

# Scientific Horizon Scanning at the HFEA

Annual Report 2006

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# 4

## Introduction

**This report presents information on the horizon scanning function at the Human Fertilisation and Embryology Authority (HFEA). Horizon scanning in this context refers to the process of searching for upcoming issues that could impact on the work of the HFEA in the future. The information within the report has been gathered to inform possible future treatments and research.**

The majority of the techniques that are included in this report are a long way from being offered in clinics. However, it is useful for us to be aware of them so that when/if they ever become available we are able to ensure that patients are suitably informed and that we have Guidance in place so that new treatments are carried out in a safe and appropriate way.

In order to be as thorough as possible we consider animal studies as well as studies involving humans. Also some of the information presented in the report is very new and therefore has not necessarily been independently validated.

### Section 2

## Scientific horizon scanning

### 2.1 Horizon scanning statement

The horizon scanning process is an early warning system that identifies, through a rigorous and systematic appraisal of scientific research, new developments that may impact on the field of assisted reproduction or embryo research so that we are aware of potential license applications and prepared, if necessary, with an Authority policy or position.

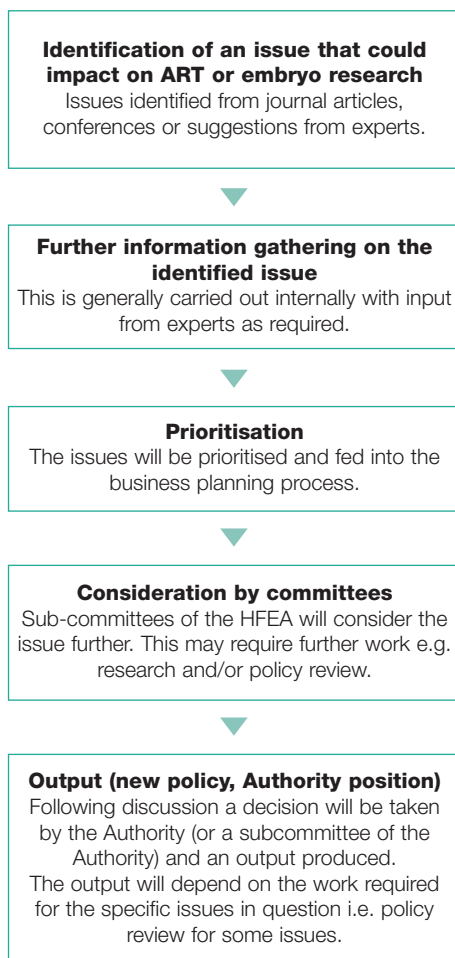
### 2.2 Purpose of scientific horizon scanning

The area of science and medicine regulated by the HFEA is fast-paced and can be controversial. As a result of this it is often necessary for the HFEA or the HFEA licence committee to make decisions about new treatments or research applications where there has not been very much research or previous consideration. In order to ensure that this is avoided as much as possible, a horizon scanning process was established. The purpose of scientific horizon scanning is to ensure that, as much as is possible, prior to an application being received by a licence committee, the HFEA has had time to consider the legal, ethical and scientific implications of any technique that scientists or clinicians may wish to use in HFEA-licensed research or treatment.

# The horizon scanning process

## 3.1 Overview

The figure below represents an overview of the HFEA horizon scanning process.



## 3.2 Internal

The internal horizon scanning process is carried out by the HFEA policy team. This involves regular identification of issues through reading journals and attending conferences. Further research into the identified issues is also largely carried out internally within the HFEA but this is often done in conjunction with external experts such as members of the Horizon Scanning Panel (see section 3.3). There is an annual meeting of the HFEA Scientific and Clinical Advances Group (SCAG) at which members of the Authority will discuss and prioritise those issues identified throughout the year. In preparation for the SCAG horizon scanning meeting, all the identified issues will be considered and recommendations for further work and priority are presented to SCAG along with details on all the identified issues. Members of SCAG decide which issues they consider to be high priority. This information is then fed into the business planning and agenda scheduling for the committee for the following year.

## 3.3 Horizon Scanning Panel

We have brought together an international panel of experts to advise on horizon scanning issues. A full list of members can be found on page 6. Members of the Horizon Scanning Panel communicate over the internet. There is also an annual meeting where members meet face to face to discuss current and future issues.

In general we send questions to members, regarding horizon scanning issues, three to four times a year. More information on the issues discussed at meetings and the questions we have asked members of the Panel can be found in section 5.

# Horizon Scanning Panel members

as of April 2007

Name	From
<b>Professor Twink Allen</b>	University of Cambridge, UK
<b>Professor Peter Andrews</b>	University of Sheffield, UK
<b>Professor Keith Campbell</b>	University of Nottingham, UK
<b>Dr Jacques Cohen</b>	IRMS, New Jersey, USA
<b>Professor Alan Decherney</b>	UCLA School of Medicine, USA
<b>Dr David Edgar</b>	University of Liverpool, UK
<b>Sir Martin Evans</b>	Cardiff University, UK
<b>Professor Chris De Jonge</b>	University of Minnesota, USA
<b>Professor Paul Devroey</b>	Free University in Brussels, Belgium
<b>Professor Hans Evers</b>	Academic Hospital, Maastricht, Netherlands
<b>Professor Stephen Hillier</b>	Centre for Reproductive Biology, Edinburgh, UK
<b>Professor Martin Johnson</b>	Department of Anatomy, University of Cambridge, UK
<b>Professor Gab Kovacs</b>	Monash IVF, Australia
<b>Professor Henry Leese</b>	Department of Biology, University of York, UK
<b>Dr Norio Nakatsuji</b>	Kyoto University, Japan
<b>Professor Andre Van Steirteghem</b>	University Hospital Brussels, Belgium
<b>Professor Alan Trounson</b>	Monash Immunology and Stem Cell laboratories, Australia
<b>Dr Maureen Wood</b>	Aberdeen Fertility Clinic, Aberdeen Maternity Hospital, UK



# Horizon Scanning Panel: summaries

## 5.1 Horizon Scanning Panel meetings

**5.1.1** To date there have been three meetings of the Horizon Scanning Panel. The meetings are held annually to coincide with the European Society of Human Reproduction and Embryology (ESHRE) annual meeting. The meetings have been held in Berlin (2004), Copenhagen (2005) and Prague (2006). A summary of the topics discussed at each meeting can be found below. For further details, please see the minutes that are included in Annex A.

**5.1.2** At the 2006 meeting the following topics were discussed:

- Presentation on progress of horizon scanning to date
- Discussion on *cdx2* null mice (introduced by Professor Alan Trounson)
- Discussion on testicular stem cells (introduced by Professor Chris Barratt)
- Discussion on oocyte and tissue freezing (introduced by Professor Gab Kovacs)

**5.1.3** The following topics were discussed at the meeting in 2005:

- Artificial gametes (introduced by Professor Keith Campbell)
- The HFEA Horizon Scanning Panel one year on

**5.1.4** The 2004 meeting in Berlin was the first meeting of the Horizon Scanning Panel and the main topic for discussion was based around the HFEA and how the Panel would work.

- Introduction to the HFEA and its function (Professor David Barlow)
- Immediate developments the HFEA needs to be prepared for (Discussion led by Professor David Barlow)
- Issues that the HFEA should be considering (Discussion led by Professor Alan Trounson)
- The practicalities as to how the expert group could function



# Horizon Scanning Panel: summaries

## 5.2 Questions asked of the Horizon Scanning Panel

This table summarises the questions that we have asked members of the Panel to date. For full details of the responses obtained, please see Annex B.

Date	Questions
October 2004	<ol style="list-style-type: none"> <li>1. What do you consider to be the biggest issue on the scientific horizon that could impact on assisted reproductive medicine and/or technology in the future (in the next one to five years)?</li> <li>2. Are you aware of any techniques that are being developed in animal models that will be potentially transferable to human ART in the future? On what time-scale do you think this will occur?</li> <li>3. What are your views on <i>in vitro</i> maturation of oocytes? In your opinion, do you think that there is sufficient knowledge about embryos created using <i>in vitro</i> matured oocytes to allow these embryos to be used in treatment services?</li> </ol>
May 2005	<ol style="list-style-type: none"> <li>1.               <ol style="list-style-type: none"> <li>a) The 1990 HFEA Act prohibits '<i>altering the genetic structure of any cell while it forms part of an embryo</i>'. Can you think of any reason why it would be desirable to alter the genetic structure of an embryo either for research or treatment?</li> <li>b) Would application of siRNA or RNAi in an embryo, in your opinion, constitute altering the genetic structure of an embryo? For what purposes could this be used in either treatment or research?</li> </ol> </li> <li>2. A recent publication from the laboratory of Magdalena Zernicka Goetz suggests that from as early as the four-cell stage, mouse blastomeres have different developmental properties depending on the initial orientation of the cell cleavage. In your opinion, does this have implications for PGD?</li> <li>3. Are there any other developments that have come to your attention in the last six months that could impact on assisted reproductive technologies or research in the near future e.g. in genetic screening, cryopreservation, embryology, IVF or human embryonic stem cell derivation?</li> </ol>
July 2005	<ol style="list-style-type: none"> <li>1. In a recent paper in RBM online (<i>Vol 11. No 2. 2005 206–218</i>) it was suggested that it could be worthwhile using host trophoctoderm to improve the chances of implantation in patients where there is a history of repeated IVF failure. Do you think that technique would ever be useful in a clinical setting? Is there any way that you could produce trophoblast vesicles <i>in vitro</i> (i.e. without the need for spare embryos)?</li> <li>2. In recent testimony before a subcommittee of the USA Senate alternative methods for obtaining embryonic stem cell lines were discussed / proposed. One of these methods was that pluripotent stem cell lines could be obtained from biopsied blastomeres. Are you aware of any research group who are working or have worked on trying to derive stem cell lines from biopsied embryos in any animal model? Is this proposal scientifically possible?</li> </ol>

# Horizon Scanning Panel: summaries

Date	Questions
December 2005	<p>Do you think it would it be possible for a totipotent cell removed from an early embryo to divide and develop into another embryo, so that there were two identical embryos one of which was derived from the removed, single totipotent cell?</p> <p>If this were possible, can you think of any reason why someone may wish to do this for research or treatment?</p>
June 2006	<p>The National Health and Medical Research Council (NHMRC) have written a discussion paper on the biological definition of an embryo.</p> <p>They are interested to hear comments on this biological definition so members were asked to respond to them directly or post a reply to the horizon scanning web forum.</p>
October 2006	<p>The HFEA has launched a public consultation, 'Donating eggs to research: safeguarding donors'. The use of eggs in research, especially where stem cell lines may be produced from donated eggs, is an issue that could have international implications. It is therefore really important that the HFEA are able to gather views from respondents outside of the UK as well as those who will be directly impacted by any policy decision in the UK.</p>
October 2006	<p>Transplantation of cryopreserved ovarian tissue is a treatment that may be introduced on a larger scale in the future. From my understanding on this there have been very few pregnancies (and even fewer live births) resulting from autotransplantation of cryopreserved ovarian tissue.</p> <p>What is your view on this treatment being offered to patients, do you feel that there is sufficient evidence of safety or efficacy? Do you have any further information on this technique to which the HFEA should be aware?</p>
November 2006	<ol style="list-style-type: none"> <li>1. Would any entity created by activating a human somatic cell nucleus within an enucleated animal (e.g. cow or rabbit) oocyte:             <ol style="list-style-type: none"> <li>a) be viable, or, at least, possibly viable?</li> <li>b) contain a complete human genome?</li> <li>c) be a human embryo?</li> <li>d) ever have the potential to develop and result in a live birth, if implanted? (N.B. the HFE Act 1990 prohibits this)</li> </ol> </li> <li>2. Given that the proteins present would be predominantly animal, would the entity created be human from the moment of activation? If not, at what stage, in your opinion, would the entity become human? How long would the animal proteins be present?</li> <li>3. What would be the significance and likely effect of the presence of animal mitochondrial DNA on any such entity's development?</li> </ol>

# Horizon scanning 2005

## 6.1 Identified

This table presents all issues that were identified through the horizon scanning process in 2005 prior to filtration and prioritisation. This will include issues that were not followed-up in any detail. This has been included for information.

