The **Academy** *of* **Medical Sciences**

Inter-species embryos

A report by the Academy of Medical Sciences

June 2007

The Academy of Medical Sciences

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This report is published by the Academy of Medical Sciences and has been endorsed by its Officers and Council. Contributions by the working group are made purely in an advisory capacity. The review group added a further 'peer-review' stage of quality control to the process of report production. Reviewers were asked to consider whether the report met the terms of reference and whether the evidence and arguments presented in the report were sound and supported the conclusions. Reviewers were not asked to endorse the report or its findings. Members of the working group and the review group participated in this report in an individual capacity and not as representatives of, or on behalf of, their affiliated hospitals, universities, organisations or associations. Their participation should not be taken as endorsement by these bodies.

All web references were accessed in June 2007.

Contents

1 Summary

Understanding the nature and potential of stem cells – unspecialised cells that can self-renew and differentiate into specialised cell types - is an important field of research that many believe could open new avenues for the treatment of human disease and injury. Studying stem cells could help shed light on disorders that underlie many diseases, ranging from developmental abnormalities in young children to some cases of degenerative disease, infertility, stroke, spinal cord injury and cancer. Learning how to control stem cell differentiation and development could allow the production of specialised cells to treat conditions in which such cells are lost, such as childhood diabetes and Parkinson's disease. Understanding how adult cells can be re-programmed to become stem cells offers the potential for a step-change in treating human disease, potentially allowing transplantation of cells and tissues containing a patient's own DNA and avoiding problems of tissue rejection. The ability to generate specialised tissues in culture, or in animal models, could also facilitate the development and testing of new drugs before they are used in patients.

Research into tissue-specific stem cells found in cord blood and several adult tissues is important and should continue. However, human embryonic stem (ES) cells, derived from very early human embryos, could provide a uniquely flexible range of research possibilities and, eventually, potential treatments. Somatic Cell Nuclear Transfer (SCNT) techniques, in which the nucleus of an adult somatic cell is transferred into an oocyte from which the nucleus has been removed, offer one way to control the genetic composition of derived hES cells – an essential step if the full opportunities for disease modelling, drug discovery or individualised stem cell therapy are to be realised. However, the availability of human oocytes for SCNT is limited by the prior needs of patients undergoing fertility treatment and the invasiveness of the donation procedure.

In this report, we describe how the use of animal oocytes represents a valid and potentially important avenue in overcoming these limitations and advancing the science of SCNT and human ES cells. Recent research has been very promising in identifying some of the chemical factors necessary to re-programme somatic cells. Increased knowledge of factors required for efficient reprogramming will come from a range of experiments, including those involving SCNT, and from a better understanding of hES cells and their pluripotency. In the longer term, such knowledge could potentially lead to methods of direct re-programming without using oocytes or early embryos (whether human or animal), but achieving that goal will require a great deal of further research.

We consider the scientific, ethical and safety issues around the creation of cytoplasmic hybrid embryos generated by SCNT involving human nuclei and animal oocytes. We also consider the scientific uses of other types of inter-species embryo, broadly split into: i) *human* embryos incorporating animal material, either nuclear genetic material (transgenic human embryos) or cellular material (human chimeric embryos); and ii) *non-human* embryos and animals incorporating human material.

The current revision of UK legislation around human embryos offers an important opportunity to consider the future research potential of inter-species embryos in their full scientific, ethical and social context. Defined limits on such research must be set out in primary legislation: the creation and use of human embryos for research should only proceed under licence from the Human Fertilisation & Embryology Authority (or its successor); human embryos used for research should not be re-implanted into a woman or animal; and human embryos used for research should not be developed beyond 14 days *in vitro*. We consider that research on cytoplasmic hybrid, human transgenic or human chimeric embryos should proceed under a similar framework of regulatory control. In this way, permissible

developments in human embryo research - with clear limits - are set out in legislation, within which an informed regulator decides on individual research proposals.

The creation and use of *non-human* animals and embryos incorporating human material (transgenic or chimeric animals/embryos) already has a long and successful research history. However, the transfer of human ES cells, or increasing amounts of human genetic material, into non-human animals and embryos is likely to present increasing regulatory and ethical challenges in the future. The current review of legislation around human embryos represents one facet of the developing framework, but further consideration should be given to the interfaces between the regulation of animal research, human embryo research and human ES cell lines. The Academy of Medical Sciences will be undertaking further work on this issue, to include a significant component of public engagement, which we hope will inform future debate.

2 Scope and objectives of study

In February 2007, the Academy of Medical Sciences convened a working group to examine research involving embryos combining human and non-human material. The membership of the working group is annexed.

The terms of reference of the working group were to:

- **Propose definitions of embryos combining human and non-human material, and identify relevant research protocols.**
- **Identify and agree key opportunities for research using such embryos, and cells derived from them, together with an assessment of how these opportunities are balanced by safety and ethical concerns.**
- **Provide recommendations where appropriate.**

Working group meetings were observed by representatives from the Medical Research Council, Royal Society and Wellcome Trust. This report was reviewed by an external panel (see Annex I) and has been endorsed by the Academy's Council.

This report is designed for policy makers in Government, research funders, universities and relevant professional and regulatory bodies, as well as all other interested parties. It reviews the nature and importance of stem cell research, current and potential sources of stem cells and cell lines, and potential uses of cells and embryos combining non-human and human material. Ethical, safety and regulatory issues relating to embryos and cells combining human and non-human material are considered in the context of previous studies involving inter-species constructs and the potential value of such research. A glossary of terms is given in Annex II.

3 Stem cells

3.1 Background

The human body is made up of somewhere between 10^{13} and 10^{14} cells, most of which belong to specialised cell types, e.g. bone, nerve or liver cells. Yet each of these different cells is descended from a single cell – the fertilised egg. As the egg cell divides and the resultant embryo develops, the cells within begin to differentiate – to specialise into lineages that will perform particular functions. Cell differentiation is usually a one-way process, so that cells committed to a specific developmental pathway do not revert back or develop into something different.

Stem cells are unspecialised cells that have the ability to proliferate indefinitely, producing both more stem cells (a process called self-renewal) and cells that commit to a pathway of differentiation into specialised cell types. Depending on the range of specialised cell types they can produce, stem cells are defined as totipotent, pluripotent, multipotent or unipotent. These terms, particularly totipotent, have evolved over the years, as new research findings have shaped their scope and application. We use totipotent to describe a cell capable of differentiating into all cell types (including cells of the extraembryonic[1] and embryonic tissues) in a manner that is specifically ordered and organised to allow embryonic development.[2]

Pluripotent cells are the descendants of totipotent cells and can differentiate into all cell types ofthe developing embryo, including some extraembryonic cells. However, pluripotent cells have lost the ability to organise themselves into a proper embryo.[3] Pluripotent stem cells can be derived from the early embryo or, with slightly different properties, from germ cells within the later embryo (EG cells) or postnatal testis (from spermatagonia). Multipotent stem cells usually produce only a closely related set of cell varieties according to the tissues in which they reside (e.g. types of blood or gut cell). Unipotent stem cells give rise to a single specialised cell type (e.g. skin epidermal stem cells or muscle satellite cells). Stem cells occur in many different tissues and at different stages of development – from the early embryo to the adult organism. It has been possible to isolate some types of stem cell and, in a few cases, to maintain them as pure populations *in vitro*.

3.1.1 Tissue-specific stem cells

Tissue-specific (also sometimes called adult) stem cells are found in many tissues such as the bone marrow, muscle and intestine. They are often multipotent and can develop into a range of cell types related to the tissue from which they are derived. For instance, blood (or haematopoietic) stem cells can divide for the lifetime of an animal and continuously produce progenitor cells that give rise to red or white blood cells, as well as various other related cell types. These progenitor cells can divide for a fixed period, but if they are committed to a line of differentiation they are no longer true stem cells.

Tissue-specific stem cells are generally thought to occupy special micro-environments or 'niches' in the tissue. Although there are intrinsic mechanisms regulating stem cell properties, the niche can often influence whether the stem cells divide, and whether they self-renew or differentiate.[4]

Tissue-specific stem cells are normally involved in tissue renewal and repair and would therefore seem to be ideal candidates for cell-based clinical therapies, especially if they can be isolated from the patient themselves (avoiding problems of graft rejection). Established treatments such as bone marrow, skin and corneal transplants, all effectively work by transplanting compatible tissue-specific stem cells.[5]

1 Extraembryonic describes cells outside the embryonic body, including those membranes involved in the embryo's protection and nutrition, which are discarded at birth without being incorporated into its body.
2 In this sense, in mammals only the fertilised egg and the cells produced by its first few divisions are therefore totipotent.
3 Although pluripotent cells can do so when combined with a normal embryo in chimeras.
4 It can be operationally difficult to distinguish between precursors and stem cells. Indeed, precursor cells have been shown in some cases to change into stem cells, especially where stem cells have been lost.
5 In some cases of cancer, allogenic transplants of bone marrow work better.

However, the scientific and medical application of tissue-specific stem cells faces several challenges:

1. Adult stem cells may have a limited repertoire, only giving rise to some cell types within a tissue or organ. This may be because other cell types in the organ have their origins in early embryonic development. For example, adult Central Nervous System (CNS) stem cells can give rise to neurons of the olfactory bulb and the dentate gyrus, but they have not (yet) been induced to form motor or dopaminergic neurons (the former needed for treating motor neuron disease, the latter for Parkinson's Disease).
2. The disease in question may itself involve a loss of stem cells, which can not therefore be isolated from the patient; the stem cells could carry a genetic defect, as would be the case for many inherited diseases; or the appropriate stem cells may be inaccessible, as is the case for CNS neural stem cells.
3. Not all adult tissues contain stem cells.

There have been reports that, under specific conditions, adult stem cells may show broader potential. For example, it has been claimed that haematopoietic stem cells can give rise to epithelial cells or brain neurons. This phenomenon is sometimes referred to as stem cell transdifferentiation or plasticity. Researchers have attempted to induce plasticity by modifying the *in vitro* culture medium or by transplanting the cells into a different organ of the body. However, the validity and reproducibility of these studies is controversial and there is no convicing evidence that such plasticity is a significant phenomenon in normal mammalian physiology. Often the number of transdifferentiated cells obtained is extremely low, and therefore of very limited practical use. It is also unclear whether such cells truly function correctly. Indeed, many of the experiments claiming to demonstrate plasticity *in vivo* were complicated by cell fusion, where the transplanted cells, or their progeny, fused with differentiated cells of the host to produce tetraploid cells (which have twice the normal number of chromosomes).[6]

Whatever the final potential of tissue-specific stem cells might turn out to be, at the moment it seems that only a very limited range of diseases may be treatable by this approach. As discussed below, there are sound practical and theoretical reasons for considering that embryonic stem cells currently provide a more flexible range of research options and routes to treatments, if we learn how to direct them down different pathways of differentiation (see 3.1.4). At this stage of research, the best option to maximise the chances of developing a wide range of effective treatments is to pursue both embryonic stem cell and tissue-specific stem cell research in parallel.

3.1.2 Cord blood and fetal stem cells

Cord blood stem cells are found in the blood of the umbilical cord and can be extracted at birth (and stored frozen). Since the 1990s, the ability of cord blood stem cells to replace bone marrow and blood cells has been used in the treatment of leukaemia and other disorders. There have been claims that cord blood stem cells have wider potential, but these remain controversial and the physiological relevance is unknown.[7] The study of cord blood stem cells has been hindered by the lack of robust methods for their culture and expansion *in vitro*,[8] as well as ways to identify and follow the fate of single cells. For example, some of the results obtained could be explained if there was more than one type of stem cell or committed progenitor in the starting cell population; it is possible, for example, that cord blood contains mesenchymal progenitors, as well as haematopoietic stem cells.

