

# Scientific Horizon Scanning at the HFEA

Annual Report 2007/08

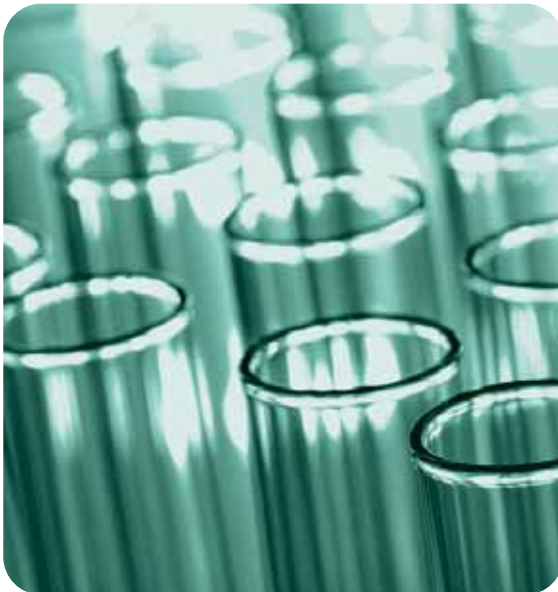
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# Scientific horizon scanning

## 1.1 Introduction

The horizon scanning process is an early warning system that identifies new scientific and clinical developments that may impact on the field of assisted reproduction or embryo research.

It was first introduced in 2004 and is carried out through a rigorous and systematic appraisal of scientific research that is conducted both within and outside the UK. The HFEA's horizon scanning process has received international recognition and has been used to brief numerous stakeholders worldwide. The horizon scanning process is being continually improved and will be reviewed this year.



## 1.2 Purpose of horizon scanning

The area of science and medicine regulated by the HFEA is fast-paced and can be controversial. As a result, it is often necessary for the HFEA to make decisions about new treatments or research applications in an area where there has been little previous research or consideration.

Horizon scanning allows the HFEA to consider the legal, ethical and scientific implications of any new technique that scientists or clinicians may wish to use in HFEA-licensed research or treatment. The Authority can then be prepared with information to make a decision on the potential licensing of techniques, or have guidance in place to ensure that new treatments are carried out safely and appropriately. The HFEA can also ensure that patients and the wider public are suitably informed.

In the past the horizon scanning process has identified issues such as deriving gametes *in vitro* from stem cells (artificial gametes). This technique could potentially allow someone unable to produce gametes naturally to have children genetically related to them. The issue has become especially important during the recent debates of the Human Fertilisation and Embryology Bill in Parliament. The horizon scanning process allows the HFEA to regularly monitor updates in this area and to inform the Department of Health and other stakeholders as necessary.

In order to be as thorough as possible horizon scanning considers animal studies as well as studies on humans. Some of the information presented in the report is very new and therefore has not been independently validated.

# The horizon scanning process

## 2.1 Overview

### Identification of issues that could impact on assisted reproduction or embryo research

Issues identified from journal articles, conferences or suggestions from experts



### Gathering further information on identified issues

Information gathered internally with input from experts as required



### Prioritisation

Issues prioritised and fed into the business planning process



### Consideration by committees

Issues considered further by sub-committees of the HFEA. This may require further work e.g. research and/or policy review



### Output (Authority position, new policy)

Issues discussed by Authority (or a sub-committee of the Authority) and a decision taken on the output e.g. policy review for some issues. The output will depend on the work required for the issue in question



## 2.2 Internal identification of issues

The internal horizon scanning process is carried out by the HFEA's Policy Team. They regularly identify and record relevant research throughout the year by reading journals and attending conferences. Common themes and issues can then be identified. The Policy Team research these issues further and also gather information from experts, such as members of the HFEA's Horizon Scanning Panel.

The HFEA's Scientific and Clinical Advances Group (SCAG) is made up of members of the Authority, including lay members, and co-opted members. It meets about four times a year to review scientific and clinical developments that may impact the work of the HFEA. SCAG members provide recommendations about these developments to inform HFEA policy formulation.