Name	Description	Use
<b>In vitro derived gametes/artificial gametes</b>		
<b>Gametes from hES cells</b>	Deriving gametes from human embryonic stem cells.	People unable to produce gametes, allow same sex couples to have children that are genetically related to them both, to overcome a shortage of oocytes for research and donation.
<b>Meiosis in somatic cells</b>	Induce meiosis in somatic cells; transfer the pronuclei to create genetically related gametes.	
<b>Replacement of sperm with a somatic cell. (This could also be done by replacing the genetic material of both the sperm and egg with somatic cell)</b>	In this process, a somatic cell is injected into an MII stage oocyte. Upon activation of the oocyte, two polar bodies would be expelled. This would result in a diploid embryo.	Men unable to produce sperm, female couples that want to have children that are genetically related to both of them.
<b>Oocyte generation from stem cells in bone marrow</b>	Bone marrow has been identified as a potential source of germ cells that could sustain oocyte production in adulthood.	People who want to delay having children or want to ensure a supply of oocytes could have bone marrow taken from them, from which germ cell progenitors could be derived.
<b>Testicular eggs</b>	A female germ cell was transplanted to a testis. Most became sperm but some developed partly into eggs.	Derive immature germ cells e.g. from embryonic stem cells and then mature them in a testes.

## Use of modified eggs

<b>Germinal Vesicle (GV) transfer</b>	Transfer of GV into the oocyte of a younger woman, where the chance of loss of fidelity of meiotic segregation is less likely to occur.	Treatment for age related oocyte dysfunction due to meiotic segregation or people with mitochondrial disease.
<b>Cytoplasmic transfer</b>	Cytoplasm, including mitochondria, from a healthy oocyte is transferred into another oocyte.	

## New techniques in genetic screening

<b>Comparative genomic hybridisation</b>	An ISH technique that allows the detection of chromosomal abnormalities where there is a net loss or gain of chromosomal material.	Chromosomal loss or gain.
<b>Genetic analysis using a chip</b>	Using microarray technology to screen for many genes or mutations in any single embryo and analysis of expression of genes required during early development.	People who are known to have specific hereditary diseases although potentially this could be used by all patients to select for embryos with specific characteristics. Embryo research.
<b>Comparative genome hybridisation using a microarray</b>	A microarray with sequences that represent the whole genome can be used to detect net loss or gain of regions of chromosomes.	Used for preimplantation screening for loss or gain of chromosomes or regions of chromosomes.
<b>PGD using cells obtained by trophectoderm biopsy</b>	Removal of trophectoderm cells from the blastocyst for PGD.	This would enable more cells to be removed because the trophectoderm does not contribute to the embryo proper. This may improve the accuracy of the test and will also be less likely to cause any long-term effects that may be associated with removing cells that form part of the embryo.

# Horizon scanning 2005

## New techniques in genetic screening (continued)

<p><b>'Intelligence genes' have been putatively linked to two regions on the genome</b></p>	<p>Regions of the genome have been linked to intelligence based on verbal IQ, performance IQ and full-scale IQ.</p>	<p>If these regions were linked or mapped to specific genes, it would be possible to screen for intelligence and associated traits such as autism, schizophrenia or reading disability.</p>
<p><b>Sequencing technique that enables quick and cheap sequencing of entire genomes</b></p>	<p>This technique allows the sequencing of an entire (microbial) genome in a significantly reduced time with equivalent levels of accuracy as are found in current standard techniques.</p>	<p>If this were introduced more widely, it is likely that more people would know their own genetic status (what susceptibility or carrier genes they have). This in turn could lead to an increase in the amount of people requesting PGD.</p>

## Genetic modification of embryos

<p><b>siRNA/ RNAi to create transgenic stable animals. (Genetic modification of embryo)</b></p>	<p>siRNA is used to knock-down a specific gene. It can be targeted specifically to a tissue type and can be inherited.</p>	<p>This could be used to treat over-expression conditions that occur in the embryo. Perhaps if a gene was found to be over-expressed in embryos where recurrent miscarriage occurs.</p>
<p><b>Altered nuclear transfer</b></p>	<p>Embryos are modified to remove a gene that is required during early development meaning that the embryos have limited developmental potential.</p>	<p>Creation of embryos that will only survive to a limited age therefore according to one argument making them more ethical.</p>
<p><b>Sperm mediated gene transfer - transgenICSI</b></p>	<p>Sperm is used as a vector for producing transgenic embryos.</p>	<p>Washed sperm is incubated in a solution of the target DNA. The sperm is then used to fertilise the oocytes. Alternatively the DNA solution can be injected into the oocyte at the same time as sperm is during ICSI.</p>

**Gamete storage and manipulation**

<b>Cryopreservation of unfertilised mature oocytes (also ovarian tissue storage)</b>	Storage of mature oocytes.	People who want to delay having children for social reasons. Women who need to store oocytes for cancer reasons. Storage can damage meiotic spindle, therefore could lead to chromosomal anomalies.
<b><i>In vitro</i> maturation (germ cell development <i>in vitro</i>)</b>	Maturation of oocytes or sperm in the lab.	Reduce the need for drugs because immature eggs could be removed, matured and used for IVF. Young cancer patients. This would also mean that more oocytes would be available for use.
<b>Vitrification</b>	A faster process for freezing embryos or oocytes.	Vitrification would be a change in process and could improve safety because only one patient's sample would be frozen at a time due to speed of the technique. May also be less damaging to the embryos/oocytes than conventional freezing.
<b>mRNA present in sperm although there is no transcriptional activity. NB see transgenicsi- perhaps it is 'soaked up'</b>	mRNA that is transcribed during spermatogenesis is present in sperm. There is no transcriptional activity in sperm therefore the mRNA is left over from spermatogenesis and will not get transcribed. The mRNA could have a purpose in early fertilisation. It could also be used for diagnostic purposes.	If it was found that the mRNA complemented the pool of mRNA present in the oocyte, mRNA could be co-injected with the sperm in ICSI to boost the potential of the embryo.

# Horizon scanning 2005

<b>Gamete storage and manipulation</b> (continued)		
<b>Preservation of fertility following cancer treatment</b>	Sphingosine 1 is a metabolite of Ceramide, which has been shown to mediate apoptosis of oocytes following cancer therapies. Using sphingosine can protect the ovaries from cancer therapies.	If sphingosine therapy was introduced, it could mean that female cancer patients could remain fertile. The safety of the treatment and the quality of the oocytes would have to be considered.
<b>Sperm sorting machine</b>	Good quality sperm is separated from poor quality sperm on the basis of negatively charged membranes being present in good sperm.	Men with reduced fertility due to smoking, age or pollution. All these factors will cause DNA damage.
<b>Activation of pig oocytes by injection of strontium and barium/ ICSI plus chemical activation</b>	Oocytes that were matured <i>in vitro</i> can be activated with barium and strontium. Using this technique they are released from MII arrest, this leads to a series of events related to oocyte activation.	Oocyte activation after <i>in vitro</i> maturation.
<b>Modification of sperm motility</b>	Adenylyl cyclases are used to alter the sperm reaction.	Could prevent premature acrosome reaction <i>in vitro</i> which means the sperm is essentially useless- this could therefore be used to improve the quality of sperm <i>in vitro</i> .

## Embryo manipulation

<b>Differentiation control in embryos</b>	Controlling the number of totipotent cells produced in an embryo to allow embryo splitting, allowing the production of more embryos.	People who do not produce many eggs and therefore there are not many embryos to replace.
<b>Making human chimeras</b>	Combining embryonic cells from the inner cell mass of two embryos or addition of embryonic stem cells to the ICM of an embryo.	Allow genetic contribution of two female partners to a child, adding desirable characteristics to an embryo (using stem cells).
<b>Hybrid embryos</b>	Using animal oocytes as donor oocytes for cloning to produce human embryonic stem cells or for embryos for research.	Overcome shortage of human oocytes.
<b>Cleavage and axis and cell contributing to embryo and anembryonic</b>	Some cells can be identified that will give rise to specific regions of an embryo as early as the 4 cell stage. This could have an impact on PGD.	Certain cells will have less of an impact on the embryo if they are removed, these cells would be chosen preferentially when cells are removed for PGD.

## Embryo Culture

<b>The effect of sub-optimal culture conditions on future development</b>	Research into the long-term effects of IVF.	Improve IVF culture conditions to reduce any long term risks associated with IVF and improve success rates.
<b>Embryo selection</b>	Improving the techniques to assess and culture embryos so the embryo with the most chance of implantation can be selected and put back.	All IVF patients could benefit from this as it could improve success rates.

# Horizon scanning 2005

<b>Embryo Culture</b> (continued)		
<b>Computer detection of nuclear structure</b>	Using a computer to detect mono - and multi-nuclearity.	Use of computer-mediated multilevel morphological analysis can improve the detection of nuclear structures in human embryos to improve embryo selection (relevant to success rates).
<b>Addition of GM-CSF to the media of <i>in vitro</i> cultured embryos</b>	Mouse embryos exposed to this growth factor prevented the restricted foetal growth and the accelerated growth after birth that is seen when mouse embryos are cultured <i>in vitro</i> .	If this was shown to be beneficial, this growth factor could be added to human culture media to improve the development of IVF children.