Fetal stem cells can be derived from several tissues of the embryo, for example from the developing nervous system following elective

6 For example, see Ying QL *et al.* (2002). *Changing potency by spontaneous fusion.* Nature **416**, 545-8.

7 Royal College of Obstetricians and Gynaecologists (2006). *Umbilical cord blood banking. Scientific Advisory Committee Opinion Paper 2*. http://www.rcog.org.uk/resources/Public/pdf/umbilical_cord_blood_banking_sac2a.pdf

8 Although there are reports that this may be possible, for example, Forraz N *et al.* (2004). *Characterisation of a lineage-negative stem-progenitor cell population optimised for ex vivo expansion and enriched for LTC-IC.* Stem Cells **22**, 100-8.

termination of pregnancy. It is these cells that have formed the basis for clinical trials of stem cell-based therapies in the brain. Clinical trials for the use of fetal stem cells in therapies for Huntington's Disease are underway and trials for Batten's Disease and for stroke are currently seeking approval. Very recently, reports have claimed that cells isolated from amniotic fluid show a high degree of multipotentiality. However, there is still uncertainty about the true nature of such cells and their physiological relevance.[9]

3.1.3 Germ cell derived stem cells

Stem cells can also be isolated from the gonads of an early foetus. These are derived from the germ cells (cells that will eventually give rise to sperm or eggs) and are pluripotent. They retain the capacity to develop into many or all cell types of the body. While in theory they are a readily available source of stem cells, and are very similar to embryonic stem cells, they are not so straightforward either to derive or maintain in culture. Moreover, they can be variable in their properties according to the precise stage of their isolation.[10] Recently, claims have been made that pluripotent stem cells can be derived from adult testis in mice, but these have yet to be independently substantiated.[11]

3.1.4 Embryonic stem cells

Embryonic stem (ES) cells are pluripotent cells derived from the undifferentiated cells of an early stage embryo known as a blastocyst. In human embryos, the blastocyst stage is reached around 5 days after fertilisation, at which time the embryo consists of 50-150 cells. Deriving ES cells involves transferring the undifferentiated cells that make up the Inner Cell Mass (ICM) into culture, where the cells are supported by nutrients and protein growth factors provided in the culture media. Once isolated, ES cells can be induced to proliferate indefinitely, forming 'immortal' ES cell lines that retain the capacity to differentiate into many different cell types (determining the pluripotency of ES cell lines is discussed in Box 1). There is considerable experience of generating mouse ES cell lines in the laboratory, but human ES (hES) cell lines are of more recent origin and there is still much to learn about their properties.

In the UK, the use of human embryos in research is currently governed by the Human Fertilisation & Embryology Act 1990 (see Box 2), which is expected to be updated by an amending Act in 2008. To date, around 400

Box 1 Determining pluripotency

Traditionally the pluripotency of mouse ES cells has been demonstrated by three main methods:

1. Differentiation *in vitro* where, by changing the culture conditions, the ES cells can give rise to a wide range of cell types.
2. Differentiation *in vivo* as tumours termed 'teratomas' or 'teratocarcinomas' after their introduction into ectopic sites (usually under the skin, the kidney capsule or into the testis) of either genetically matched or immuno-compromised mice. These tumours contain a wide range of cell types, including some organisation into discrete tissue types.
3. Determining their ability to participate in normal embryonic development after reintroduction into blastocyst stage mouse embryos, which are then implanted into the uterus of receptive female mice (surrogate mothers).

It is this last method that provides the strictest test of potential, as it is possible to screen for contributions from the ES cells to all tissues of the resulting offspring. It is against the law to perform this test in human embryos. The term 'pluripotent', when associated with hES cells, should therefore be used with the caveat that it is currently only possible to test this by *in vitro* differentiation and/or by the ability to make many tissues in teratomas in mice.

9 De Coppi P *et al.* (2007). *Isolation of amniotic stem cell lines with potential for therapy*. Nature Biotechnology **25**, 100-6.
10 This is at least in part due to progressive changes in genomic imprinting – which affects the activity of many genes.
11 Guan K *et al.* (2006). *Pluripotency of spermatogonial stem cells from adult mouse testis.* Nature **440**, 119-1203.

hES cell lines have been derived worldwide, almost all of which come from early embryos (blastocysts) donated by patients undergoing *in vitro* fertilisation (IVF) treatment. A small number of embryos are donated by patients following Preimplantation Genetic Diagnosis (PGD), which is used to test embryos for serious genetic disease. Stem cells derived from PGD embryos will therefore often carry mutations for genetic diseases. These cell lines will not be useful for therapy, but they can provide valuable tools for research into the mechanisms of disease caused by the genetic fault and could potentially be used in the discovery and testing of drugs. Apart from embryos resulting from the limited number of diseases tested for by PGD, the genetic constitution of donated embryos (and derived hES cell lines) is a matter of chance and cannot be chosen for individual research programmes.

ES cell research might eventually increase our understanding of the biochemical states that underlie pluripotency, how cell fate decisions are reached, the stability of the differentiated state, and mechanisms of cell regeneration and re-programming. hES cells from individual patients could provide valuable tools in

Box 2 Embryo research under the UK Human Fertilisation & Embryology Act 1990

The creation and use of human embryos in research is permitted in the UK under the Human Fertilisation & Embryology (HFE) Act 1990. Embryo research is subject to legally defined limits and licensing on an individual project basis by the Human Fertilisation & Embryology Authority (HFEA).

The Act permits research on human embryos only for strictly defined purposes:

- Promoting advances in the treatment of infertility.
- Increasing knowledge about the causes of miscarriage.
- Increasing knowledge about the causes of congenital disease.
- Developing more effective techniques of contraception.
- Developing methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation.
- Or for such other purposes as may be specified in regulations.

In 2001, the Human Fertilisation and Embryology (Research Purposes) Regulations extended the purposes for which an embryo could be created to include:

- Increasing knowledge about the development of embryos.
- Increasing knowledge about serious disease.
- Enabling any such knowledge to be applied in developing treatments for serious disease.

Research on somatic cell nuclear transfer is allowed under these provisions (see section 4). Embryos created by somatic cell nuclear transfer (in common with other embryos created outside the body for research) can be kept only up to 14 days (shortly before the appearance of the primitive streak). In addition, it is a criminal offence to implant embryos created by somatic cell nuclear transfer into a woman, under the Human Reproductive Cloning Act 2001.

The Human Tissue and Embryos (Draft) Bill, published by the Department of Health in May 2007, set outs proposals to update the 1990 Act.[12]

12 http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsLegislation/DH_074718

studying the nature and underlying causes of different diseases, as well as the efficacy and safety of treatments. In theory, a genetic fault present in a patient could be repaired during ES cell culture prior to treatment. This has already been successfully demonstrated in a mouse model.[13] In principle, it may one day be possible to control cell differentiation by manipulating the environment in which the ES cells are grown. To some extent, this can already be achieved to produce certain cell types, but in general this is a long way off and still requires much additional research.

One of the long-term aims of stem cell research is to develop cell-based therapies, i.e. to use hES cells to replace damaged cells and tissues in patients. However, successful transplantation of hES cells will have to overcome the problem of immunological rejection common to all types of foreign organ and tissue transplants. One solution is to develop large banks of genetically diverse hES cells to increase the chances that adequate matches can be found for all patients. Another avenue would be to genetically modify hES cells to reduce immunogenicity. In the long term, researchers hope to derive hES cells using a patient's own genetic material, thus ensuring that transplanted material is immune-compatible, thereby avoiding the risk of graft rejection and reducing the need for immunosuppressants.

13 Rideout WM *et al.* (2002). *Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy*. Cell **109,** 17-27.

4 Somatic Cell Nuclear Transfer (SCNT)

SCNT involves the transfer of the nucleus of, for example, an adult somatic cell[14] into an oocyte from which the nucleus has been removed. As yet unknown factors in the oocyte cytoplasm re-programme the somatic cell nucleus so that the resultant cell regains totipotency. An electric pulse is applied to the oocyte to activate early development and cell division. After a few days in culture, a proportion of the early embryos derived in this way form blastocysts, from which ES cell lines can be derived. The nuclear genetic material of blastocysts formed by SCNT is identical to the donor of the specialised cell, not the donor of the oocyte. The oocyte does provide some genetic information in the form of the mitochondrial DNA, but the genes in the nucleus are of overriding importance, the nuclear genes being responsible for determining the vast majority of traits in the developing and adult organism (see Box 3 and section 8.1.1).

This process, in which a somatic nucleus is re-programmed or dedifferentiated by transfer into an oocyte cytoplasm, brings the significant advantage that the genetic make-up of the resulting ES cells can be controlled, depending on the somatic nucleus used. This could include nuclei with specific genotypes, and those that are immunologically compatible with individual patients. SCNT therefore has the potential to generate hES cells of defined genotype that can address issues of genetic diversity, causes of disease, development of pharmaceuticals and transplant rejection.

Implantation of a blastocyst derived using SCNT could potentially lead to a live-born offspring - a clone of the nuclear donor. This technique was most famously used by researchers at the Roslin Institute to create Dolly the sheep.[15] Since the birth of Dolly, live cloned offspring have been created from several other mammalian species, including mice, goats, pigs, rats and cats. However, the success rate of live births is very low and a variety of abnormalities have been found in cloned animals.[16] Applying this technique of reproductive cloning to humans is illegal in the UK and elsewhere. The international regulatory context around stem cell research is discussed in Box 4.

Derivation of pluripotent mouse ES cells from a cloned SCNT blastocyst has been successfully demonstrated by a number of research groups.[17] Furthermore, the ES cells derived from SCNT embryos appear identical to ES cells derived from normal fertilised embryos.

Box 3 DNA-containing structures within a cell

A cell consists of a membrane enclosing a gel-like substance known as the cytoplasm. Within each cell is a nucleus containing the chromosomes – the DNA that encodes the genetic material of the cell. Each time a cell divides, the DNA also divides so that each daughter cell contains a full copy of the genetic material.

There are many different organelles performing different functions within the cytoplasm. Amongst these are the mitochondria, which are concerned with energy metabolism. Each mitochondrion contains a large number of proteins, predominantly derived from the genes in the nucleus. However, each mitochondrion also contains DNA encoding some of the mitochondrial proteins. Like the nucleus, mitochondria have the capacity to divide and be passed on to daughter cells. However, unlike the nucleus, mitochondrial replication is not strictly tied to cell division, and the numbers of mitochondria in a cell can increase or decrease depending on the nature of the cell and its environment.

14 A somatic cell is generally taken to mean any cell forming the body of an organism – it does not include cells of the germline.
15 Campbell KH *et al.* (1996). *Sheep cloned by nuclear transfer from a cultured cell line.* Nature **380**, 64-66.
16 National Research Council (2002). *Scientific and Medical Aspects of Human Reproductive Cloning*. National Academy Press. Washington D.C.
17 For example, Wakayama T *et al.* (2001). *Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer.* Science **292**, 740-743.

However, successful production of ES cells is still very inefficient (less than 5%). Several research groups are attempting to derive ES cells from cloned human embryos, which is proving to be extremely challenging. To date there are very few published reports of embryos being produced by SCNT using human somatic cell nuclei and enucleated human oocytes, and none of these developed sufficiently far to be used for ES cell derivation. One apparent exception was thought to be the work of the Korean research group led by Woo Suk Hwang, who claimed that several cloned human blastocysts had been derived via SCNT. However, it now appears that evidence of derived hES cell lines was fabricated and this work has been discredited.