SCAG has an annual meeting to prioritise issues identified through the horizon scanning process. The Policy Team present information to SCAG on all the issues identified throughout the year, along with recommendations for further work and prioritisation. Members of SCAG discuss the issues and agree which should be considered high priority. Issues are prioritised using a systematic approach that looks at whether:

- the technique is transferable to humans for research or treatment;
- the diffusion of the technique is likely to be rapid;
- there will be public interest or concern;
- there will be ethical or legal considerations;
- the technique is within the remit of the HFEA.

The prioritised issues are then fed into the business planning and agenda scheduling to be considered by SCAG over the coming year.

## The horizon scanning process

### 2.3 Horizon Scanning Panel

The HFEA has brought together an international panel of experts to advise on scientific horizon scanning issues. The Horizon Scanning Panel is a virtual group that communicates with each other and the HFEA predominantly via email. Generally the Policy Team send questions to members four to five times a year. The questions are on issues identified by the horizon scanning process and on ad hoc issues that arise throughout the year requiring a scientific view point. There is also an annual meeting where members meet face to face to discuss current and future issues.

### 2.4 Horizon scanning outputs

Each issue that has been identified as high priority during the horizon scanning process will be considered in depth at one of SCAG's committee meetings throughout the year. This may lead to the issue being referred to the Authority to decide on a policy review or on a new Authority position.

All issues that have been identified during the horizon scanning process, along with any outputs from issues considered in that year, are presented in the annual horizon scanning report. The report is launched at the HFEA's annual horizon scanning meeting at the European Society for Human Reproduction and Embryology (ESHRE) conference in July each year. It is circulated widely, including to key stakeholders in the research field and the Department of Health. It is also put on the HFEA website. The HFEA is intending to produce a lay version of the horizon scanning report to inform the wider public.



## 3.1 Members

Name	From	Area of expertise
<b>Professor Twink Allen</b>	Paul Mellon Laboratory of Equine Reproduction, UK	Equine reproduction
<b>Professor Peter Andrews</b>	University of Sheffield, UK	Embryonic stem cells
<b>Professor David Barlow</b>	University of Glasgow, UK	Reproductive medicine
<b>Professor Keith Campbell</b>	University of Nottingham, UK	Animal cloning
<b>Professor John Carroll</b>	University College London, UK	Reproductive physiology
<b>Dr Jacques Cohen</b>	Institute for Reproductive Medicine and Science of Saint Barnabas, USA	Assisted reproductive technology/ Preimplantation genetic diagnosis
<b>Professor Alan Decherney</b>	National Institutes of Health (NIH), USA	Obstetrics and gynaecology
<b>Professor Chris De Jonge</b>	University of Minnesota, USA	Assisted reproductive technology
<b>Professor Paul Devroey</b>	Free University of Brussels, Belgium	Assisted reproductive technology
<b>Professor David Edgar</b>	University of Liverpool, UK	Embryonic stem cells
<b>Sir Professor Martin Evans</b>	Cardiff University, UK	Vertebrate development genetics
<b>Professor Hans Evers</b>	Academic Hospital Maastricht, The Netherlands	Assisted reproductive technology
<b>Professor Bart Fauser</b>	University Medical Center Utrecht, The Netherlands	Assisted reproductive technology
<b>Dr Joyce Harper</b>	University College London, UK	Preimplantation genetic diagnosis
<b>Professor Stephen Hillier</b>	University of Edinburgh, UK	Reproductive endocrinology, IVF research and ovarian pathophysiology
<b>Professor Outi Hovatta</b>	Karolinska Institute, Sweden	Preimplantation genetic diagnosis
<b>Dr Mark Hughes</b>	Genesis Genetics Institute, Detroit, USA	Preimplantation genetic diagnosis
<b>Professor Martin Johnson</b>	University of Cambridge, UK	Preimplantation mammalian development
<b>Professor Gab Kovacs</b>	Monash IVF, Australia	Assisted reproductive technology
<b>Professor Henry Leese</b>	University of York, UK	Early mammalian embryology
<b>Dr Norio Nakatsuji</b>	Kyoto University, Japan	Embryonic stem cells
<b>Professor Alan Trounson</b>	California Institute for Regenerative Medicine, USA	Embryonic stem cells
<b>Dr Maureen Wood</b>	Aberdeen Fertility Clinic, UK	Cryopreservation
<b>Professor André Van Steirteghem</b>	University Hospital Brussels, Belgium	Assisted reproductive technology
<b>Professor Stéphane Viville</b>	Université Louis Pasteur, Strasbourg, France	Preimplantation genetic diagnosis