<b>Stem cell derivation and cloned embryos</b>		
<b>Generation of pluripotent stem cells from neonatal mouse testis</b>	Stem cells are extracted from neonatal mouse testis.	If this were shown to be true in humans, this is an alternative source of stem cells. This could be relevant in treatments with HLA-matched embryos.
<b>'Stembrids'</b>	This technique involves fusing a somatic cell with a human embryonic stem cell.	An alternative source of stem cells that does not require the use of donated oocytes.
<b>Stem cells from an embryo whose development continues</b>	Making stem cells from a portion of the embryo before it is transferred so that the child has personalised embryonic stem cells should they require them for treatment during their life.	Ensure suitable stem cells for a child for future treatment.
<b>Injection of hES cells into anembryonic trophoblast vesicles</b>	Human embryonic stem cells are injected into trophoblast vesicles to produce artificial embryos.	To create multiple cloned embryos.
<b>Embryonic stem cells from parthenotes</b>	Artificially activating an egg to develop and deriving embryonic stem cells from the parthenote embryo.	This technique could be used for more ethical production of stem cells.

## 6.2 Prioritised

These were the issues that were identified during 2005-2006 and were prioritised for consideration in 2006-2007.

- Stembrids (fusion of somatic cells with stem cells to attempt to reprogram and produce a new line of stem cells without the need for eggs and to produce embryos).
- Deriving stem cell lines from individual blastomeres (cells of an early embryo).
- Microarrays for embryo selection.
- Microarrays for PGD.
- *In vitro* derived gametes (derivation of gametes from alternate sources e.g. stem cells).
- Germinal Vesicle (GV) Transfer (transfer of GV into a different enucleated oocyte).
- *In vitro* maturation of oocytes (development *in vitro* of immature oocytes - generally from the germinal vesicle stage).
- Vitrification (alternative cryopreservation technique).

## 6.3 Committee consideration and outputs.

### 6.3.1 Vitrification

SCAG concluded that there are no particular concerns regarding this technique that are above those for freezing, other than potential direct contact with a non-sterile product, but consider proper training and protocols to be essential.

November 2005

### 6.3.2 Stembrids

Members of SCAG felt that the research into these cells was at a very early stage and as yet these cells have not been shown to be an alternative to embryonic stem cell lines derived directly from an embryo. Differences in the cell types could arise because of the limited reprogramming ability of embryonic stem cells compared to that of an oocyte. Further research is needed to demonstrate the value and potential of these cells and this research is awaited with interest.

February 2006

### 6.3.3 *In vitro* derived gametes

This issue was specifically discussed at the 2005 Horizon Scanning Panel meeting. The view of the Horizon Scanning Panel was that sperm would be more likely derived artificially in the near future than oocytes and the most likely source would be from human embryonic stem cell lines.

June 2005

Members of SCAG were of the opinion that the development of gametes from embryonic stem cells is not yet at a stage for therapeutic use but it is very likely that the HFEA will receive a research licence application, for the production of male gametes, within a year. An HFEA licence would be needed if researchers wanted to test the viability of gametes by fertilisation. It was suggested that some research groups may culture human sperm germ cells *in vivo*, e.g. in mice and pigs. They were of the opinion that the development of eggs from stem cells should be considered by SCAG again in a year and that the HFEA would not receive a licence application for this within the next couple of years.

September 2006

This issue was considered by the Authority's Ethics and Law Committee who agreed that both researchers creating embryos from *in vitro* derived gametes, and the creation of embryos for the purpose of deriving stem cells to differentiate into gametes would require a licence from the HFEA.

The Committee agreed that the key issues for the use of *in vitro* derived gametes in treatment are the scientific unknowns, consent issues and definitions of parenthood, which the Committee suggested should be addressed by Parliament.

January 2007

## Horizon scanning 2005

### 6.3.4 In vitro maturation (IVM)

SCAG concluded that no studies demonstrate the technique is dangerous and there is no evidence of abnormalities in the children who have been born following this procedure. However, there is insufficient evidence to be absolutely certain of its safety.

Long-term follow-up studies of the children born following IVM treatment are required to determine if there are any possible effects of using this technique in treatment services. If the technique is offered to patients it should be offered with all relevant information and parents should be strongly encouraged to take part in long-term follow-up studies.

The Group also recommended that clinics would have to demonstrate that they have gained expertise in maturing eggs in the laboratory before being licensed to offer this treatment to patients.

February 2006

### 6.3.5 Germinal vesicle transfer (GVT)

SCAG members agreed that currently there is not enough evidence for the safety and efficacy of the technique in order to use it clinically. It is not known what the long-term consequences of having a mixed population of mitochondria are and more evidence is needed from animal studies. SCAG concluded that this technique needs to be reviewed at regular intervals and new research needs to be monitored.

November 2005

This issue was reconsidered by SCAG the following year. It was decided that the studies on germinal vesicle transfer which have been published since November 2005, support SCAG's previous conclusions on GVT which was that there is currently not enough evidence for the safety and efficacy of the technique in order to use it clinically.

September 2006

### 6.3.6 Microarrays in assisted reproduction

This issue was considered by SCAG at their September meeting. It was suggested that the use of microarray technology for aneuploidy would be nothing more than an advance in the technology used within already identified aims.

Members were of the opinion that this technology may be available for therapeutic use in three to five years. However, the problem with carrying out this technology on embryos is that there may be mosaicism of gene expression amongst the blastomeres.

Members suggested that through the use of this technology it could be possible to identify genes involved in pathways required for embryogenesis. This could then be used to assess embryos non-intrusively e.g. study metabolites in culture media.

September 2006



### 6.3.7 Deriving stem cells from individual blastomeres

The Group came to the conclusion that currently the use of this technique in humans is not realistic, the success rate and number of people who will benefit from it will be low. Also, if it is possible to produce therapeutic lines, embryos will be created specifically for this purpose. SCAG recommends that this is monitored. The Group will consider this technique again in a year.<sup>1</sup>

April 2006

The Ethics and Law committee considered this at a meeting in July. They felt that parallels can be drawn between this technique and cord blood banking for future treatment. In both situations there is a possibility of patients feeling pressure to bank the cells or cell lines for the future health of their child. For this reason it would be important to ensure that patients were made aware that there are very few conditions that may be treated with this.

Members also raised issues regarding informed consent and ensuring that patients understood as far as possible exactly what the treatment involved. It was pointed out to members that the Act does not refer specifically to embryo biopsies, but this is licensed under Schedule 2 1(1)(d), and interpretation of this by the House of Lords allows HLA typing in certain circumstances.

Members felt that further legal advice on the mechanism by which this technique might be licensed was necessary to facilitate further discussion by the Committee and that it would be important to raise this issue with officials at the Department of Health, so that it may be considered as the new Act is being drafted.

July 2006

<sup>1</sup>A research paper was published following SCAG consideration of this issue and this issue is being reconsidered in light of the new data.

# Horizon scanning 2006

## 7.1 Identified

This table presents all issues that were identified through the horizon scanning process in 2006 prior to filtration and prioritisation. This will include issues that will not be followed-up in any detail. This has been included for information.

Name	Description	Use
<b>Gamete storage and selection</b>		
<b>Sperm Desiccation</b>	Desiccation of mouse sperm in the presence of trehalose allows the sperm to be stored and later rehydrated for use in ICSI.	As an alternative to cryopreservation for storage of sperm from donors or cancer patients prior to therapy.
<b>Microarray to analyse gene expression of oocytes</b>	Enough RNA can be amplified from a single human oocyte to analyse the expression of multiple genes on a microarray.	Could be used to identify what makes a 'good' oocyte, i.e. one that is most likely to fertilise and implant. However, this would involve the destruction of the oocyte itself so would only really be useful for research purposes unless a non-invasive test could be developed.
<b>Sperm DNA integrity testing</b>	The degree of DNA damage in human sperm is measured using flow cytometry or TUNEL staining.	Can be used as a prognostic tool, where there is an inverse correlation between sperm integrity and rates of fertilisation and implantation, and embryo quality.
<b>Magnetic selection of apoptotic sperm</b>	Magnetic cell sorting eliminates apoptotic sperm, which are less able to penetrate oocytes.	The selection of non-apoptotic sperm could be used to enhance the results of IVF.
<b>Zeta method of sperm selection based on membrane charge</b>	Utilises the natural electrostatic charge of mature sperm to immobilise mature sperm and wash away immature sperm and debris.	Could be used to select mature sperm and enhance results of IVF/ICSI. A similar issue was identified last year (sperm sorting machine) and SCAG considered this then – awaiting results from a trial.

## Gamete storage and selection (continued)

<p><b>Qualitative RT-PCR for same-day detection of HIV in sperm</b></p>	<p>A PCR based technique that can detect HIV RNA in human semen and its components in the same day.</p>	<p>Processed semen from patients could be tested and validated on the day of collection allowing the immediate use of semen when desired. This issue was considered already by SCAG in September 2006.</p>
<p><b>Marker of oocytes able to fuse with sperm</b></p>	<p>The protein CD9 is expressed in mouse oocyte at the optimum time for fusion with sperm and is indispensable for this fusion event.</p>	<p>If a non-invasive method of CD9 detection was developed this could be used to screen for viable oocytes.</p>

## In vitro derived/artificial gametes

<p><b>Follicle-mimicking 3D co-culture method of <i>in vitro</i> maturation</b></p>	<p>Co-culturing human oocytes with somatic cells provides a suitable environment for maturation. In addition there is growing evidence that the use of hormones in maturation results in poor oocyte quality so this method provides a hormone-free alternative.</p>	<p>An alternative method for <i>in vitro</i> maturation of oocytes, which may result in oocytes of a better quality. This technique could be utilised by women undergoing IVF treatment and is successful in women undergoing natural cycles such as those suffering from polycystic ovary syndrome.</p>
<p><b><i>In vitro</i> spermatogenesis</b></p>	<p>Germ stem cells are isolated from patient and cultured <i>in vitro</i> to develop into mature sperm cells.</p>	<p>Could be used to produce mature sperm from patients with non-obstructive azoospermia for use in ICSI.</p>
<p><b>Testicular stem cell transplantation (TSCT)</b></p>	<p>In chicken, transplantation of male germ cells into the testes of sterilised recipients results in production of heterologous sperm capable of fertilising ova and producing progeny expressing donor genes.</p>	<p>If this technology was transferred to the human it could provide a potential method of preserving fertility in pre-pubertal cancer patients.</p>

# Horizon scanning 2006

<b>In vitro derived/artificial gametes</b> (continued)		
<b><i>In vitro</i> growth</b>	<i>In vitro</i> growth (IVG) of oocytes refers to the development, in the lab, of oocytes from immature (preantral) follicles (earlier than the stage from which <i>in vitro</i> maturation is carried out).	This technique could allow eggs to be matured from ovarian tissue <i>in vitro</i> without the need to transplant. If developed this technology would also mean that future IVF cycles could be carried out without the need for stimulation.
<b>Culture media conditioned by testicular cells supports differentiation of oocytes</b>	Oocytes can be derived from mouse embryoid bodies when provided with the appropriate growth factors present in conditioned testicular cell media.	This technique could be used to produce <i>in vitro</i> derived gametes for women who are unable to produce mature oocytes. This technique is relevant to the issue of <i>in vitro</i> derived gametes which was identified previously. This will be included in the information when IVDG gametes are reconsidered in June 2007.