Box 4 Human ES cells: the international context

Human ES cell research is developing within a complicated global patchwork of regulation, politics, ethics and funding, which can make international collaborations difficult (UK regulations are discussed in Box 5). There are several guidance documents in existence, notably '*Guidelines for Human Embryonic Stem Cell Research*' from the US National Research Council and Institute of Medicine (2005), '*Guidelines for the Conduct of Human Embryonic Stem Cell Research*' from the International Society for Stem Cell Research (2006) and the Hinxton Group's consensus statement on human ES cell research (2006). The International Stem Cell Forum has funded a database detailing the current regulatory frameworks governing stem cell research in over 50 countries.[18]

There are many countries that permit the derivation of hES cells from 'spare' embryos from IVF treatment, including the UK, Sweden, France, Spain, Switzerland, Czech Republic, Israel, Brazil, Iran, Japan, China and South Korea. In Australia, following the Lockhart Legislative Review, the generation of embryos for research or derivation of ES cells lines - including SCNT – is now permitted under licence. A few countries have prohibited all human embryo research and hES cell derivation including Italy, Germany, Austria, Norway, Ireland, Poland and Slovakia.

In the US, federal funding of human embryo research is not permitted, although it can be used for research involving hES cells lines derived before 2001. Privately funded human embryo research and derivation of hES cell lines is not regulated under federal law. Several US states permit and fund hES cell research, including California, New Jersey, Massachusetts, Connecticut, Pennsylvania and Illinois. Restrictive legislation (above and beyond any regulations around funding) has been passed in Michigan, Arizona, Louisiana, Minnesota, North and South Dakota.

18 http://www.stemgen.org

5 Sources of oocytes for SCNT

Undertaking SCNT research on human cells requires somatic cell nuclei, which are readily available from most tissues, but also human oocytes, which are much more limited. Many scientists and others consider the availability of human oocytes to be a major limitation to this research. Current and potential future sources of oocytes are discussed below.

5.1 Oocyte donation

The donation of eggs from IVF programmes is currently the primary source of oocytes used in the UK, but it will always be limited. Altering the IVF procedure to induce more oocytes than are needed for reproductive purposes is rightly considered unethical, since it alters medical practice to the potential detriment of the patient. The possibility of altruistic third party donation of oocytes from women not undergoing fertility treatment also raises safety and ethical considerations that are summarised in a recent report from the US National Academy of Sciences.[19]

The retrieval of oocytes from a woman involves hormone treatment that causes 10-20 oocytes (instead of the usual single oocyte) to mature in the ovaries at the same time. The treatment involved can have a variety of health effects, the most serious – if preventative steps are not taken - being Ovarian Hyper Stimulation Syndrome (OHSS). In its severest form, OHSS can be life threatening when excess fluid accumulates in the abdominal cavity and sometimes the chest. Data from women undergoing IVF indicate that 0.1-0.2% experience severe OHSS. The risks for egg donors should be even lower, since the stimulation procedure can be abandoned if the early response to stimulation is seen to be excessive.

Concern has been raised that the use of fertility drugs may lead to increased risk of hormone-dependent cancers, e.g. breast, ovarian and uterine cancers.[20] More research is needed, but epidemiological studies currently suggest this risk to be either very low or absent. There is at present no evidence on the possible effects of ovarian stimulation on a woman's long-term fertility.

Removing oocytes from a donor requires the insertion of a needle through the wall of the vagina and into the ovary, performed under heavy sedation or anaesthesia. While both surgery and anaesthesia carry inherent risks, experience with IVF patients shows these risks to be very low; one study of several hundred thousand surgeries showed that only 0.002% of women experience complications. Again, there are no data to suggest that stimulation of the ovary or egg retrieval surgery affect a woman's future fertility.

Ethical considerations are raised if substantial payments are made for donated oocytes.[21] For instance, the nature of informed consent in such circumstances could be problematic (although it should be noted these problems are not exclusive to this field). The HFEA conducted a public consultation on the issue of oocyte donation for research in 2006, asking respondents to assess the risks and benefits and any safeguards that would need to be in place. In February 2007, the HFEA announced that it had agreed '*to allow women to donate their eggs to research, either as an altruistic donor or in conjunction with their own IVF treatment'*.[22] The HFEA went on to say that '*given that the medical risks for donating for research are no higher than for treatment, we have concluded that it is not for us to remove a woman's choice of how her donated eggs should be used'*. In allowing this activity, the HFEA has set out safeguards involving a clear separation between the researchers and people carrying out treatment, the provision of detailed information on the realistic outcomes of the research, and a requirement for the person obtaining consent to be independent of

19 National Academies of Science (2007). *Assessing the medical risks of human oocyte donation for stem cell research: workshop report.* National Academies of Science. Washington USA.

20 Check E (2006). *Ethicists and biologists ponder the price of eggs*. Nature **422**, 606-7; Pearson H (2006). *Health effects of egg donation may take decades to emerge*. Nature **422**, 608.

21 ISCF Ethics Working Party Letter to the Editor (2007). *Oocyte Donation for Stem Cell Research.* Science **316**, 368-369.

22 See http://www.hfea.gov.uk/cps/rde/xchg/SID-3F57D79B-EE78FF9E/hfea/hs.xsl/1491.html

the research team. The HFEA has also stated that payment of donors over and above any expenses incurred is not permitted, although it should be noted that IVF costs may be reimbursed under egg sharing schemes.

A recent *Science* editorial made the point that '*the long waiting list of women needing donated eggs to have babies demands that scientists wanting such eggs for stem cell research act with great restraint.'*[23] **Given the nature and complexity of the procedure, we consider it improbable that donation will provide an adequate source of human oocytes to meet future research needs.**

5.2 Oocytes matured from oophorectomies or fetal ovaries from pregnancy terminations

It is possible that oocytes could be harvested from adult ovaries donated either posthumously or after removal for clinical reasons, or from fetal ovaries obtained from pregnancy terminations. While it is possible to mature fully grown human oocytes in culture, most of the oocytes obtained in this way are very small and contained in primordial follicles. Research in other mammals has shown that it is possible to grow and mature such oocytes in culture and to achieve fertilisation and normal development, although the process is inefficient. Success has been limited in humans and requires an intermediate xenograft of the ovarian tissue (into a non-human host) or very long-term cultures for oocyte growth. **More research is needed into how human oocytes can be grown and matured *in vitro*.**

5.3 Derivation of oocytes from non-reproductive material

ES cells, being pluripotent, are able to differentiate into germ cells as well as other cell types. This has been clearly demonstrated *in vivo* in mouse chimeras and a relatively recent report claims that cells resembling oocytes can be formed from mouse ES cells in culture.[24] If this approach could be extended to human ES cells, it could provide a renewable source of oocytes that could reduce the demand for donated eggs.[25] However, it is still not clear whether the reported ES cell-derived gametes are functional. **As yet, we have no information as to whether oocyte-like entities derived from mouse ES cells are capable of re-programming somatic cell nuclei in the same way as normal oocytes and more research is needed.**

5.4 Use of non-human animal oocytes

Several research groups are exploring the possibility of using animal oocytes in SCNT and there are at least three UK groups that would like to pursue this approach, two of which have already applied to the HFEA for a licence. This would involve the transfer of a human somatic nucleus into an animal oocyte, (e.g. from a cow or rabbit) from which the nuclear material has been removed. If this were successful, the nuclear genome would be entirely human (but with some animal mitochondria, at least initially). The cell would then be induced to divide to the blastocyst stage, at which point cells could be extracted to form ES cell lines. There are several reports of inter-specific nuclear transfer leading to blastocyst stage embryos. To date, there is one report from a laboratory in China of putative ES cell lines produced after transfer of human nuclei to rabbit oocytes,[26] but this work has yet to be reproduced. The scientific, ethical and regulatory issues associated with using animal oocytes are explored in further detail in section 8.1.

23 McLaren A (2007). *Free-range eggs?* Science **316**, 7
24 Hubner K *et al.* (2003). *Derivation of oocytes from mouse embryonic stem cells.* Science **300**, 1251-1256.
25 Evans M (2005). *Ethical sourcing of human embryonic stem cells-rational solutions?* Nature Reviews Molecular Cell Biology **6**, 663-7.
26 Chen Y *et al.* (2003). *Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes.* Cell Research **13**, 251-263.

5.5 Re-programming cells without oocytes

5.5.1 Use of fertilised eggs

It has very recently been reported that fertilised mouse eggs can be used instead of oocytes for SCNT if they are blocked in the process of mitosis, a stage during cell division when the replicated chromosomes are lined up on the spindle waiting to be separated so that they end up in the two daughter cells. At this stage the chromosomes are not surrounded by a nuclear membrane and it appears that the re-programming factors are dispersed in the cytoplasm.[27] By removing the chromosomes and replacing them with those of the donor somatic cells, it was shown that the latter can be reprogrammed and the resulting embryos could give rise to cloned embryos. This work was technically difficult and it is not clear how readily it could be adapted to human fertilised eggs. However, **since fertilised eggs are generally more available than unfertilised eggs,[28] this is a promising approach and we predict that UK research groups will soon want to explore these techniques.**

5.5.2 'Direct' re-programming

A key research goal is to re-programme adult somatic cell nuclei directly – not by transfer to oocytes, but by other means, e.g. fusion with pluripotent ES cells or exposure to factors from such pluripotent cells.[29] A very new, but exciting, possibility has arisen from research in mice showing that it is possible to re-programme fibroblasts (a type of differentiated cell found in the skin and elsewhere) into ES-like cells.[30] These cells, termed 'iPS' for induced pluripotent cells, are derived using retrovirus vectors to insert extra copies of four transcription factors characteristic of ES cells - Oct4, Sox2, Klf4 and cMyc - into fibroblasts.

Research has shown that at least some iPS cell lines can contribute extensively to chimeric mice after they are introduced into blastocyst stage mouse embryos. These are promising results, but the low efficiency of reprogramming (on average 1 in 10,000 cells) and length of time involved (14-20 days) suggest that other factors could be found that might increase efficiency. This work offers the hope that similar methods can be applied to directly re-programme human cells into ES-like cells. However, hES cells are different in many ways from mouse ES cells and there is no guarantee that the same 4 factors would have the same effect. Moreover, any future human applications would bring safety concerns around using retroviral vectors and altered expression of genes such cMyc, which is an oncogene and can cause cancer.

Increased knowledge of factors required for efficient reprogramming will come from SCNT experiments and from a better understanding of hES cells and their pluripotency. **In the longer term, such knowledge could potentially lead to methods of direct re-programming without using oocytes (whether human or animal) or early embryos, but achieving that goal will require a great deal of further research and it would be premature to assume at this stage that this approach will prove successful.**

27 Previous failed attempts to use fertilised eggs had involved removing their nuclei, and presumably therefore the reprogramming factors.

28 e.g. Supernumerary fertilised eggs left over from IVF and stored frozen; abnormal fertilised eggs that have more than two pronuclei.

29 Takahashi K & Yamanaka S (2006). *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.* Cell **126**, 663-676.

30 Okita K *et al.* (2007). *Generation of germline-competent induced pluripotent stem cells.* Nature June 6 (e-publication ahead of print*);* Wernig M *et al.* (2007). *In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state*. Nature June 6 (e-publication ahead of print*);* Maherali N *et al.* (2007). *Directly reprogrammed fibroblasts show global epigenetic remodelling and widespread tissue contribution.* Awaiting publication.

