent evidence suggesting that the efficiency of producing nuclear transferred ES cell lines was very low (8.8%). In this study using the mouse model, the authors obtained 398 blastocysts from 1016 reconstructed eggs (39.2%) using tail-tip and cumulus cells. From these 398 blastocysts only 35 cell lines (8.8%) were developed. (Wakayama et al 2001).

30. It would seem illogical to disallow the creation of embryos for stem cell research through in vitro fertilization clinics and at the same time allow the creation of therapeutically cloned embryos for ES cell research. The use of frozen spare embryos from fertility treatments would be a use of an embryo that would be disposed of anyway. Thus, the deliberate creation of human embryos for research via any means must be disallowed. Once therapeutic cloning is allowed it would be easy for someone misguided enough to get to the next step and allow them to be implanted to produce a fully cloned human being. The US congress under the Bush Jr administration recently banned federal and private funding for therapeutic cloning research (NIH, June 2001). Interestingly, guidelines proposed recently allowed Canadian scientists to derive stem cells from human embryos left over from fertility treatments or fetal tissue obtained from elective abortions. However, the 10 member Canadian panel opposed the donation or sale of sperm or eggs to create embryos for the sole purpose of generating stem cell lines. It also urged a moratorium on creating human embryos by therapeutic cloning stating that the underlying science was flimsy and that the practice would inevitably lead down a slippery slope to human cloning (Kondro, 2001). Very recently, Germany also paved the way allowing researchers to import ES cell lines from other countries for research. However, the creation of human embryos solely for research as well as therapeutic cloning was disallowed (Steghauss – Kovacs, 2001).

Cross-species hybrid cells

31. One speculative means to the same end as therapeutic cloning is to produce non-viable human embryos within cow eggs for ES cell research. The concept is the same as

nuclear transfer except that the donor human cell is introduced into an enucleated cow egg instead of a human egg. Although theoretically feasible, one would have to be quite sure that the use of the animal egg as a host for the human cell has no adverse effect on the eventual human cell lines. Even though it would avoid the creation of a viable human embryo, the mixing of human and animal genetic material at such a profound level would raise major clinical and ethical objection by most people. Thus, cross-species experimentation must be strongly discouraged.

What happens once a HES cell line has been established?

32. Once an ES cell line is established the cells can go on proliferating forever in an undifferentiated state and can be made immortal. Thus the need for more embryos or cell lines is not necessary. A few cell lines are adequate for the whole world. At any stage of proliferation if a single ES cell is transferred to the uterus of a woman, it cannot develop into a complete human being. If transferred in large numbers into the human body without directed differentiation there is the risk of producing teratomas (tumours). Thus it is imperative that ES cells be first directed into specific cell types and tested in animal models before transplantation into humans.

33. Once ES-cell derived heart, nerve, blood etc cell types have been produced, their usefulness in curing disease though transplantation therapy should first be tested on laboratory and larger animal models before direct human transplantation. For this reason therefore transfer of human ES-derived cells to animals (xenografting) to ensure safety and efficacy must be allowed. Thus xenotransplantation of human ES derived cells to specific laboratory and large animals such as mice, rats, primates and pigs must be permitted for reasons of convenient testing, accurate assessment of functional clinical outcome, genetic closeness to the human and histocompatability. For research, the ES or ES-derived cells should not be sold to other researchers but instead distributed free so as to expedite the clinical benefits to mankind as soon as possible.

Clinical trials

34. The same regulations governing any clinical trial should be applied to ES-derived cells. Before applying such cells in the human, these newly derived cells must be screened for microbes and safety. There must be adequate counseling, informed consent, privacy and confidentiality regarding the clinical application. The participants should not be entitled to

any benefits or share in the fruits of success of the clinical trial. The possible cure of the specific illness in itself is a benefit. During the counseling process, the participant should be informed that the procedure is at his/her own risk and there would be no compensation for such risks.

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