<b>IVF/ICSI technologies</b>		
<b>Strontium activation with round spermatid injection</b>	Mouse oocytes are activated with strontium in combination with injection of sperm at the round spermatid stage. This technique results in live-birth outcomes.	This could be used to treat certain types of male infertility where the sperm does not mature correctly and only sperm at the round spermatid stage is produced.
<b>IVF within microfluidic channels</b>	Microfluidic devices have been developed that allow the minaturisation of IVF, where smaller volumes of media are required to flow over the embryos. Thus, lower total numbers and concentrations of sperm are required. Tested in mice.	Microfluidics could provide an alternative to conventional IVF.
<b>Reduced co-incubation time of embryos and sperm</b>	Good fertilisation rates have been demonstrated to result from shorter incubation times in human IVF.	Could improve IVF success rates.

## IVF/ICSI technologies (continued)

<b>Removal of zona pellucida disrupts methylation</b>	<p>It has been demonstrated in mouse that removing the zona pellucida between 1-8hpf disrupts methylation, which is important for correct epigenesis/imprinting.</p>	<p>This could have implications for assisted hatching – this information will be considered when the assisted hatching fact-sheet is reviewed.</p>
<b>Laser-assisted ICSI</b>	<p>A laser is used to open and thin the zona pellucida of human oocytes prior to ICSI.</p>	<p>The combination of these 2 techniques results in higher fertilisation rates for couples with repeated failed ICSI attempts.</p>

## Embryo selection

<b>Proteomic analysis of pre-implantation embryos</b>	<p>Mass spectrometry is used to get a protein expression profile of pre-implantation human embryos.</p>	<p>Could be used in research to identify genes involved in viability/implantation potential.</p>
<b>Analysis of the secretome/ metabolome of pre-implantation embryos</b>	<p>A system for identifying the molecules found in the spent culture medium of pre-implantation human embryos. For example, secreted proteins (the secretome) or by-products of metabolism (the metabolome).</p>	<p>An alternative, non-invasive assay of embryo viability. Used to identify the embryo with the best chance of implanting.</p>
<b>Cytochip</b>	<p>Embryos could be tested for up to 30,000 conditions at once using this chip.</p>	<p>Pre-implantation genetic diagnosis of <i>in vitro</i> fertilised embryos.</p>

# Horizon scanning 2006

<b>Embryo manipulation</b>		
<b>Cloning and RNAi</b>	Maternal and embryonic transcripts of genes of interest can be temporarily knocked down.	Could be used to knockdown genes with negative effect on viability or implantation for CNR, for example, knockdown of CDX2 for stem cell derivation from CNR embryos. The most likely use for this would be to circumvent legislation where the potential of an entity determines whether or not it is regulated.
<b>Embryo culture from blastomere biopsy (embryo splitting)</b>	Half of the total blastomeres from 2-, 4-, 6- and 8-cell stage mouse embryos can be removed and cultured to develop into another embryo. Embryos can be split up to 3 times serially, although efficiency is greatly reduced by the 3rd split.	Could be used to increase the number of embryos available for transfer in a single IVF cycle or for storage instead of going through another cycle. Identical embryos could prove useful for PGD and research into disease. Option of creating 'back-up' embryos in case of organ transplant. Currently embryo splitting is banned.
<b>Genetic Screening</b>		
<b>Sperm of patients with balanced translocations is likely to express apoptosis markers</b>	If a non-invasive marker were identified that could test whether sperm were undergoing apoptosis, it would be possible to select sperm without translocations.	Requires identification of suitable marker, but then could screen for balanced translocations prior to ICSI.
<b>Mutations inherited through RNA</b>	Abnormal RNA accumulates in mouse sperm and transmits mutations to offspring even when genomic DNA does not contain mutations.	Implications for testing RNA in sperm for mutations prior to ICSI.

## Genetic Screening (continued)

<b>Preimplantation genetic haplotyping (PGH)</b>	DNA fingerprinting of the human embryo is used to identify chromosomal mutations in diseases where the specific mutation is unknown.	Widens the scope of PGD to diseases where the exact mutation is unknown. This technique is already being used.
<b>Sperm analysis for PGD</b>	Haplotype analysis technique carried out on single human sperm.	Allows analysis of sperm on regions around mutation for linkage analysis.
<b>Multiple displacement amplification (MDA)</b>	A technique which allows amplification of the genome for PGD, prior to PCR.	MDA produces greater amounts of DNA and this allows more PCR analyses to be carried out on one embryo to diagnose more single-gene defects.

## Stem cell derivation and cloned embryos

<b>Testicle stem cells</b>	Mouse spermatogonial stem cells cultured under certain conditions acquire the properties of embryonic stem cells.	These cells could provide an alternative source of embryonic stem cells that does not involve the destruction of embryos. This was discussed at the horizon scanning meeting in Prague. It was felt that the functionality of the cells should be proven before the cells could be thought to be an adequate alternative to hESC.
<b>Use of 'dead' embryos for derivation of human embryonic stem cell lines</b>	Cells from human embryos that have stopped dividing can be used to create hESC lines.	An alternative source of embryonic stem cells that does not involve the destruction of 'live' embryos.
<b>Human embryonic stem cell lines derived from single blastomeres</b>	A single blastomere is biopsied from a developing human embryo and cultured overnight to derive stem cells.	Allows derivation of human embryonic stem cell lines without destroying embryos. Could be used to set up stem cells lines for every individual resulting from IVF. This was already considered but it was suggested at a previous meeting that we reconsider due to new data.

# Horizon scanning 2006

<b>Stem cell derivation and cloned embryos</b> (continued)		
<b>Commercialisation of stem cells</b>	The setting up of companies that supply stem cells.	Increased availability of human stem cell lines for researchers – academics and commercial companies. HFEA might have to licence large scale production.
<b>One-step micromanipulation technique of somatic cell nuclear transfer (SCNT)</b>	A one-step micromanipulation technique for SCNT in rhesus monkey has been developed that is twice as efficient as the previously used electrofusion technique.	Allows the routine production of SCNT blastocysts and increases probability of transferring technology to humans.
<b>Autologous SCNT</b>	A somatic nucleus from a cow cell is transferred to an enucleated cow oocyte of the same individual rather than unrelated.	Results in significantly higher developmental competence and thus higher pregnancy and birth rates in cow.
<b>Epigenesis in SCNT embryos</b>	Cloned rhesus monkey embryos display increased methylation and acetylation of genomic DNA.	Potential danger in transferring this technology to humans and implications for any stem cells derived from SCNT embryos.
<b>Laser technique method to isolate ICM from blastocysts</b>	New method for ICM isolation using laser drilling to eliminate the trophectoderm of mouse blastocysts.	Alternative method for isolating ICM for human embryonic stem cell isolation.
<b>Identity of protein network that defines 'stemness'</b>	Have identified a network of proteins that are responsible for giving mouse stem cells their pluripotency.	Potential to reprogram adult cells to provide an alternative source of stem cells.

**Transplantation**

<b>Xenografting of immature testicular tissue</b>	Testicular tissue from pre-pubertal and adult humans is xenografted into mouse. Spermatogonia continue to be produced.	Potential application in restoring fertility to pre-pubertal cancer patients. If this were to be carried out there would be significant ethical concerns.
<b>Womb transplants</b>	US doctor claims he will perform human womb transplant by 2007. Womb is likely to come from a dead donor.	Restore fertility to patients with abnormal/damaged/absent uterus.

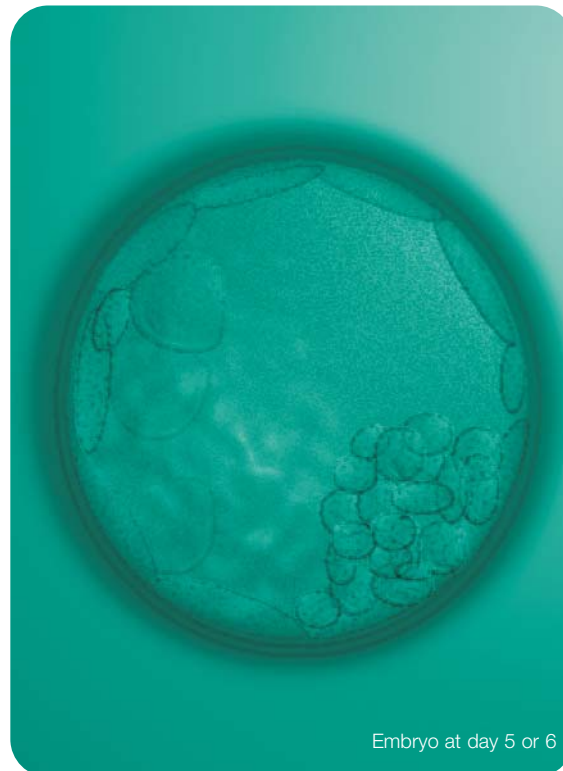
**7.2 Prioritised issues from 2006**

The following issues were identified in 2006 and prioritised for further consideration in 2007. Any outputs following consideration will be available in the horizon scanning report of 2007.

- **In vitro growth of oocytes** (the development, in the lab, of oocytes from immature (preantral) follicles - earlier than the stage from which *in vitro* maturation is carried out).
- **Embryo selection using Metabolomic techniques** (techniques to analyse viability of preimplantation embryos by protein or metabolite expression in the culture media).
- **Derivation of hESC from single blastomeres** (although this was considered previously, it was reconsidered again in light of new data).
- **Preimplantation Genetic Diagnosis (PGD) for lower penetrance conditions** (conditions that people may wish to consider doing PGD for)<sup>2</sup>.

The following issues are ongoing from 2005.

- **Germinal Vesicle transfer** (transfer of GV into the oocyte of a younger woman).
- **In vitro derived gametes** (derivation of gametes from alternate sources e.g. stem cells).



Embryo at day 5 or 6

<sup>2</sup>The HFEA recently carried out a consultation on PGD for lower penetrance conditions – Choices and boundaries. Further information on this can be found on our website <http://www.hfea.gov.uk/en/489.html>

# Assessment of the horizon scanning process

## 8.1 Summary

The establishment of the horizon scanning process has allowed issues to be identified and researched. The success of the horizon scanning process is only likely to be determined after several years because the issues that we aim to identify are those that will impact on the work of the HFEA in several years time.