6 The history of human-animal constructs

Using SCNT techniques to transfer a human nucleus into an animal oocyte would generate an inter-species construct – an embryo containing both human and animal material. Much of the remainder of this report considers the issues raised by this and other types of inter-species constructs. In this section we briefly outline the history of inter-species constructs and their use for research.

Superficially, we are accustomed to thinking of different animal species as being completely separate from each other. But we know that this is not entirely true; for example, a mule is a hybrid resulting from mating a donkey and a horse. It is surprisingly difficult to define the word 'species' in a way that is easily applicable to many biological situations. Most textbooks use the term to describe organisms that can interbreed at maturity to produce fertile offspring.

In the laboratory, it is possible to create cells *in vitro* with genetic contributions from different species. Techniques in which human and animal (usually mouse or hamster) cells were fused to produce 'inter-specific cell hybrids' were developed as research tools in the 1960s and were the basis for early mapping studies on human genes in the 1970s, eventually leading to the highly successful Human Genome Project. Thousands of such cell lines have since been generated, involving many different species combinations, and have contributed extensively to knowledge of human genetics and cell biology.

The introduction of human gene sequences into mouse cells *in vitro* is a technique now practised in virtually every biomedical research institution across the world, for instance to express a specific gene to obtain a protein. The identification of oncogenes by transferring DNA from human cancer tumours into mouse cell lines is one among many examples of a technique that has become integral to molecular biology. In these cases it is the cross species difference that is essential in identifying the relevant gene. These techniques have also been extended to provide important clinical treatments, for example the human protein erythropoietin (EPO), which is used to treat anaemia resulting from chronic renal failure or cancer chemotherapy, is produced in hamster cell lines. 'Humanised' mouse antibodies (see below) are also now becoming an important new source of effective treatments for cancers.

Detailed investigation of gene sequences has shown there to be remarkable levels of conservation between widely divergent species. This is most strikingly shown by the fact that some gene sequences from human cells can function in yeast cells and vice versa, demonstrating the stability of these basic building blocks of life over very long periods of evolutionary divergence. At the genetic level, the differences between mammalian species rest upon exceptional differences within large areas of similarity, and the critical areas that determine species characteristics have not yet been fully identified.

This conservation has been exploited through the insertion of genetic material from one species into the developing embryo of another, creating 'transgenic' animals. The deliberate addition of human genes to mouse embryos to create mouse strains mimicking aspects of human disease is an important technology in biomedical research. Thousands of different mouse strains have been created in this way, often permitting research and testing that could not reasonably be carried out in humans.[31] In one example amongst many, mice have been produced that recapitulate the brain lesions and memory loss associated with Alzheimer's Disease, and have been used to test pharmacological and vaccine strategies for treating this debilitating disease. Analogous experiments form a key part of research into HIV, hypertension and very many other diseases. One of the most notable recent

31 Schultz *et al.* (2007). *Humanized mice in translational biomedical research.* Nature Review Immunology **7**, 118-130.

advances has been the creation of a mouse strain that carries an almost complete extra copy of human chromosome 21, designed to facilitate research into Down's syndrome.[32]

In a recent paper, a UK research group demonstrated the precise replacement of a large segment of mouse genome, containing multiple gene loci, with a corresponding segment of the human genome.[33] The researchers replaced the mouse α globin regulatory domain with the human region, and were able to establish that the human genes were expressed in an appropriate developmental stage and tissue-specific manner. As a next step, the researchers intend to generate mice with different mutations in the human gene regulatory sequences, in order to create disease models of thalassaemia.

Other examples include mice that have been generated with human immune systems – so called huMab mice. In these 'humanised' mice the large antibody-encoding genes have been replaced by their human counterparts. The two genes encoding the heavy and light chains of the antibodies are very large and complex loci, requiring the replacement of more than 1 million bases of mouse DNA with the human equivalent. These mice have been used to address a variety of research questions, but have also been used to generate 'humanised' monoclonal antibodies, which are increasingly utilised to produce antibody-based drugs such as the cancer treatment avastin.[34]

32 O'Doherty A *et al.* (2005). *An aneuploid mouse strain carrying human chromosome 21, with Down's syndrome phenotypes.* Science **309**, 2033-2037.
33 Wallace HAC *et al.* (2007). *Manipulating the mouse genome to engineer precise functional syntetic replacements with human sequence.* Cell **128**, 197-209.
34 There are currently 23 approved therapeutic monoclonal antibodies: 12 are humanised, 2 are fully human, 5 are chimeric and 4 are mouse.

7 Definitions

7.1 Inter-species constructs

Given the long history of inter-species constructs and the large body of work involving transgenic animals and cell hybrids (see previous section), it is important to be clear about the exact nature of the entities under discussion. There is a reasonably well-established scientific nomenclature concerned with cells, embryos and animals containing material from more than one species. This field has not previously generated a significant public discussion, and we welcome the attention now being drawn to it. While appreciating that wider public usage often requires some evolution of scientific language, it seems helpful to start by broadly defining the terms currently accepted in the scientific literature.

There are two factors that should be taken into account when discussing biological entities containing material from more than one source:

1. Whether the sources of material are from the same species (intra-specific) or from different species (inter-specific).
2. Whether the entities involved are cells, embryos or later stages.

Definitions are given in Table 1.

7.2 Embryos and embryonic stem cells

It is operationally important to distinguish between the creation and use of human embryos for research, which fall under HFEA regulation, and research involving hES cells *in vitro*, which is not directly regulated by the HFEA (but is overseen by the UK Stem Cell Bank Steering Committee – see Box 5). The crucial point is that very early embryos are totipotent, i.e. the cells can self-organise in a way that, in the appropriate environment, enables embryonic development. Currently, SCNT techniques use an enucleated oocyte to de-differentiate (re-programme) a somatic cell, such that the combined egg and nucleus become totipotent. As mentioned previously, the identification of appropriate re-programming factors brings the possibility of reproducing such re-programming in the test tube, so obviating the need for nuclear transfer into an egg. Importantly, while the presence of such factors in the test tube would provide an environment for cell re-programming, it does not necessarily follow that embryonic development would be supported. It is notable that a recent report of the conversion of mouse fibroblasts to pluripotency by gene transfer in the test tube produced ES-like cells and not embryos, i.e. the cells were not totipotent (see section 5.5.2).[35]

Box 5 UK Regulations on human stem cells

In the UK, the use of human stem cells in research is overseen by the Steering Committee of the UK Stem Cell Bank, which was established in 2003 in response to Government recommendations to facilitate '*the sharing of quality controlled human stem cell lines by the clinical and research communities*'. The role of the Steering Committee is to ensure that research is conducted within an ethical framework that is transparent to the public and in keeping with HFEA regulations. The Steering Committee has published a Code of Practice to explain its role and to provide guidance on best practice to those working with human stem cells.[36] The oversight mechanisms governing research on established human stem cell lines and the Code of Practice are voluntary. However, compliance is a condition of any license issued by the HFEA to create or use human embryos to generate stem cells.

35 Takahashi K & Yamanaka S (2006). *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors*. Cell **126**, 663-676.

36 The Code of Practice for the Use of Human Stem Cell Lines can be downloaded from http://www.ukstemcellbank.org.uk

	Cells and cell lines	Embryos	Animals
Hybrid	Created by the fusion of different cell lines, e.g. human cells fused with mouse cells. Each hybrid cell initially contains roughly equal genetic contributions from each species. Routinely used in research.	Created by the fusion of gametes from two different species. Each hybrid cell of the embryo contains roughly equal genetic contributions from each parent. Most hybrid embryos cannot develop successfully.	An animal resulting from the development of a hybrid embryo, i.e. created by mating between two different species, e.g. a mule. Each cell of the animal contains roughly equal genetic contributions from each parent.
Chimera	Not used.	Created by inserting one or more cells from one embryo or ES cell line into another early embryo. Often known as 'primary' chimeras. The genetic material is not combined within individual cells. Can be intra- or inter-specific. Mouse chimera embryos are routinely used in research.	An animal resulting from the development of a 'primary' chimeric embryo, or a 'secondary' chimera animal, into which cells have been introduced at a later stage of development (e.g. post-natally). The genetic material is not combined within individual cells. In primary chimeras, the progeny of donor cells will be in many or all of the tissues, but in secondary chimeras, the progeny may only be present in a few tissues. Can be intra- or inter-specific.[37]
Transgenic	Cells into which genetic material from a different genotype or species has been inserted. Routinely used in research.	An embryo whose cells contain genetic material transferred from another individual of the same (intra-specific) or different species (inter-specific). Several techniques have been used, including pro-nuclear microinjection of DNA and via the production of chimeras using genetically altered ES cells.[38]	An animal created by inserting genetic material from another individual of the same (intra-specific) or different species (inter-specific), e.g. a transgenic mouse. Routinely used in research.[38]
Cytoplasmic hybrid	Created by combining the nucleus (with small amounts of cytoplasm and mitochondria) of one species with the cytoplasm (including the mitochondria) of another species.	Created by transferring a somatic cell nucleus from one species into the enucleated oocyte of another species.	An hypothetical animal resulting from the development of a cytoplasmic hybrid embryo.

As discussed later, beyond ensuring their ethical provenance, we do not see any requirement for statutory regulation of the maintenance and experimental use of hES cells while they remain in tissue culture (see section 10.1).

7.3 Entities combining human and animal material

Following the definitions described in Table 1, we set out the different kinds of entities combining both human and non-human animal material below. We emphasise that, apart from cytoplasmic hybrid and true hybrid embryos, the categories outlined below can include both inter- and intra-species (e.g. human-human) entities.

Human embryos incorporating animal material

1. **Cytoplasmic hybrid embryos** – Embryos resulting from replacing the nucleus of an animal oocyte with the nucleus of a human cell.
2. **Human transgenic embryos** – Human embryos into which animal DNA has been integrated.
3. **Human chimeric embryos** – Human embryos into which one or more animal cells have been integrated.
4. **Hybrid embryos** – Embryos resulting from fusion of human and animal gametes.

Non-human embryos and animals incorporating human material

5. **Non-human transgenic embryos/ animals** – Non-human embryos or animals into which human DNA has been integrated.
6. **Non-human chimeric embryos** – Non-human embryos into which one or more human cells have been integrated at an early stage of development ('primary' chimeras).
7. **Non-human chimeric animals** – Animals resulting from the development of 'primary' chimeras or animals into which human cells have been introduced at a later stage of development, e.g. post-natally ('secondary' chimeras).

We first consider categories 1-4 set out above, before discussing non-human embryos and animals in section 9.

37 In its broadest definition, chimeras include human patients who have received transplanted cells or organs. Some twins are intra-specific chimeras due to the continued presence of blood cells from their co-twin that were exchanged during pregnancy.

38 This is often done by injecting purified DNA directly into the nucleus of fertilised eggs. Some transgenic animals, including farm animal species, have been generated by first introducing the genetic alteration into somatic cells, which have then been used for SCNT and reproductive cloning. Many transgenic mice have been derived via the production of chimeras using genetically altered ES cells. This involves transferring the gene into an ES cells in culture line, a proportion of which take up the 'foreign' gene. These modified cells are then transferred into recipient embryos. These embryos are chimeras, with some embryonic cells carrying the transgene and others not. Such embryos develop into chimeric animals which, when they breed, will produce some germ cells carrying the transgene. Animals formed from such modified germ cells will not be chimeric, since they will be wholly formed of cells carrying the genetic modification, but they will be transgenic since all their cells carry the 'foreign' gene.