However in the short-term through the horizon scanning process, we have identified and considered several issues which later have been highlighted in the press or had further developments as suggested by the horizon scanning process. Examples of such issues are vitrification, stembrids and hybrids.

## 8.2 Comments from external people

*"We welcome the setting up of an international Horizon Scanning Expert Panel as a positive step in improving the HFEA's use of evidence."*

House of Commons Science & Technology Committee

*"Many techniques used in animal ART have the potential to be translated into treatments for humans. The HFEA's initiative in getting an international panel together and providing a web forum as a dynamic environment for sharing ideas in real time is fantastic."*

Chris de Jonge - Horizon Scanning Panel member

## 8.3 Internal benefit of the HFEA horizon scanning process

### 8.3.1 Vitrification

We have had many enquiries from centres about using vitrification. The horizon scanning process has enabled inspectors to give informed and consistent advice on this issue. There was also some press interest in this issue at ESHRE 2006 – members of the press office were able to use the view of SCAG to inform their responses to the press.

### 8.3.2 Stembrids

There has been some coverage in the press about stembrids and as the press interest was after the consideration by SCAG, the press officers were able to refer to the view of SCAG when discussing the issue with journalists.

## 8.4 Further refinement of the horizon scanning process

As the horizon scanning process develops it will be necessary to further refine the criteria that we use to identify and prioritise issues. One issue that was identified but not prioritised was that of hybrids. This has subsequently become a significant issue for the HFEA and one that could have been incorporated into the business plan for the year earlier had it been considered to be a priority issue.

One element of the horizon scanning process that makes planning and prioritising issues very difficult is that of timing. When a development is likely to occur in this field is often impossible to predict. This is something that we try to ask members of the Horizon Scanning Panel, but even for experts in the field it is very difficult to put an accurate timing on when a given technique may be applicable to humans.

In order to improve the horizon scanning process and its ability to identify and prioritise those issues that will have an impact on the business of the HFEA, the process is continually being monitored and developed. One way that we will attempt to do this is to look at those issues that have become significant for the HFEA and retrospectively try to identify common features which we can then use prospectively in the future to improve the process by which we identify and prioritise issues.

# Horizon Scanning Panel meetings

## Summary of the first meeting of the Horizon Scanning Expert Panel held on 29 June 2004 in Berlin

### PRESENT:

Members	Executive	Observers
David Barlow	Katy Berry	Claire Ainsworth
Jacques Cohen	Charles Lister	(New Scientist)
Alan DeCherney	Angela McNab	
Paul Devroey	Chris O'Toole	
Hans Evers	Tim Whitaker	
Maybeth Jamieson		
Henry Leese		
Andre Van Steirteghem		
Alan Trounson		
Maureen Wood		

## 1 Apologies

1.1 Apologies were received from David Edgar, Martin Evans, Stephen G. Hillier and Martin Johnson.

## 2 Discussion

### Issues on the Horizon

2.1 The HFEA was briefly introduced. An expert group is required to keep the HFEA abreast of scientific developments that may impact on reproductive medicine, this will allow advance Policy making. Horizon scanning requires both short term (1 year) and longer term (5 years) concepts. New clinical ideas and applications as well as developments in gamete technology need to be considered within the scope of the Horizon scanning group.

2.2 The HFEA Horizon Scanning Expert Panel will have a unique opportunity to contribute to the HFEA and British Policy making.

2.3 It was discussed that one issue on the horizon involves human embryonic stem cells produced from leftover embryos in clinics and Good Manufacturing Practice (GMP). If the stem cells were used for any clinical treatments they would be required to comply with GMP. This will have a significant impact on the labs and the clinics that work with human embryonic stem cells.

2.4 It was noted that the EU Tissue directive will require clinics to attain standards that will push them in the direction of GMP although not all clinics will be able to attain this readily.

2.5 Genetic techniques are performed in IVF clinics and it is from clinics that new techniques are likely to emerge. It was suggested that the logical place for embryonic stem cells to be produced is at the IVF clinic at the end of an IVF program and then be transferred to a lab for differentiation. Embryos that are homozygous mutant for a specific gene will be used to produce 'mutant' embryonic stem cells which will be valuable for understanding various hereditary conditions better and for studying potential treatments.

## Horizon Scanning Panel meetings

2.6 The creation of artificial gametes is probably not an immediate issue because there are still many problems associated with this technology (chromosomal chaos, imprinting) although the differentiation of gametes from human embryonic stem cells is probably closer on the horizon.

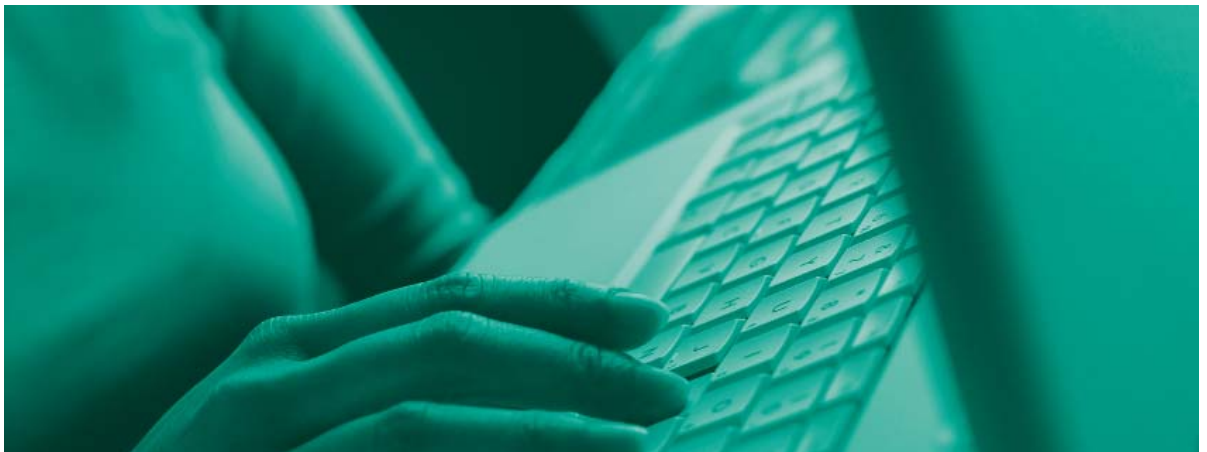
2.7 The membership of the group was discussed and it was suggested that the group might benefit from having a geneticist and Joe Leigh Simpson name was proposed. It was also suggested that the group may benefit from having a lawyer and more women involved.

2.8 There are no other groups with similar functions on which to model the HFEA horizon scanning experts group correspondence. A suggestion was put forward and agreed upon by the members.

- HFEA will be alerted to potential horizon scanning issues by other members of the HHSEP by group email. Any email sent will also be directly sent to other members of the group. This will allow other members of the groups to be informed of any issues raised.
- A Questionnaire / *Pro forma* will be sent out twice a year to the members of HHSEG with current key horizon scanning issues being raised and a response will be returned from each member.
- All responses and received information will be summarised and conveyed to the members of the HHSEP.
- Annual (or biannual) meeting of the group to coordinate with the ESHRE conference.

2.9 It was agreed that the minutes of the meeting, and the terms of reference will be sent to the members.

**Action: HFEA executive**



# Horizon Scanning Panel meetings

## Minutes of the second meeting of the HFEA Horizon Scanning Expert Panel held on 19 June 2005 in Copenhagen

### PRESENT:

Panel Members	Authority members	Executive
Keith Campbell	David Barlow	Katy Berry
Alan Decherney	Chris Barratt	Trish Davies
Chris De Jonge	Peter Braude	Charles Lister
Paul Devroey	Maybeth Jamieson	Peter Mills
Hans Evers	Suzi Leather	Tim Whitaker
Gab Kovacs		
Stephen Hillier		
Alan Trounson		
Andre Van Steirtgem		

### 1. Introduction by Suzi Leather

**1.1** Suzi Leather, Chair of the Human Fertilisation and Embryology Authority, welcomed members of the Horizon Scanning Panel to the meeting in Copenhagen.

**1.2** It was noted that the contribution of the Horizon Scanning Panel was highly valued by the HFEA. The HFEA is involved in licensing and policy development and therefore must be aware of new developments; the horizon scanning process allows this to happen. The expert panel was praised by the UK Parliament's Science and Technology Select Committee in a report about reproductive technologies and the law.

**1.3** Many of the issues raised by members of the Horizon Scanning Panel have been taken forward by subcommittees of the HFEA. There will be a meeting of the HFEA's Scientific and Clinical Advances Group (SCAG) in September where priority issues that have been raised will be discussed for possible incorporation into the 2006-2007 business plan.

**1.4** It was noted that to date, information gathered from the Panel had been in the form of a yearly meeting and a questionnaire sent out twice a year. This means that there is infrequent contact between the HFEA and the Panel and between Panel members. In an attempt to improve the communication of the Panel, the HFEA have developed a web forum exclusively for the use of Horizon Scanning Panel members. This was introduced later in the meeting.

### 2. Discussion on Artificial Gametes

**2.1** This item was introduced by Keith Campbell. He started by discussing what was meant by artificial gametes and suggested that it could mean "a process that bypasses the normal units of genetic inheritance." Artificial gametes could be used to treat male infertility, female infertility, same sex parents and allow an individual to produce both male and female gametes and use these to make an embryo. This process has been described as 'tantamount to cloning'. It was noted that this was not cloning because there would be meiosis and therefore the embryo would not be identical to the parent. Following this introduction, various ways to produce artificial gametes were described.

## Horizon Scanning Panel meetings

### 2.2 Replacement of sperm by a somatic cell

The first technique that was described involved a sperm with a somatic cell. It was noted that this is being attempted. In this process, a somatic cell is injected into an MII stage oocyte. Upon activation of the oocyte, two polar bodies would be expelled. One polar body containing DNA derived from the oocyte and the other containing half of the genetic material contained within the previously-diploid somatic cell. This would result in a diploid embryo.

2.3 This has been carried out by one of the members of the Panel, and he noted that the imprinting in these embryos was not correct and when analysed it was shown that all these embryos were chromosomally abnormal. The embryos were chaotic and it is uncertain whether these issues could be resolved. One member noted that the same was said for cloning.

### 2.4 Germinal Vesicle Transfer

Germinal Vesicle Transfer (GVT) was suggested as the second way that an artificial gamete could be derived. In this process the nucleus of an oocyte at the germinal vesicle stage can be removed and placed in an enucleated donor oocyte at the same stage. This treatment could be used to treat people with mitochondrial disease or older women. GVT has been performed in bovine oocytes.