8 Human embryos incorporating animal material

8.1 Cytoplasmic hybrid embryos

As mentioned earlier, there is a growing interest in the UK and elsewhere in exploring the use of cytoplasmic hybrid embryos as part of SCNT to derive stem cell lines (section 5.4). There are varied opinions about the likely success of this research, and much of the discussion has focused on the role and behaviour of the mitochondria in cytoplasmic hybrid embryos and any stem cells that are derived from them. **Although it is not yet clear how useful approaches involving cytoplasmic hybrid embryos will be, there is widespread agreement within the scientific community that uncertainties will only be resolved by actually carrying out the necessary experiments.**

8.1.1 The behaviour and role of mitochondria[39]

As described in Box 1, in addition to the DNA in the cell nucleus, almost all cells contain a small amount of DNA within the mitochondria. Mitochondria contain over 1000 proteins, the vast majority of which are encoded by the nuclear DNA. However, the 13 essential polypeptides of the respiratory chain – the cellular complex involved in energy production - are encoded by the multi-copy, circular DNA molecules within the mitochondria (mtDNA). Mitochondria are therefore under the control of both the nuclear and mitochondrial genomes. While their primary function is to generate energy for the cell, mitochondria also have roles in steroid synthesis and programmed cell death (apoptosis). However, these functions depend on gene products encoded solely in the nucleus.

There has been considerable discussion about the relative amount of human and animal mtDNA that would be present in cytoplasmic hybrid embryos, and the implications for defining their nature and status. Two factors that will affect the relative amount of human and animal mtDNA in these embryos are:

1. ***The amount of mtDNA present in the oocyte and transferred with the nucleus***
   The number of mitochondria varies between different types of cell and the mechanisms for regulation are not well understood. It appears that mitochondrial numbers vary as germ cells develop, and increase in the final stages of oocyte growth in order to fuel fertilisation. There are estimated to be about 100,000 mitochondria present in the mouse oocyte at the time of fertilisation.[40] Evidence from transgenic mouse studies using pronuclear transfer indicates that approximately 20% of mtDNA is transferred with the nucleus/ pronucleus.[41] Recent studies in human oocytes suggest that the amount of mtDNA transferred may be less – around 5%.[42] However, pronuclei are unusually large nuclei that are closely surrounded by a considerable number of mitochondria. This may lead to a higher donor/host ratio of mitochondria than is likely to be the case with SCNT.

2. ***The replication of oocyte mtDNA***
   Once the oocyte starts to divide to form an embryo, the mitochondria will also need to proliferate. This process will depend on the ability of nuclear encoded products to interact with the part of the mtDNA (the D-loop region) that is required for replication and gene expression. It will also be affected by the ability of products encoded by both the nuclear and mitochondrial genomes to work together within the mitochondria and so support proper mitochondrial function. It might be expected that a mismatch between human nuclear factors and the animal D-loop sequence would lead to defective transcription and/or replication of the animal mtDNA. The more divergent the

39 Some of the material in this section is drawn from St. John J & Lovell-Badge R (2007). *Human-animal cytoplasmic hybrid embryos, mitochondria, and an energetic debate.* In preparation.

40 Cao L *et al.* (2007). *The mitochondrial bottleneck occurs without reduction of mtDNA content in female mouse germ cells.* Nature Genetics **39**, 386-390.

41 Inoue K *et al.* (2000). *Generation of mice with mitochondrial dysfunction by introducing mouse mtDNA carrying a deletion into zygotes.* Nature Genetics **26**, 176-181.

42 Craven, Herbet and Turnbull (unpublished data).

species involved, the more likely it will be that the donor nucleus would not support the replication of the oocyte mtDNA.[43] Thus, there may be preferential replication of any human mtDNA transferred with the nucleus. In studies involving transgenic animals generated by pronuclear transfer, it has been shown that the perinuclear mitochondrial genomes (i.e. those that are close to the nucleus) are selectively replicated compared with those that are more dispersed in the cytoplasm.[44]

Studies on animal embryos generated by SCNT have shown that, in practice, the mtDNA can come from either the oocyte only, or a combination of the donor cell and recipient oocyte. For instance, studies carried out in China involving human-rabbit cytoplasmic hybrid embryos reported that the derived ES cells contained both human and rabbit mitochondria[45], while human-cow embryos had apparently eliminated all human mtDNA content prior to blastocyst formation.[46] **It is therefore conceivable that a cell line derived from a cytoplasmic hybrid embryo might have mitochondria derived from either or both of the parental cell types.**

It is feasible to test many of the assumptions about the role and behaviour of mitochondria in cytoplasmic hybrid embryos, including the relative amounts of human and animal mtDNA present at different stages and mitochondrial function. With regard to the latter, mitochondrial defects (for instance due to incompatibility between the mitochondrial subunits encoded by the human nuclear DNA and the animal mtDNA) may not be apparent under the high glucose concentrations typical of cell culture, where cells are mainly glycolytic and not dependent on normal mitochondrial function. However, such defects could be detected by growing the cells in galactose medium, which causes the cells to switch to oxidative metabolism and so exposes those cells with a respiratory chain defect.

It may eventually be possible to isolate cells from cytoplasmic hybrid embryos that contain only human genetic components, which may be desirable for research and could conceivably be suitable for future therapeutic use. For instance, it may be useful to transfer as much material as possible from the human somatic cell to the animal oocyte, or to explore methods to select against oocyte mtDNA before nuclear transfer. We stress that the account given here remains largely conjectural, and further insights can only be gained through continued research.

8.1.2 Safety issues [47]

As with all new technologies, it is necessary to consider safety issues involved in the development of cytoplasmic hybrid embryos. For example, in evidence to the Commons Science & Technology Committee, the Scottish Council on Human Bioethics stated that '*animals may harbour in their organs, cells and genome, microbiological and other entities which may cross the species barrier and develop in the host*', citing the examples of CJD and HIV. The Council questioned whether, through this research, humankind could be subjected to '*the risk of devastating and uncontrollable pandemics*'.

The field of xenotransplantation (the transplantation of tissues or organs from one animal species into another) has previously highlighted the potential risk of infectious diseases being spread from a non-human donor animal to a human recipient. The most often-cited examples are porcine endogenous retroviruses - viral genomes carried in pig chromosomes that can infect human cells in

43 Recent *in vitro* studies in which human cells without mtDNA were fused with enucleated primate cells suggested that, with increasing evolutionary divergence, a barrier is reached where the animal mtDNA cannot be maintained. Thus, chimpanzee and gorilla mtDNAs were replicated and transcribed in human cells, but mtDNAs from orang-utan and more evolutionary distant species were not. See Kenyon L & Moraes CT (1997). *Expanding the functional human mitochondrial DNA database by the establishment of primate xenomitochondrial cybrids.* PNAS **94**, 9131-9135.

44 Meirelles F *et al.* (1997). *Mitochondrial genotype segregation in a mouse heteroplasmic lineage produced by embryonic karyoplast transplantation*. Genetics **145**, 455-451.

45 Chen Y *et al.* (2003). *Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes.* Cell Research **13**, 251-263.

46 Chang KH *et al*. (2003). *Blastocyst formation, karyotype, and mitochondrial DNA of interspecies embryos derived from nuclear transfer of human cord fibroblasts into enucleated bovine oocytes*. Fertility and Sterility **80**, 1380-1387.

tissue culture (although there is no evidence that they cause disease in humans).

In the context of cytoplasmic hybrid embryos, the mitochondria and the cytoplasm represent potential sources of retroviruses within the animal oocyte. Studies of mammalian mitochondrial DNA do not appear to show the presence of any endogenous retroviral genomes, although it is conceivable that these may exist. If it was judged worthwhile, this could be checked by analysing the complete mtDNA sequences of species that might provide oocytes for SCNT – most likely cow or rabbit. If endogenous retroviral sequences are absent, this could eliminate concern about mitochondria passing retroviruses on to humans via cytoplasmic hybrid embryos.

The nuclear genomes of cows and rabbits do contain endogenous retroviral genomes.[48] It is therefore possible that rabbit or bovine oocyte cytoplasm may contain RNA transcripts or express endogenous retroviruses encoded by their nuclear genome. Such viruses might conceivably re-integrate into the transferred human nucleus. While this scenario is not impossible, on balance we consider it to be highly unlikely. To ascertain whether such an event presents a genuine problem, expression profiles for endogenous retroviruses could be sought for oocytes from potential recipient species. If present, it could then be ascertained whether the retroviruses are replication-competent or, much more likely, defective. It is important to remember that cytoplasmic hybrid embryos would not be re-implanted into women or animals. We also emphasise that, at this stage, researchers are not seeking to use cell lines derived from cytoplasmic hybrid embryos for clinical treatment purposes, but solely for research. If, at some future stage, the therapeutic use of cell lines derived from such embryos were to be contemplated, screening for endogenous retroviruses could be undertaken.

We do not consider the risk to laboratory workers or the wider public of endogenous retroviruses from cytoplasmic hybrids to be any greater than that associated with regular cell culture procedures (or indeed exposure to animal tissues in other circumstances). There are hundreds (if not thousands) of human and murine cell lines and hybridomas in routine use in laboratories that release retroviruses, including endogenous retroviruses able to infect other human cells in culture. Of course, safety factors will require more in depth consideration if and when stem cell technology generates products that might have clinical applications in humans. **For now, provided standard laboratory procedures are adhered to, there is no obvious reason for undue concern about experimental work on cytoplasmic hybrid embryos for fear of retroviral infections.**

8.2 Transgenic, chimeric and hybrid human embryos

We know of no existing proposals to transfer animal DNA or cells into human embryos to create either transgenic or chimeric human embryos. However, we consider it likely that researchers will at some stage have good reasons to conduct experiments involving genetic manipulations (e.g. the insertion of exogenous DNA) of human embryos *in vitro*. These techniques could facilitate the investigation of gene function in very early embryogenesis (i.e. up to the 14 day limit), thus aiding research into re-programming, stem cell derivation, early cell commitment, differentiation and early embryo development.

It is clear that despite many similarities, there are considerable differences between the early developmental processes of mammalian embryos of different species, for instance trophectoderm and epiblast development. While many of these experiments could be performed with animal embryos, extrapolation of the results to the human requires verification in the human embryo *in vitro*. One outcome of such studies may be more objective criteria for

47 The working group is very grateful to Professor Robin Weiss FRS FMedSci for contributing to this section.

48 For instance, a replication-defective, endogenous beta-retrovirus of rabbits is described in: Griffiths DJ *et al.* (2002). *Novel endogenous retrovirus in rabbits previously reported as human retrovirus.* Journal of Virology **76**, 7094-7102.

classifying human embryos for use in assisted reproduction.

A type of construct likely to be useful will be the incorporation of reporters such as GFP (Green Fluorescent Protein), which could be used to label cells and so enable tracing of cell lineages. Testing whether genes identified in mouse embryos as important in specifying early cell lineages (trophectoderm, extraembryonic endoderm, ICM and epiblast) are also required in human embryos could be achieved via RNA interference (RNAi), which might a involve insertion of hybrid transgenes. RNAi, dominant negative or 'gain of function' experiments could be required to test genes required for morphogenesis and patterning of the early embryo. In the future, it is possible to envisage using non-permanent genetic manipulation technologies to optimise and/or provide reporters of embryo quality, prior to use in assisted reproduction.

Another experimental use of transgenic techniques would be to facilitate derivation of hES cells, trophoblast stem (TS) cells and extraembryonic endoderm (XEN).[49] The effect of the transgene on the derivation of these cells could then be studied *in vitro*. Such methods could be used to manipulate early development in a way that facilitates the derivation of useful stem cell lines, for example to express 'toxic' genes that could eliminate unwanted cell types, or to stimulate or repress mitochondrial replication.

Importantly, many of these experiments will involve the creation of human-human transgenic embryos, i.e. the manipulation of DNA that does not involve the insertion of animal material. It is therefore important that regulation does not focus exclusively on the source of the exogenous DNA or cells (see section 10). It can also be foreseen that researchers may need to introduce hES cells into human embryos *in vitro* to determine their relationship to normal embryo cells and to investigate how pathways to different lineages are triggered. Such embryos would be human-human chimeras.

We are not aware of any current scientific reasons to generate true hybrid embryos (by mixing human and non-human gametes) *in vitro*. However, given the speed of this field of research, the emergence of scientifically valid reasons in the future cannot be ruled out.

8.3 Ethical considerations

As with most forms of stem cell research, there are strongly held views both for and against the creation of human embryos incorporating non-human material. Many of those in opposition are against any form of research involving human embryos. However, others who generally agree with the basis of UK legislation (i.e. that the human embryo is a morally significant entity that must be treated with respect but that research on human embryos is important and is permissible up to 14 days' development) have expressed ethical concerns about this area of research. The following sections summarise some of the specific ethical issues that have been raised in this area;[50] issues relating to *non-human* embryos containing human material are discussed in later sections.

8.3.1 Subversion of the animal-human species distinction

Some have argued that the creation of human embryos containing animal material is unacceptable because it subverts the animal-human species distinction and undermines human dignity and human rights. It is important to distinguish between the creation of a human embryo incorporating animal material that will not exist beyond 14 days, from the possibility of bringing such an embryo to term. Let us begin with the first case. Here the proposed research involves the creation of cells that will only be maintained up to 14 days *in vitro*, and will never be permitted to become human-animal hybrid or chimeric creatures. For good reasons, implanting such an embryo into a woman is illegal in the UK, and we do not want to see this changed. We do not consider the creation of such cells per se to pose any

49 Rossant J (2007). *Stem cells and lineage development in the mammalian blastocyst.* Reproduction, Fertility and Development **19**, 111-8.

50 Some of these issues are outlined by David Archard, Professor of Philosophy and Public Policy at Lancaster University and HFEA member, in a 2007 HFEA discussion paper.

threat to human dignity. We have previously described the long history of laboratory work involving the mixing of human and non-human cells, and the value of such work in generating knowledge and tackling human disease. **In our judgement, no moral (nor any other) harm has derived from the many inter-specific hybrid cell lines that have been created.**

But what if, for some specific and substantial medical or scientific reason, the possibility of permitting a human embryo containing animal material to come to term is eventually contemplated? Such a situation is clearly more morally charged than the creation of embryos within the 14 day rule. However, that is not to say there are no existing examples of incorporating animal material into a human - the most obvious being xenotransplantation. The use of pig tissue in operations to repair damaged heart valves in human patients is now in widespread practice. Few have argued (and none cogently) that transplanting a pig heart valve into a human compromises the humanity or dignity of the recipient.

On a more fundamental level, we judge it unlikely that 'human dignity', a phrase used to emphasise the special moral status and importance of human beings, derives simply from species membership. If the concept of 'human dignity' has content, it is because there are factors of form, function or behaviour that confer such dignity or command respect. Either hybrid creatures would also possess these factors or they would not. If they do possess these factors, they would also have a specific type of dignity analogous or identical to human dignity that other creatures lack; if not, they would not. Either way, the distinction between creatures that possess dignity and those that do not remains as it is now.

The hypothetical possibility of allowing human embryos incorporating animal material to come to term might be thought to threaten dignity in two distinct ways: either the dignity of the hybrid creatures would suffer because they are not fully human, or human dignity would suffer because of the creation of creatures that are close to, but not quite, human. Regarding the first possibility, we again emphasise that dignity arises from the qualities possessed by a creature, rather than species membership per se. This focus on the possession of qualities also applies to the second possibility. Our dignity does not depend on our distance from all other creatures, but on the intrinsic nature of our endowments.[51]

We stress, however, that comments on the development of human embryos containing animal material past the 14 day limit are comments on possibilities that are not proposed or even envisaged at this time. If such proposals are ever made, they will require deep and detailed consideration.

8.3.2 The 'yuk factor'

In responding to the UK Government's 2005 consultation on the review of the HFE Act, some members of the public expressed unease with the possible creation of 'hybrid' embryos.[52] There is an important need here to distinguish between legitimate concerns and discomfort arising merely from unfamiliarity. Although moral intuitions may vary, this is an area of significant moral concern to many people. Two moral claims have been highlighted as forming part of the 'yuk factor' response: a horror of the idea of playing God and the transgression of a fundamental taboo.

Each of these claims is, in some way, associated with issues of 'naturalness' i.e. scientists are wrong to attempt to manipulate nature in this way because such manipulation is unnatural. We find these arguments difficult to sustain. Not only is it very difficult to specify what 'unnatural' means, but it is not clear why 'unnaturalness' should be bad; IVF is an 'unnatural' process, but it has few contemporary opponents.[53] Vaccination and antibiotic therapy, and nearly all of modern medicine, represent a scientifically informed intervention in nature. Indeed all technological

51 To give a non-human analogy, the existence of mules does not decrease or compromise the dignity of horses.

52 Responses can be downloaded from: http://www.dh.gov.uk/Consultations/ResponsesToConsultations/ResponsesToConsultationsDocumentSummary/fs/en?CONTENT_ID=4132358&chk=CnrKSR

53 Although IVF was widely disapproved of before the birth of Louise Brown, the first human produced by IVF.

innovation is in a sense unnatural. The claim that a particular practice is bad because it is unnatural may be a rationalisation of a prior decision that something is wrong - a decision whose basis is not always transparent.

Understanding the views, hopes and concerns of the public (or publics) is a crucial facet of this debate and we welcome the consultations being conducted by the HFEA and Medical Research Council (MRC). We note that a 2003 MORI study sponsored by the MRC, Wellcome Trust and others showed around 70% support for the use of human embryos for medical research to find treatments for serious diseases and for fertility research.[54] However, public reaction to the specific issue of creating of inter-species embryos was not tested.

8.3.3 Slippery slopes

It has been argued that, while the research currently proposed may be acceptable, sanctioning it starts society on a slippery slope towards something unacceptable. This concern is usually expressed as 'yes, but what next?' The principal response to this argument is that this is not a slope down which we will, or need to, slide. As the frameworks that have developed around IVF have shown, regulation is key to public assurance, as is a clear articulation of what activities would remain prohibited, if this research were sanctioned. **We do not consider that concern about slippery slopes is a good argument for prohibiting valuable research; it is a good argument for rigorous and ethically informed regulation.**

8.4 Legal and regulatory background

Research involving human embryos incorporating animal material has been the subject of a great deal of debate in the UK, which has mainly focused on the review of the Human Fertilisation & Embryology Act (HFE Act) 1990. The initial Government Command Paper reviewing this Act, published in December 2006, contained a proposal '*that the creation of hybrid and chimera embryos in vitro should not be allowed*'.[55] Although the balanced tone of the Command Paper was widely welcomed, disappointment was expressed within the scientific community at this proposal, specifically the ban on research involving cytoplasmic hybrid embryos.

Two UK research teams have already applied to the HFEA for licences to create cytoplasmic hybrid embryos for the purpose of generating ES cell lines. The HFEA did not approve these applications and announced a '*public consultation as to whether, in principle, licences for these sorts of research could be granted*.'[56,57]

In January 2007, the House of Commons Science & Technology Committee launched an inquiry into '*Government proposals for the regulation of hybrid and chimera embryos'*. This followed their 2005 report '*Human reproductive technologies and the law*' and was prompted by the proposals contained in the Command Paper and the applications to the HFEA. The Committee's report, published on 5 April 2007, concluded that '*the creation of human-animal chimera or hybrid embryos, and specifically cytoplasmic hybrid embryos, is necessary for research*'. They found '*the Government proposals prohibitive, notwithstanding the provision of powers to allow future regulation in this area at an unspecified date*'. They were also '*critical of the Human Fertilisation and Embryology Authority for delaying assessment of applications for licenses to create cytoplasmic hybrid embryos for research*'.

The Government's Command Paper presenting a draft Bill updating the HFE Act was published on 17 May 2007. The Paper's introduction announced the Government's intention to accept the principle that legislation should provide for the creation of inter-species entities for research purposes, subject to the usual requirements for embryo research. This list of

54 See http://www.mrc.ac.uk/NewsViewsAndEvents/InvolvingThePublic/Consultations/Useofhumanembryosinmedicalresearch2003/index.htm.
55 The Command Paper can be downloaded from http://www.dh.gov.uk/assetRoot/04/14/13/15/04141315.pdf
56 See http://www.hfea.gov.uk/cps/rde/xchg/SID-3F57D79B-921EE528/hfea/hs.xsl/1478.html
57 The HFEA's consultation was launched on 26 April 2007.

inter-species entities specifically did not include 'true' hybrids created by mixing human and animal gametes, but did include:

- *'Cytoplasmic hybrid (cybrid) – an embryo created by replacing the nucleus of animal egg or cell with a human cell or the nucleus of a human cell.*
- *Human transgenic embryos – an embryo that has been altered by the introduction of any sequence of nuclear or mitochondrial DNA from an animal.*
- *Human-animal chimera – a human embryo that has been altered by the introduction of one or more animal cells.'*

In the Command Paper, the Minister asked the Parliamentary pre-legislative scrutiny committee to consider whether the proposal to permit the creation of these entities for research purposes should be effected on the face of the Bill, or through secondary regulations. This decision has not been reached at the time of writing (see section 10.1).

9 Non-human embryos and animals incorporating human material

Whilst much of the current debate focuses on *human* embryos incorporating animal material (discussed in section 8), we consider that discussion of the converse situation – already an active and productive field of research – is helpful for a full appreciation of the issues involved. We think it possible that this area of research will generate more active discussion as the science progresses over the coming years. The following sections therefore examine issues relating to *non-human* embryos and animals incorporating human material.

9.1 Non-human transgenic animals

As discussed in section 6, there are thousands of examples of transgenic animals, mostly mice, containing human DNA, mainly used as models of human gene function and human disease. Virtually all of these models currently involve the insertion of a single human gene into a mouse, but it is likely that the amount of human genetic material incorporated into transgenic mouse strains will increase as the technology develops.

The creation of transgenic animals (including those incorporating human DNA) is regulated by the Home Office under the Animal (Scientific Procedures) (A(SP)) Act 1986 . An animal comes under the remit of the Act at the mid-point of gestation (see Box 6).

9.2 Non-human chimeric embryos and animals

A range of situations can be envisaged involving the transfer of human cells into non-human embryos and animals at different stages of post-fertilisation, fetal or post-natal development, generating 'primary' or 'secondary' chimeras (see Table 1 for definitions).