2.5 It was noted that it is possible to do PGD for mitochondrial diseases.

### 2.6 Replace oocyte and sperm with somatic cells

This is similar to the technique described in 2.2 except that the genetic material of both the sperm and the oocyte are replaced with somatic cells and a pseudo meiotic event occurs. This is likely to result in serious imprinting problems. It was noted that this was also likely to result in a very low efficiency.

2.7 This was followed by some discussion on the relative developments of the technology and societal change. There was some disagreement on which is likely to change first. It is important to understand new developments to be able to educate people about the reality of any technologies that may be introduced.

### 2.8 Development of gametes from embryonic stem cells

This is the most likely way that artificial gametes will be derived for future use. Gametes could be derived from any human embryonic stem cell line but for treatment this technique involves producing a human embryonic stem cell line from an embryo created by therapeutic cell nuclear replacement. The stem cell line can then be differentiated down the pathway of germ cell production. It was noted that the final development could be either *in vitro* or *in vivo*. There has been some success in mice where germ cells were replaced into testes for further development. By deriving gametes in this way, there is less likely to be issues resulting from imprinting problems. One member noted that this technique did not produce artificial gametes and should be referred to as *in vitro*-derived gametes.

2.9 Because both sperm and eggs could be derived from the same human embryonic stem cell line, it would be possible for a person to have a child with themselves. It was suggested that there could be people who would consider doing this. This would have a dramatic response from the public and is likely to be viewed as abhorrent. This would not be considered cloning but is comparable to an embryo derived from a consanguineous relationship.

2.10 It was noted that there should be discussion about this, but it should be made clear that it is not generally supported. It would potentially be possible to ban 'self-fertilisation'.

2.11 When asked to put a likely timing on the introduction of these techniques, one member suggested that it could be anywhere between five weeks and fifty years.

# Horizon Scanning Panel meetings

**2.12** One member raised the issue of 'stembrids'. Work on stembrids was presented by Yuri Verlinsky at the recent International Symposium on PGD in London (May 2005). This technique involves fusing a somatic cell with a human embryonic stem cell. The somatic cell DNA underwent some reprogramming. In producing embryonic stem cell lines the limiting factor is often the availability of donated oocytes. If it were possible to derive further stem cell lines from embryonic stem cells this would not be an issue because of the unlimited availability of human embryonic stem cells.

**2.13** There has been some evidence from a different group of reprogramming of a somatic cell when it was fused to an embryonic stem cell if the two nuclei are kept together initially with the embryonic stem cell nuclei being removed later. It is not certain if the reprogramming that occurs is global.

**2.14** The difficulties surrounding consent and deriving gametes from human embryonic stem cells was raised. It will become difficult to control the use of embryonic stem cells after they have left the stem cell bank. This could mean that gametes are derived from human embryonic stem cells; these would be tested by using them to produce an embryo. It would be important that there is correct consent for this purpose.

**2.15** The issues surrounding the use of artificial gametes were likened to the use of gametes derived from aborted fetuses. One member noted that the HFEA should be careful not to be driven by the media and that just because something was possible, it does not mean that it should be allowed.

**2.16** It was noted that if sperm was derived *in vitro*, it would require a licence to be stored if it were to be used in treatment. However, if the *in vitro* derived sperm was used fresh, it would not require a licence and therefore could potentially fall completely outside of the remit of the HFEA. This would also be the case if the sperm that was derived from the embryonic stem cells were immature. The newly created Human Tissue Authority will have responsibility for the regulation of immature gametes.

**2.17** In conclusion, members noted that sperm would be more likely to be derived artificially in the near future than oocytes.

### 3. Development of the Panel

**3.1** Members were asked to comment on the Panel and its future development. It was suggested that more face to face meetings would be beneficial and it was agreed that the Panel would meet twice a year instead of once a year. The venue of the second annual meeting is to be confirmed. The American Society of Reproductive Medicine (ASRM) meeting was suggested.

**3.2** The web forum was demonstrated and members agreed that it could be useful. Membership to the Panel will be set up for all members upon our return to the UK.

### 4. Date of the next meeting

**4.1** The date of the next meeting is to be confirmed.

# Horizon Scanning Panel meetings

## Minutes of the third annual meeting of the HFEA Horizon Scanning Expert Panel held on 19 June 2006 in Prague

### PRESENT:

Members	Authority	Executive	Observers
Alan Decherney	David Barlow (Chair)	Katy Berry	Francine Manseau
Chris De Jonge	Sam Abdalla	Charles Lister	
Gab Kovacs	Chris Barratt	Angela McNab	
Alan Trounson	Melanie Davies	Tim Whitaker	
Andre Van Steirteghem	Maybeth Jamieson		
Maureen Wood			

### 2. Introduction – the horizon scanning process: after the Panel

2.1 Katy Berry introduced the horizon scanning process and discussed how the HFEA had taken forward some of the issues that have previously been identified. A general overview of the process was presented. Issues are identified either through the Panel, publications, conference attendance and conversations with researchers and scientists. These are researched further and then prioritised by a sub-committee of the HFEA, the Scientific and Clinical Advances Group (SCAG). The high priority issues are then included in the business planning process for the following year. The issues are then considered by the Authority or one of the subcommittees of the Authority (Scientific and Clinical Advances Group or the Ethics and Law committee) and a decision is taken or output is produced.

2.2 Examples of some of the issues identified during 2005, although not necessarily taken forward, were presented to give members an idea of the kind of topics that are useful to hear about. The examples presented were: trophectoderm donation, germ cells in the bone marrow, artificial gametes, IVM and genetic modification of gametes. This was then followed by a list of those issues that were prioritised for consideration in 2006-2007 these were: stembrids, deriving stem cells from individual blastomeres, microarrays for embryo selection, microarrays for PGD, *in vitro* derived gametes, IVM, vitrification and germinal vesicle transfer.

It was noted that as some of these techniques were very close to being used or are being used, these issues were considered sooner. The outputs for the techniques that have been considered to date were presented (these will be available elsewhere on the webforum).

# Horizon Scanning Panel meetings

**2.3** Members of the Panel were told that the HFEA is intending to produce a horizon scanning annual report. The first complete cycle of horizon scanning (from issues identification to output) is coming to an end in the Autumn of this year and this will be when the first annual report will be available. This report will be made available to members when it is complete.

### 3. Discussion on *cdx2* null mice

**3.1** Alan Trounson introduced the discussion on this subject. He noted that to clone a human embryo from which to produce a stem cell line is likely to require many oocytes he suggested a number in the region of 200. One alternative that has been suggested is the use of animal oocytes to circumvent the ethical issues around obtaining the large numbers of human oocytes that are likely to be required. This has been carried out by a group in China and the follow-up work on stem cell lines derived from interspecies CNR show that the cells had a very slow turnover, which could be caused by incompatibility between the mitochondrial and nuclear DNA.

**3.2** There have been some reports of reprogramming of somatic cells using already-derived human embryonic stem cells. To do this it has been necessary for both nuclei to be present. By allowing mixing of cellular components through holes made in the two cells (somatic cell nucleus and a nucleus from the human embryonic stem cell line), it could be possible to avoid having to separate the nuclei from the somatic and the nuclear cells. Alternatively, it could be possible to separate the somatic and stem cell nuclei by using a stem cell line that has specifically been developed to be triploid. This will mean that following reprogramming of the somatic cell, the nucleus from the stem cell can easily be separated based on differential mass using centrifugation.

**3.3** Embryos that are null for the *cdx2* gene do not form a trophectoderm (CDX2 is required for trophectoderm formation) but do form a small clump of cells equivalent to the inner cell mass. It is possible to derive an embryonic stem cell line from the *cdx2* null ICM-like cells. If the expression of *cdx2* was temporarily removed (using RNA interference – RNAi) from a somatic cell, it would be possible to carry out SCNT using this cell to form the ICM-like cells and

from these an embryonic stem cell line. The knocked-down expression of *cdx2* is only temporary so there are no long-term consequences in the stem cell line.

**3.4** It was noted that this technique may get round the legislation in countries where SCNT is illegal because the entity may not be considered to be an embryo. It was noted that in the UK it would not be necessary to circumvent the legislation because SCNT is permitted. If someone did want to carry out this technique, it would probably require a licence from the HFEA.

**3.5** It was suggested that this entity should not be referred to as an embryo as it would never be able to develop. If it were not called an embryo then people would be happier for the entity to be used in research.

### 4. Discussion on testicular stem cells

**4.1** This topic was introduced by Chris Barratt who told the group about a recent paper where cells that were thought to be pluripotent were isolated from testicular tissue (Guan et al Nature 440 -2006). The group was asked to comment on whether they thought that these cells would be equivalent (or equivalent enough) to embryonic stem cells.

**4.2** It was noted that this work is interesting but only if someone else is able to repeat the results. Although the cells have been analysed with specific markers the real test will be in the function of the cells.

**4.3** It was noted that if it were possible to derive stem cells from these tissues it would be more likely to be accepted in the USA than cells derived from embryos. The stem cells from testicular tissue were compared to those stem cells that are found in cord blood. The cells in cord blood were not as pluripotent as previously suggested. The group suggested keeping a watching brief on these cells as an alternative source of pluripotent cells.

# Horizon Scanning Panel meetings

## 5. Discussion on oocyte and tissue freezing

**5.1** This topic was introduced by Gab Kovacs. He noted that people are being offered oocyte and tissue cryopreservation to preserve fertility either for people suffering from cancer or for those people that would like to delay having children. Members were asked to comment on the appropriateness of offering this service when it is not reliably established.

### Oocyte cryopreservation

**5.2** It was noted that, to date, there were probably only about 100-150 children born following egg freezing. A member estimated that for someone to complete their family (2 children) using frozen embryo transfer it would be necessary to collect 44 eggs. This number would be even greater if it was oocytes that were frozen rather than embryos. One member noted that about 1-2% of thawed oocytes resulted in a live birth.

**5.3** Conventional oocyte freezing was compared to vitrification. Following freezing about 1-2% of oocytes result in a live birth compared to 9-10% for vitrification (6/64). The survival rates and fertilization rates of oocytes following freezing are improving but the live birth results are still low.

### Tissue cryopreservation

**5.4** It was noted that in the UK ovarian tissue cryopreservation is not regulated by the HFEA unless there are mature oocytes present. However a licence is required by the Human Tissue Authority (HTA) to transplant the tissue back in to the patient. There are some tissue samples that have been in storage for many years where there is not information such as ethics committee approval because of the time the tissue has been in storage.