This issue of 'primary' chimeras has been brought into sharper focus with the recent publication of a study investigating the contribution of hES cells to mouse blastocysts.[58] As described in Box 1, the potency of hES cells has been studied in tissue culture or in teratomas, although these methods cannot demonstrate the potential for developing many tissue types integrated into an embryonic structure. Mouse ES cells have been shown to give rise to every cell type when inserted into mouse blastocysts. A research group at the Rockefeller University explored the use of mouse blastocysts to demonstrate the potential of hES cells. The authors claimed that hES cells could engraft into mouse blastocysts, where they proliferate and differentiate *in vitro* and persist in mouse/human embryonic chimeras that can implant and develop in the uterus of pseudo-pregnant surrogate mice.[59] However, few human cells were found within post-gastrulation stage embryos, suggesting

Box 6 UK regulation of research involving animals

Research involving protected animals (all vertebrates, excluding humans, but including octopi), including transgenic animals, is subject to licence by the Secretary of State for the Home Office under the Animals (Scientific Procedures) Act 1986. Section 2 of the Act includes within the definition any protected animal from the mid-point of the gestation or incubation period for the relevant species.

There is no legislation that specifically applies to research involving non-human embryos *in vitro*, but the 1986 Act applies to any procedure involving a living animal, e.g. the hormonal stimulation of oocyte maturation or implantation of a blastocyst, as well as the production or breeding of any genetically altered animal.

58 James D *et al.* (2006). *Contribution of human embryonic stem cells to mouse blastocysts.* Developmental Biology **295**, 90-102.
59 Five days following transfer, implanted embryos were recovered from the uterus of foster mothers and examined for hES cell contribution.

that they were at a considerable disadvantage compared to the surrounding mouse cells.

The authors of this study claim that this work provides a potential model system in which to study the developmental potential of hES cells and their derivatives. They also point out that, if hES cells can be reconciled with mouse embryogenesis *in vivo*, engrafting hES cells prior to gastrulation would provide an accessible platform for studying the emergence of many human cell types.

There is not, as yet, scientific consensus on the value of this experimental system. Nevertheless, it is likely that researchers will seek to develop these techniques further, for instance by increasing the duration for which the chimeric embryo is maintained, and transferring hES cells into post-implantation stage embryos *in utero* (for instance using high resolution ultrasound imaging). Indeed, this is already carried out in mouse embryos to look at the contribution of mouse progenitor cells to the CNS or enteric nervous system in mouse models of CNS defects and Hirschsprung Disease. It might eventually be feasible to use pre-gastrulation stage mouse embryos, which could provide improved testing for the pluripotentiality of hES cells.

Approaches involving 'secondary' chimeras, i.e. the transfer of human cells into animals at a later stage of development, are already in widespread use in studies of human and mouse pluripotent and tissue-specific stem cells. For instance, transplantation of hES cells to immunodeficient mice is a technique used to assess their capacity for differentiation in multiple cell types, thereby verifying that the cells are indeed pluripotent. It is also common practice to investigate the potential of human neural stem cells to integrate appropriately into mouse or rat brain as a test of their potential, safety and usefulness.[60]

As described in Box 6, experiments involving chimeric animals ('secondary' chimeras) are regulated in the UK by the Home Office under the A(SP) Act 1986. Techniques to obtain gametes or other material from adult animals to generate chimeric animal embryos ('primary' chimeras), or the implantation of such embryos into the uterus of a recipient female animal, would be regulated by the Act. The embryos themselves would become regulated under the Act if they were allowed to progress beyond mid-gestation.

There has already been some useful examination of the regulatory issues raised by the generation of chimeric animals, although this has formed part of the frameworks governing stem cell research more broadly. Of note is the regulatory framework proposed by the International Society for Stem Cell Research (ISSCR), an independent, non-profit organisation involving groups from 29 countries (including UK, USA, China, South Korea, Russia, Germany and Sweden).[61] Their 2006 '*Guidelines for the conduct of human embryonic stem cell research*' are designed to encourage uniform global research practices, conducted to rigorous standards of ethics. They represent an important source of self-regulation by researchers working in the field.

The guidelines highlight two points of concern regarding chimeric animals containing human cells: the degree of the resulting chimerism and the type of tissues that are chimerised.[62] The ISSCR guidelines point out that the earlier that human stem cells are introduced during animal development, the greater the potential for their widespread integration. They note that the introduction of a greater number of cells later in development may have an equivalent effect. There is also the issue of whether implanted cells might migrate through the animal's body.

As described in Box 7, research involving chimeric animals falls into category two of the ISSCR's framework for regulatory oversight of stem cell research. It states that, when considering applications for this type of

60 Lindvall O & Kokaia Z (2006). *Stem cells for the treatment of neurological disorders.* Nature **441.** 1094-6.

61 http://www.isscr.org/about/index.htm

62 International Society for Stem Cell Research (2006). *Guidelines for the conduct of human embryonic stem cell research.* Version I at http://www.isscr.org/guidelines/ISSCRhESCguidelines2006.pdf

research, the responsible regulatory body should pay special attention to:
'A) the probable pattern and effects of differentiation and integration of the human cells into the non-human animal tissues; and B) the species of the animal, with particular scrutiny given to experiments involving non-human primates. Experiments that generate chimerism of the cerebral cortex or germline should be subjected to especially careful review.'

The California Institute for Regenerative Medicine (CIRM) has also issued guidelines for all CIRM-sponsored research.[63] These guidelines state that research involving the introduction of hES cells into non-human animals at any stage of embryonic, fetal, or post-natal development is '*permissible after additional review and written approval by an Embryonic Stem Cell Research Oversight committee (ESCRO)*' and '*provided the investigators evaluate the probable pattern and effects of differentiation and integration of the human cells into the non-human animal tissues*'.

The CIRM guidelines prohibit: the introduction of hES cells into non-human primate blastocysts; the introduction of any ES cells into human blastocysts; and the breeding of an animal into

Box 7 ISSCR framework

The ISSCR sets out a framework for hES cell research involving three categories:

Category 1 includes experiments that are permissible after review by existing local committees, including research with pre-existing hES cell lines that are confined to cell culture or involve routine and standard research practice.[64]

Category 2 research is permissible only after additional and comprehensive review by a specialised body. Such forms of research require provision of greater levels of scientific justification, consideration of social and ethical aspects of the research and reasons for not pursuing alternative methods. This category includes:

- Research involving the derivation of new hES cell lines by any means.
- Research in which the identity of the donors or blastocysts, gametes or somatic cells from which stem cells are derived is readily ascertainable.
- Mixing human totipotent cells or pluripotent stem cells with pre-implantation human embryos (with the caveat that such experiments are not permitted to progress for more than 14 days of development *in vitro*).
- Transplanting totipotent or pluripotent cells of human origin into living human subjects.
- **Research that generates chimeric animals using human cells, including (but not limited to) introducing totipotent or pluripotent human stem cells into non-human animals at any stage of post-fertilisation, fetal or post-natal development.**

Category 3 research is not permissible at the current time. Such experiments include:

- *In vitro* culture of any post-fertilisation human embryos, regardless of derivation method, for longer than 14 days.
- Implanting any products of research involving human totipotent or pluripotent cells into a human or non-human primate uterus.
- Research in which animal chimeras incorporating human cells with the potential to form gametes are bred together.

63 http://www.cirm.ca.gov/meetings/pdf/2005/08/083005_item_7a.pdf
64 Such as assays of teratoma formation in immune-deficient mice.

which hES cells have been introduced at any stage of development. The CIRM guidelines further state that an ESCRO committee should include representatives of the public and '*persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research*'.

10 Discussion and conclusions

We concur with the widely held view that stem cell research is likely to lead to major advances in our understanding of human development and the control of specialised cell functions. This will give insights into disease processes, and may lead to entirely novel therapies in which the specialised cells lost in some diseases can be replaced.

The field of tissue-specific (adult) stem cell research holds much promise, but its challenges and limitations mean that ES cell research currently provides a more flexible range of options for research and development. In this context, **research into generating human embryos via SCNT, allowing the derivation of hES cells lines with a controlled genetic make-up, should be pursued, both for nearer term research possibilities and the longer term potential of therapeutic benefits.**

This is a challenging field of science, in an early stage of development. It is also rapidly evolving, which makes it difficult for scientists, let alone non-specialists, to predict what will become possible. At this early stage, it is important that scientists are given every opportunity to bring their intuitions to bear on the problems of stem cell science. **We emphasise that the fine balance of judgement around which particular experiments are more or less likely to be successful is best exercised by the peer review process of science funding, involving experts in the field with up-to the-minute knowledge about this rapidly evolving field.**

It is important to consider research on inter-species embryos in the context of the long history of scientific research involving other inter-species constructs, which has both advanced knowledge of human biology and led to the development of clinical therapies. Mouse-human hybrid cell lines were the basis for early mapping studies on human genes in the 1970s, eventually leading to the successful Human Genome Project; animal cells have been widely used to produce human therapeutic proteins; and transgenic mice expressing human genes have led to key insights into understanding and treating diseases ranging from Alzheimer's Disease to cancer. No insurmountable ethical or safety issues have emerged over three decades of this research.

The lack of ready availability of cell lines and other model systems for experimental use is a serious obstacle to progress. In addition, excessive regulatory hurdles and adverse publicity run the risk of discouraging the best young scientists from entering this field. We clearly recognise that no field of science can prosper without the moral, as well as financial, support of society. **To benefit from the potential fruits of stem cell research it is therefore necessary not only to pursue the science, but also to ensure that the methods and goals of the science are clearly communicated, well understood, and supported by the society in which we work.**

We first discuss issues regarding *human* embryos incorporating animal material, before considering *non-human* embryos and animals containing human material.

10.1 Human embryos incorporating animal material

We consider one of the major limiting factors in pursuing hES cell research to be the availability of hES cell lines of defined or controlled genotype, which is in turn dependent on the availability of donated human embryos and oocytes. We believe that the clinical demands of assisted reproduction, and the invasive nature of the procedures involved, mean that donated human embryos and oocytes are unlikely to ever fulfil the research need. **We consider there to be a broad consensus**

view amongst the scientific community that exploring the use of animal oocytes represents a valid and potentially important avenue towards advancing the science of SCNT; animal eggs could provide an essentially unlimited supply of oocytes with which to hone the techniques and skills of SCNT, allowing more rapid progress and sparing the use of valuable human eggs.

Recent research has been very promising in identifying the chemical factors necessary to re-programme somatic cells. Increased knowledge of factors required for efficient reprogramming will come from SCNT experiments and from a better understanding of hES cells and their pluripotency. **In the longer term, such knowledge could potentially lead to methods of direct re-programming without using oocytes (whether human or animal) or early embryos, but achieving that goal will require a great deal of further research and it would be premature to assume at this stage that this approach will prove successful.**

We have considered, with expert advice, some of the safety issues raised in relation to this work, including the possible activation of endogenous animal viruses. If the therapeutic use of cell lines derived from such embryos should ever be contemplated, it would be prudent to scan the mitochondria and cytoplasmic RNA of the species to be used as oocyte donors for replication competent RNA viruses. **However, provided good laboratory practice is rigorously followed, we do not believe that *in vitro* laboratory research involving cytoplasmic hybrids or other inter-species embryos raises any significant safety risks over and above regular cell culture, either to researchers or to the public at large.**

Although we are not aware of any existing proposals, we consider it likely that researchers will at some stage have good reasons to conduct experiments involving either the insertion of exogenous DNA or the genetic manipulation of human embryos *in vitro*. Such work may, for example, help to identify genes that are important in specifying early cell lineages, or those that are critical in specifying the earliest stages of embryonic development. This work could also lead to direct benefits for infertility treatments. **We emphasise that many of these experiments will involve the manipulation of DNA that does not involve the insertion of animal material.**

We have considered the ethical issues raised in relation to cytoplasmic hybrid embryos and related constructs. We appreciate the sincerely held beliefs of those who consider all research involving human embryos to be inherently unethical. However, UK legislation permits licensed research on human embryos up to 14 days, and **our considered view is that there are no substantive ethical or moral reasons not to proceed with research on cytoplasmic hybrid, human transgenic or human chimeric embryos under a similar framework of regulatory control.** The creation of true hybrid embryos is prohibited in current legislation. The reasons for banning the creation of hybrid embryos for *in vitro* experimental use, while permitting research involving other types of human embryo incorporating animal material, are not clear to us, but we are not aware of any current scientific reasons to create such entities.

We emphasise that the 14 day limit should apply to organised human embryos, i.e. human embryos in which embryonic development can be triggered and supported, generating an entity potentially capable of implantation and initiation of gastrulation. **Beyond ensuring ethical provenance of hES cells, we can see no reason to impose statutory regulation on the *in vitro* use of hES cells, including hES cells that have undergone some differentiation (such as an embryoid body in which the cells exist in a disorganised multi-layered tissue culture).**

In a rapidly moving scientific field, it is impossible to create an exhaustive list of experimental techniques that should or should not be permitted in primary legislation; research will always give rise to situations that could not have been anticipated in advance. **We are concerned that a general prohibition on research involving human embryos incorporating animal material, subject to exceptions for particular entities requiring Parliamentary approval, will not provide sufficient flexibility for research to proceed in a timely and effective manner.**

A system whereby permissible developments - with clear limits - are set out in primary legislation, giving reasonable flexibility within which an informed regulator decides on individual research proposals, has served the UK well in the past. In this way, the HFEA (or its successor) is empowered to consider all research proposals involving human embryos that are not specifically excluded by the legislation. This does not preclude the power for Parliament to regulate future developments. We support the House of Commons Science & Technology Committee proposal that the Secretary of State may invoke regulations to prohibit a particular research procedure, subject to an affirmative process by both Houses of Parliament.

We support the following key principles for the regulatory framework in this field:

- **Research involving the creation and use of human embryos incorporating animal material should be permitted under licence by the HFEA. The creation and use of such embryos should only be licensed where there is a clear and important research need.**
- **The re-implantation into a woman of any human embryo generated by SCNT should be prohibited in law.**
- **The re-implantation into a woman of any human embryo containing animal genetic or cellular material should be prohibited in law.**
- **Human embryos used for research purposes - whether generated by the fertilisation of a human oocyte or by SCNT – should not be developed *in vitro* beyond 14 days.**
- **The 14 day limit should apply to organised human embryos, i.e. human embryos that have (or are predicted to have) the capacity to proceed through normal stages of further development, including gastrulation and the acquisition of a correctly patterned body plan. It should not apply to the culture of isolated cells or tissues derived from embryos, or to embryos that have acquired a disorganised state prior to 14 days.**

We further emphasise that cells, including potential therapeutic stem cells, derived from SCNT or inter-specific embryos should not be implanted into any humans without further detailed regulatory and saftey consideration.

10.2 Non-human embryos and animals incorporating human material

While the area of legislation currently under consideration in the UK covers research involving *human* embryos, including those incorporating animal material, we believe that regulatory questions will increasingly arise from research involving *non-human* embryos and animals incorporating human material. The interface between regulatory regimes governing human embryos, hES cells and animal research will become increasingly important.

As discussed previously, the ISSCR has proposed a useful regulatory framework to oversee research involving the transfer of human stem cells into non-human animals. We support the ISSCR framework, including the caveats relating to the proportion of human stem cells transferred, the likely integration into critical tissues such as the germline and CNS, and the transfer of human cells into

non-human primates. We also support the emphasis placed by the ISSCR and CIRM on the need for such experiments to be overseen by a committee incorporating expertise in stem cell research and other relevant scientific areas, as well as ethical, legal and public representation. Establishing such a framework in the UK would require input from several different stakeholders, including the UK Stem Cell Bank Steering Committee, the Home Office, scientists, animal welfarists and others.

However, the origins of the ISSCR framework lie in the regulation of human stem cell research; the creation of transgenic animals incorporating human genetic material (not involving human stem cells) is not considered. As the science of transgenics has progressed, researchers have constructed ever more ambitious transgenic animals; mice have now been created that carry almost an entire copy of human chromosome 21, containing many hundreds of genes. It seems likely that the process of engineering ever larger amounts of human DNA into mice will continue, and **it will be necessary to consider the appropriate conceptual and regulatory framework for transgenic and chimeric animals that contain significant amounts of human genetic material.**

We consider that current UK activity in this area is adequately covered by existing mechanisms for regulating animal research. However, it would be sensible to start considering the types of regulatory regime that may be necessary in the future. Animal welfare will continue to be a primary concern in regulating these experiments, including consideration of non-experimental conditions such as their housing and handling, and any welfare issues raised by the particular transgenic modification. However, the presence of significant amounts of human material may raise further ethical and social issues that do not fall within the remit of the Home Office and A(SP) Act.

For both transgenic and chimeric animals, it will be important to develop a system of oversight that is both proportionate and appropriate. The entities created and the processes involved in this research fall along a spectrum; in scientific terms, differences will not be categorical, but of degree. **It is therefore essential that the regulatory framework operates on a case-by-case basis in which individual judgements can be exercised on a wide variety of intermediate cases. Such matters should not be rigidly defined in primary legislation.**

It will be important to ensure that this area of science does not come under dual regulation. In the introduction to the proposed revisions to the HFE Act, the Government makes a welcome pledge to prevent the regulation of inter-species embryos by two bodies, and excludes those embryos that are within the remit of the Home Office under the A(SP) Act 1986. It is important that the interface between regulation of human embryos, and that applicable to animal embryos, is managed in such a way that potentially useful research is not excluded without clear reason. **As a first step, it is essential that proper channels of communication and consultation are established between those bodies regulating human embryos, human stem cells and animal research.**

Looking forward, there is a need to develop general guidance as to how different entities along the human/animal spectrum are treated for the purposes of law and regulation. Further public discussion of these issues over the coming years will be important. The progress of science may eventually help in producing some more objective definitions, but these are in essence matters of public values and judgements. **As a follow up to this report, the Academy will be undertaking further work on this issue, to include a significant component of public engagement, which we hope will inform future debate.**

Annex I: Report preparation

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This report was prepared by an Academy of Medical Sciences Working Group. Members participated in a personal capacity, rather than as a representative of their organisations.

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Annex II: Glossary

This glossary is designed to help readers understand some of the terms used in this report; it is not presented as a definitive list of terms.*

Blastocyst	The mammalian embryo at the time of its implantation into the uterus (see 3.1.4).
Central Nervous System (CNS)	The largest part of the nervous system, including the brain and spinal cord.
Chimera/ Chimeric	See Table 1.
Chromosome	In a eukaryotic nucleus, one of the threadlike structures carrying genetic information arranged in a linear sequence.
Cytoplasm	The gel-like substance enclosed by the cell membrane.
Cytoplasmic hybrid	See Table 1.
Diploid	The state in which each type of chromosome (except the sex chromosomes) is represented twice. This is the normal state of all cells of the body, except the germ cells (sperm and eggs), which have only a single (haploid) set of chromosomes.
Embryonic stem cells	Pluripotent stem-cell lines derived from early embryos before formation of the tissue germ layers (see 3.1.4).
Enteric nervous system	The part of the nervous system that directly controls the gastrointestinal system.
Epithelial	Relating to the epithelium, the outside layer of cells that covers all the surfaces of the body, including the skin.
Eukaryote	Any organism having as its fundamental structural unit a cell type that contains specialised organelles in the cytoplasm, a membrane-bound nucleus enclosing genetic material organised into chromosomes, and a system of division by mitosis or meiosis; characteristic of all life forms except bacteria, and other primitive microorganisms.
Gamete	A haploid mature sexual reproductive cell, e.g. a sperm or egg, which can unite with another cell to form a new organism.
Gastrulation	A phase early in the development of animal embryos, during which the morphology of the embryo is dramatically restructured by cell migration.
Germ cell	A sex cell or gamete; a reproductive cell that fuses with one from the opposite sex in fertilisation to form a single-celled zygote.
Haematopoietic	Pertaining to a cell able to produce all types of blood cells.
Haploid	The state in which each type of chromosome is represented once, i.e. half the diploid number.
Hybrid	See Table 1.
Inner Cell Mass	The mass of cells inside the embryo that will eventually give rise to the definitive structures of the fetus.
In vitro	The technique of performing a given experiment in a test tube or other non-living environment. Contrasted with an *in vivo* experiment, which is performed within a living organism.
Meiosis	Part of the process of gamete formation, consisting of chromosome conjugation and two cell divisions, in the course of which the diploid chromosome number becomes reduced to the haploid.

* Many of the descriptions are drawn from Smith AG (2006). *A Glossary for Stem Cell Biology*. Nature 29 June 2006 doi:10.1038/nature04954; and King RC & Stansfield WD (1997). *A Dictionary of Genetics*. Fifth Edition. Oxford University Press, UK.

Mesenchymal	Relating to the mass of embryonic tissue that later differentiates into blood vessels, blood-related organs and connective tissues.
Mitochondria	Organelles in the cytoplasm of nearly all eukaryotic cells, containing genetic material and many enzymes important for cell metabolism and energy production.
Mitosis	The normal form of cell division in all body tissues, characterised by the separation of replica copies of each chromosome with one part being retained in each of two new cells resulting from the original cell.
Multipotent stem cells	Can form multiple lineages that constitute an entire tissue or tissues, e.g. haematopoietic stem cells (see 3.1).
Niche	Cellular microenvironment providing support and stimuli necessary to sustain stem cell self-renewal (see 3.1.1).
Nucleus	A membrane-bound structure, usually spherical, present in all eukaryotic cells, which contains DNA in the form of chromosomes.
Oncogene	A gene that induces uncontrolled cell proliferation.
Plasticity	Unproven notion that tissue-specific stem cells may broaden potency in response to physiological demands or insults (see 3.1.1).
Pluripotent stem cell	Able to form all the body's cell lineages, including germ cells, and some or even all extraembryonic cell types. Includes embryonic stem cells (see 3.1).
Potency	The range of commitment options available to a cell.
Primitive streak	A structure that forms during the early stages of mammalian embryogenesis; one of the first signs of gastrulation; characterised as a furrow in the midline of the emrbyonic disc.
Progenitor cell	Generic term for any dividing cell with the capacity to differentiate. Includes putative stem cells in which self-renewal has not yet been demonstrated.
Self-renewal	Cycles of division that repeatedly generate at least one daughter equivalent to the mother cell with latent capacity for differentiation. This is the defining property of stem cells.
Somatic cell	Any cell forming the body of an organism, not including germ cells.
Somatic Cell Nuclear Transfer (SCNT)	The transfer of the nucleus from an adult somatic cell into an oocyte from which the nucleus has been removed (see section 4).
Stem cell	A cell that can continuously produce unaltered daughters and also has the ability to produce daughter cells that have different, more restricted properties (see 3.1).
Teratoma	A type of tumour that contains several different tissue types.
Tissue-specific stem cells	Derived from, or resident in, a fetal or adult tissue, with potency limited to cells of that tissue. These cells sustain turnover and repair throughout life in some tissues (see 3.1.1).
Totipotent stem cells	Sufficient to form entire organism. Totipotency is seen in zygote and plant meristem cells; not demonstrated for any vertebrate stem cell (see 3.1).
Transgenic	See Table 1.
Unipotent stem cells	Form single lineages, e.g. spermatogonial stem cells (see 3.1).
Zygote	The diploid cell resulting from the union of the haploid male and female gametes.

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