## 6. Any other issues

**6.1** One member raised the issue that it is possible that women may wish to continue storing embryos not to be used in fertility treatment but in the event of an already existing sibling requiring a tissue matched donor. If any of the embryos in storage were a match, it could be possible for stem cells to be derived from the embryo to be used to treat the sick sibling. It was noted that the HFEA's Ethics and Law committee could consider this issue although in the UK the legislation (HFE Act) defines statutory storage limits.

**6.2** There was some discussion about public banking of cell lines for future treatment being a more practical solution rather than individuals trying to store enough tissue for their own use. By using stem cell therapy, patients may be able to tolerate transplanted tissue/cells.

**6.3** The issue of paid oocyte donation was raised. Charles Lister clarified that the current position at the moment was that the EU directive outlaws payments but allows expenses and inconvenience. The HFEA have just carried out a review of sperm, egg and embryo donation (SEED) review. One of the outcomes of the review was that donors may be reimbursed all demonstrable out-of-pocket expenses incurred within the UK in connection with gamete or embryo donation. In addition to reimbursement of out-of-pocket expenses, donors may be compensated for loss of earnings (but not for other costs or inconveniences) up to a daily maximum commensurate with jury service but with an overall limit of £250 (or the equivalent in local currency) for each 'course' of sperm donation or each cycle of egg donation.

# Horizon Scanning Panel Questionnaire Response Summaries

October 2004

## October 2004 horizon scanning questionnaire

The questionnaire consisted of three questions; two general horizon scanning questions and one about *in vitro* maturation (IVM). Since horizon scanning is an ongoing process we would be happy to receive responses to the questionnaire or information on any horizon scanning issue at any time.

### The questionnaire

1. What do you consider to be the biggest issue on the scientific horizon that could impact on assisted reproductive medicine and/ or technology in the future (in the next one to five years)?
2. Are you aware of any techniques that are being developed in animal models that will be potentially transferable to human ART in the future? On what time-scale do you think this will occur?
3. What are your views on *in vitro* maturation of oocytes? In your opinion, do you think that there is sufficient knowledge about embryos created using *in vitro* matured oocytes to allow these embryos to be used in treatment services?

## Summary of responses

The responses to the first two questions have been summarised according to topic because there was some overlap in the issues and topics raised. The third question about *in vitro* maturation of oocytes is summarised separately.

### Questions 1 and 2

#### Issues relating to gametes

Several experts commented that the development of germ cells *in vitro* is an issue that could have an impact on ART in the future. This was specifically brought up in association with development of both sperm and oocytes from embryonic stem cells. It was noted that generation of oocytes from embryonic stem cells could potentially overcome a shortage of oocytes for use in derivation of further embryonic stem cell lines, if the embryos from the stem-cell

derived oocytes were able to develop to the blastocyst stage.

One member of the Panel mentioned that *in vitro* sperm maturation is being developed in animals and could potentially be transferable to humans within five years. Another issue raised was that of germ cell transplantation which, in the opinion of the expert, could potentially have an impact on ART within 5 years. It was noted that *in vitro* maturation and cryopreservation of oocytes will require risk assessment for higher rates of chromosomal anomalies.

### Responses from experts relating to gametes

- Improvement on the freezing protocols for oocytes, will require analysis for abnormalities following fertilisation including chromosomal abnormalities.
- Germ cell development *in vitro*.
- Generation of germ cells from embryonic stem cells.
- Gender separation of spermatozoa by fluorescence activated cell sorting (FACS).
- Derivation of oocytes from mouse embryonic stem cells ~10 years, several groups are trying to reproduce experiments of such oocyte production from ES cells. If we could produce oocyte-like cells from human ES cells, which could at least develop to the blastocyst stage, there would be an unlimited supply of oocyte-like cells to reprogram somatic cells and derive ES cells by using the somatic cell nuclear transfer to produce stem cells that have the identical genetic make-up as the patient. Without this it would be very difficult to supply enough oocytes for this technique to be possible.
- Derivation of germ cells and male gametes from embryonic stem cells (~10 years).
- *In vitro* sperm maturation (5 years).
- Germ cell transplantation (~3-5 years).

# Horizon Scanning Panel Questionnaire Response Summaries

October 2004

## Issues relating to embryos

Controlling differentiation of embryos to maintain proliferation of a cleaving embryo to gain large numbers of cells for embryo splitting was an issue raised by one of the Panel members.

The production of human chimeras was mentioned in two contexts. The first, stem cells with desirable characteristics can be added to the ICM of an embryo to enhance the genetic 'quality' of an embryo. The second reason for potentially producing chimeras would be to allow lesbian partners to have equivalent genetic contribution to a child by combining embryos created from fertilised eggs from each partner.

It was suggested that expression analysis of developmentally important genes in embryos *in vitro* could predict the implantation potential of an embryo prior to implantation. This would allow selection of embryos with high chances of implantation on characteristics other than morphology. Many of the expert Panel stressed the importance of research in the area of embryo culture, with regards to both the use of automated culture systems and also the effect of sub-optimal culture conditions on embryos.

Research in this area would potentially produce more sophisticated techniques to select the best embryo to transfer. Improved assessment of the best embryos to transfer will improve success rates so that single embryo transfer will be more attainable.

New technology such as comparative genome hybridisation or microarray technology (gene chips) could have an impact on screening of preimplantation embryos for chromosomal abnormalities or developmental gene expression.

## Responses to experts relating to embryo development

- Controlling the differentiation of the cells within the cleaving embryo so as to maintain proliferation and gain large numbers of cells for potential embryo splitting.
- Making human chimeras so as to have equivalent genetic contributions from two women (lesbian partners).
- The impact of sub-optimal culture conditions on the health of individuals born to ART procedures.
- Embryonic stem cells and their insertion into the ICM of embryos to make desirable chimeras, or their injection into anembryonic trophoblast vesicles to create multiple cloned vesicles to create multiple cloned embryos.
- Improved ways of growing embryos and the use of automated culture methods (5 years).

## Ethical issues

Two experts commented on ethical issues in terms of the future with embryos selection; the use of IVF for selecting characteristics of embryos in fertile couples and how far 'scientific perfection' should be allowed and regulated.

## Responses from experts regarding ethical issues

- Investigation and discussion about scientific perfection and health risk for the baby, and also how far such medical practice should be allowed and regulated.
- The continued use of IVF to select for certain characteristics in the offspring, irrespective of infertility.

# Horizon Scanning Panel Questionnaire Response Summaries

October 2004

## Question 3

### Use of IVM in treatment

A large majority of Panel members considered there to be insufficient research and information on the procedure for use in treatment. However, it was pointed out that there had been successful pregnancies from IVM oocytes and the technique is promising. It was suggested by one member that it could be possible to resolve the major issues surrounding IVM within five years.

It was noted that before IVM be used in treatment, further research would need to be carried out. The research would need to focus on the effects of imprinting or epigenetic reprogramming. Maternal imprinting occurs during the maturation of the oocyte; if maturation occurs *in vitro*, it may have an impact on this process and could lead to an increase in the cases of rare imprinting diseases. A study of the potential of embryos derived from IVM oocytes to form embryonic stem cells was suggested as a way to understand more about the potential of these embryos.

The general feeling from the expert Panel was that further research into the effect of IVM on oocytes and the potential of the embryos was needed before being offered as treatment to patients.

### Responses from experts regarding the use of IVM oocytes for treatment

- “I think this field is still immature and lacking enough data to allow clinical treatment.”
- “A promising technique, but still in the experimental, pre-clinical phase of development.”
- “There is now a wealth of data and background information on IVM of bovine oocytes, including pregnancy rates and pregnancy failure rates after transfer of *in vitro* produced embryos to recipients.”
- “It is premature to introduce IVM of human oocytes clinically without adequate investigation using non-human primate models. One of the most resounding issues regarding this technology is imprinting and whether normal patterns will be retained with *in vitro* growth.”

- “Knowledge of epigenetic influences on oocyte development is insufficient to allow such embryos to be used for treatment. More basic research on signalling between somatic and germ cells and translational research on the optimisation of *in vitro* culture conditions for oocyte maturation is still required.”
- “*In vitro* maturation rates and clinical success seems to be slowly increasing particularly considering the data that is made available through Danish clinics...A new approach with human IVM must be carefully considered, particularly in the light of imprinting disease studies in humans after IVF, which are mainly concerned with a dysfunction of maternal allele modification. IVM may increase the rates of these very rare diseases.”
- “...despite the fact that several pregnancies resulting from IVM have been reported, progress has been slow in developing and optimizing techniques for human application (and for that matter also for domestic species). The underlying problem is still our incomplete knowledge of how the oocyte acquires developmental competence during its growth within the follicle. The culture systems that have been developed to support the development of immature oocytes have benefited from increasing knowledge of at least some of the endocrine/autocrine/paracrine factors involved. But only when we have a more in-depth/comprehensive understanding of what is required during development to make a viable oocyte, will we perhaps be able to develop *in vitro* culture systems for routine clinical application.”
- “As with any new technique outcome should be carefully monitored. More prolonged *in vitro* culture warrants further experimental study.”
- “*In vitro* maturation of oocytes is clearly necessary in any valid ART program.”

# Horizon Scanning Panel Questionnaire Response Summaries May 2005

## May 2005 horizon scanning questionnaire

The questionnaire followed the same format as the previous questionnaire and consisted of three questions: one regarding altering the genetic structure of an embryo, one regarding blastomere developmental properties and one general horizon scanning question.

### The questionnaire

1.
  - a) The 1990 HFE Act prohibits '*altering the genetic structure of any cell while it forms part of an embryo.*' Can you think of any reason why it would be desirable to alter the genetic structure of an embryo either for research or treatment?
  - b) Would application of siRNA or RNAi, in your opinion, constitute altering the genetic structure of an embryo? For what purposes would this be used in either treatment or research?
2. A recent publication from the laboratory of Magdalena Zernicka-Goetz suggests that from as early as the four cell stage, mouse blastomeres have different developmental properties depending on the initial orientation of the cell cleavage. In your opinion, does this have implications for PGD?
3. Are there any issues that you are aware of from the last 6 months that could have an impact on assisted reproductive technologies or research in the near future e.g. in genetic screening, cryopreservation, embryology, IVF or human embryonic stem cell derivation?

## Summary of responses

### Question 1

This question was put to members in order to gain information regarding possible future techniques that might constitute altering the genetic structure of an embryo.

In particular, the possible implications of siRNA and RNAi techniques for the ART field as their use in human embryos becomes more realistic. The HFE Act prohibits altering the genetic structure of any cell while it forms part of an embryo but it is useful to gauge opinion regarding whether using these RNA techniques would strictly constitute breaking this prohibition.

### Question 1a)

One expert suggested that before considering this prohibition we need to be clear what "genetic structure" means in law.

A number of reasons were given as to why it would be desirable to alter the genetic structure of an embryo. With regard to treatment it may be useful if a specific gene defect can be corrected by insertion of a 'replacement' gene at the preimplantation stage (e.g. in the case of dominant, inherited genetic abnormalities, cystic fibrosis, diabetes). This is not a practical proposition at present, as techniques with necessary specificity and efficiency for this application have not been developed, but it is conceivable that appropriate technology might be developed in the future.

With regards to research, the following suggestions were given as to why it would be desirable to alter the genetic structure of an embryo:

- Introducing or knocking out a gene to test a hypothesis<sup>3</sup> about early human development and differentiation. These experiments could be particularly informative/essential as human embryos differ greatly from say mouse model embryos and we know relatively little about the fundamental cellular processes involved in human embryogenesis.
- Increase understanding of long term control of gene expression by altering the structure of DNA and surrounding proteins (e.g. by introducing episomal DNA).

<sup>3</sup> Hypothesis about early human development and differentiation = e.g. a proposal regarding the function of a gene that is subject to experimental test.

# Horizon Scanning Panel Questionnaire Response Summaries May 2005

However, it was also suggested that because, at present, there is no method to avoid genetic alterations to embryos being passed on to the next generation it should probably be avoided.

## Question 1b)

All the experts, except one, were of the opinion that application of siRNA or RNAi does not strictly constitute altering the genetic structure of an embryo as the intent is to influence gene expression. Also, if applied using transient systems, the resulting manipulation of gene expression is reversible. One expert expressed the opinion that if the potential outcome of using this technology would significantly further our understanding of early human development and the embryo is not allowed to develop past 14 days, its use should not be a major problem.

The following possible uses of the technology were suggested:

- Investigating the function of a specific gene in early human embryo development, for example by observing developmental responses to gene silencing.
- Rejuvenating older eggs.
- Transient suppression/expression of particular genes during early development for certain treatment or research.

## Question 2

The paper by Zoenicka-Goetz examines the developmental potential of individual mouse cells at the four-cell stage by making chimeric embryos with four cells of the same type (type of cell is defined by order of division and position in relation to polar body). This question was put to Panel members as the paper suggests that as early as the four cell stage, blastomeres have different developmental potential. This raises concerns regarding embryo biopsy for PGD as the findings suggest that removal of one cell could have more of an effect on development than others.

A number of Panel members considered this publication to be controversial and that progress in this research must be watched. Work of other research groups does not support these findings; there is evidence to show that removing specific blastomeres does not lead to any loss of developmental potential. In addition, existing PGD techniques have not shown any problems. But in past work failures on blastocyst removal tended to be ascribed to experimental shortcomings, rather than restricted developmental potential.

One Panel member commented that this research is unlikely to be of practical relevance because blastomere biopsy for PGD would not normally be done as early as the four cell stage. However, removal of any cellular material at any stage of preimplantation embryonic development is likely to impact subsequent development to greater or lesser extent and risk benefit analysis will always be required in every situation.

Another Panel member suggested that these findings will also have relevance for the outcome of embryo freezing. The poor outcome from transfer of embryos with 50% of cells intact (especially transfer of four cell embryos with only two intact blastomeres) may reflect a failure of cell potential rather than simply a small number of intact cells.

It was also noted that before any implications for PGD can be assessed, it will be necessary to assemble evidence from other mammalian species and ideally from human blastocysts, to show that the same is true in humans. It should be possible to characterise human blastomeres at equivalent stages well enough to be able to selectively remove individual cells in order to follow their lineages that arise on subsequent tissue culture.

# Horizon Scanning Panel Questionnaire Response Summaries May 2005

## Question 3

This question gave Panel members the opportunity to inform the HFEA of recent developments in areas of science that may have an impact on assisted reproductive technologies or research in the near future.

Vitrification as a new method of cryopreservation was identified by two members as a development which may soon become a reality for clinical practice. The technique has been successful in animal species and embryo vitrification packs have recently been marketed.

One Panel member was of the opinion that there should be discussion by the HFEA and recommendations about the appropriateness of egg freezing and ovarian tissue freezing and its role in preserving fertility. Another Panel member suggested that there will be growing pressure for oocyte cryopreservation for social reasons.

Due to the progress in human somatic nucleus transfer technology by the Korean group therapeutic cloning was highlighted as an important issue by two Panel members.

- The Korean group have shown that therapeutic cloning is a feasible way to avoid immunological rejection in cell therapy using hES cells, therefore regulation of such work should be facilitated.
- The rapid advance of the Korean group relative to that in the UK (Newcastle) is likely to be at least in part due to the availability of fresher oocytes in Korea and that any restrictions and ethical considerations relating to the provision of such material in the UK need to be discussed now.
- Somatic cell dedifferentiation for stem cell production instead of oocytes is a possibility in the future. The Panel member suggested that development of these techniques would greatly ease the ethical objections to therapeutic cloning.



# Horizon Scanning Panel Questionnaire Response Summaries July 2005

## July 2005 horizon scanning questionnaire

### The questionnaire

In recent testimony before a subcommittee of the USA Senate alternative methods for obtaining embryonic stem cell lines were discussed / proposed. One of these methods was that pluripotent stem cell lines could be obtained from biopsied blastomeres.

I am unaware of any published data that shows that that human embryonic stem (hES) cell lines have been derived from a single cell taken from biopsied embryos and I am only aware of one article reporting the establishment of an embryonic stem cell line from eight cell stage mouse embryos (Delhaise et al., 1996 Eur J Morphol).

Are you aware any research groups who are working or have worked on trying to derive stem cell lines from biopsied embryos in any animal model? Is this proposal scientifically possible?

### Responses

"At the present time I feel that the ability to isolate ES cells is very limited, when suggesting the use of a biopsied embryo as starting material this further limits the possibility based on present success rates. However it is possible that various tricks could be used to increase cell numbers and therefore increase the possibility of isolating an ES cell like line. Therefore I feel that this possibility cannot be excluded on a scientific basis."

### The questionnaire (continued)

Please find attached a recent article published in Nature where the group established stem cell lines from individual blastomeres. This exemplifies how quickly things can move on in this area.

Given that this technique has now been established in a mouse model, can we assume that it will be possible to do in human embryos in the near future. What problems/limitations could prevent this from happening in humans?

### Responses

"If indeed the technology can be transferred from mouse to human and be condoned by the scientific and lay communities then there is the possibility of creating individualized hESC repositories, i.e. IVF for the purpose not only of creating offspring but also for establishing a personalized (familial) cell line for possible future therapeutic purposes."

"Verlinsky in Chicago says that they have derived human ES cell lines from human blastomere . Details of their lines are, however, sketchy."

"The paper by Chung et al. shows that mouse ES cell lines can only be derived from single blastomeres by co-cultivation with pre-existing ES cells. If this technique is to be applied to the derivation of human ES cells two obvious questions are (i) will pre-existing human ES cell lines offer the same necessary support for self-renewal while cell numbers build up from a single human blastomere (likely) and (ii) which pre-existing human ES cell lines should be used or can other cells substitute? The latter question involves scientific, clinical and ethical considerations."

# Horizon Scanning Panel Questionnaire Response Summaries

December 2005

## December 2005 horizon scanning questionnaire

### The questionnaire

Do you think it would it be possible for a totipotent cell removed from an early embryo to divide and develop into another embryo, so that there were two identical embryos one of which was derived from the removed, single totipotent cell?

If this were possible, can you think of any reason why someone may wish to do this for research or treatment?

### Responses

"I do not recall for certain what has been done in this regard but as far as I remember, separated blastomeres from two cell stage mouse embryos can each give rise to viable embryos, which of course would be identical twins. I also thought this had been achieved with blastomeres from the four cell stage - but not later; however, I also have the memory that using chimera techniques it was shown that blastomeres from eight cell stage retain totipotency, but the proposition was that their size precluded them forming viable embryos. I do not know about other mammals.

I cannot think why anyone would want to do this with respect to human assisted conception. However, for experimental purpose I imagine there may be reasons for doing this, for example:

- To optimise techniques for deriving ES lines one might think of someone separating blastomeres from two or four cell stage embryos, producing genetically identical blastocysts, and then attempting to derive ES lines from them using different techniques.
- Likewise one might do the same to investigate some aspect of early embryonic development by allowing such 'twin' embryos to develop under different conditions, or perhaps after different genetic manipulation, for example, to understand regulation of early cell lineage allocation in human embryos: is differentiation to trophoctoderm, epiblast and primitive endoderm different in humans from mouse? Answers could be pertinent to understanding problems in early development."

"I agree with the previous comments and list.

Another reason to do such experiments might be to test ideas emerging about the onset of polarity in the early embryo. I'm not up to date with this topic (I'm sure some Panel members are) - but I think some are saying polarity is established at the two-cell stage; others, at the eightcell.

Either way, this raises the possibility that blastomeres removed by embryo biopsy for diagnostic purposes may not be equivalent and that this might lead to consequences (at present not revealed) for offspring conceived by ART."

# Horizon Scanning Panel Questionnaire Response Summaries

November 2006

## November 2006 horizon scanning questionnaire

### The questionnaire

1. Would any entity created by activating a human somatic cell nucleus within an enucleated animal (e.g. cow or rabbit) oocyte:
  - a) be viable, or, at least, possibly viable?
  - b) contain a complete human genome?
  - c) be a human embryo?
  - d) ever have the potential to develop and result in a live birth, if implanted? (N.B. the HFE Act 1990 prohibits this)
2. Given that the proteins present would be predominantly animal, would the entity created be human from the moment of activation? If not, at what stage, in your opinion, would the entity become human? How long would the animal proteins be present?
3. What would be the significance and likely effect of the presence of animal mitochondrial DNA on any such entity's development?

### Question 2

The general view of Panel members was that at some stage after embryonic genome activation all proteins produced (with the exception of those coded by the animal mitochondrial genes) would be human. In humans embryonic genome activation does not happen until between the four and eight cell stage. Therefore, until this stage, the embryo is relying on proteins and genetic messages that were present in the oocyte i.e. from the animal.

### Question 3

Members who felt able to answer this question were of the view that the mixture of human and animal mitochondrial DNA would have a negative effect on the development of this entity, reducing its viability. Members felt that the work on animal cybrids (the fusion of an enucleated somatic cell with a somatic cell) would be worth reviewing. These entities may be more viable if the animal mitochondrial DNA is eliminated.

## Summary of responses

### Question 1

Panel members agreed that the entity would contain a complete human genome. One panel member was of the opinion that the entity may contain human mitochondrial DNA, as well as nuclear DNA, as mitochondria transferred with the donor nucleus may be preferentially replicated.

There were mixed views but it was generally thought that these entities are only likely to be viable and have the potential to result in a live birth if they are constructed from related species.



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