## Horizon Scanning Panel

### 3.2 2007 Horizon Scanning Panel meeting

The Horizon Scanning Panel meetings are held annually to coincide with the European Society of Human Reproduction and Embryology (ESHRE) conference. The 2007 meeting was held at the ESHRE conference in Lyon. The following topics were discussed:

- **The horizon scanning process**

Members were given a general overview of the horizon scanning process. They looked at how issues are identified and prioritised, and how views from the Horizon Scanning Panel members feed into this process.

- **Hybrid and chimera embryos**

(introduced by Professor Alan Trounson)

Hybrid and chimera embryos refer to a range of different types of human-animal admixed embryos. Members discussed the potential of interspecies cytoplasmic hybrid embryos being used to derive stem cell lines. Alternative sources of embryonic stem cells were also discussed.

- **Metabolomics**

(introduced by Dr Jacques Cohen)

Metabolomics involves assessing the viability of embryos by analysing protein or metabolite expression in the culture media. Members discussed how it could be used to select the most viable embryos on a non-invasive biochemical basis, providing additional information to that based on their morphology.

- **Derivation of stem cell lines from blastomeres and arrested embryos**

(introduced by Dr Jacques Cohen)

These are two alternative methods of deriving embryonic stem cells that do not involve destroying viable embryos. Members discussed the potential of these techniques.

The minutes of this meeting can be found at Annex A.

### 3.3 Questions asked of the Horizon Scanning Panel

Specific issues arose throughout the year which the HFEA required input from the Horizon Scanning Panel. Members were asked to respond to questions on interspecies cytoplasmic hybrids, reproductive immunology, *in vitro* derived (artificial) gametes and preimplantation genetic screening. Details of the questions and a summary of responses can be found at Annex B.



# Outcomes of 2006-07 horizon scanning process

## 4.1 Issues prioritised for further consideration in 2007-08

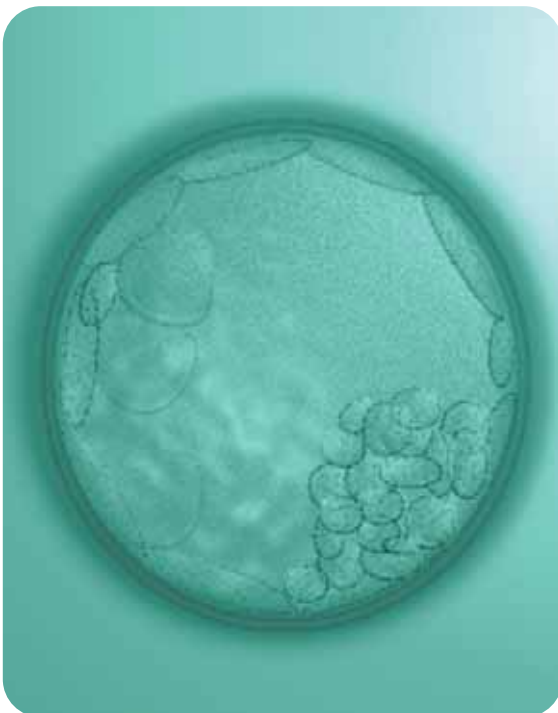
The following new issues were identified through the 2006-07 horizon scanning process and prioritised for further consideration by the HFEA in 2007-08:

- ***In vitro* growth of oocytes**

Oocytes from immature (preantral) follicles are developed in the laboratory. This is carried out at an earlier stage of oocyte development than the technique of *in vitro* maturation. This technique could allow women who have stored their ovarian tissue, for example female cancer patients, to obtain eggs from their stored tissue for use in treatment.

- **Embryo selection using metabolomic techniques**

The viability of preimplantation embryos is assessed by analysing the protein or metabolite expression in the culture media. This could allow the most viable embryos to be selected on a non-invasive biochemical basis, providing additional information to that based on their morphology.



- **Derivation of human embryonic stem cells from single blastomeres**

A single cell is removed from a preimplantation embryo and used to derive stem cells. The remaining embryo continues to develop. Stem cells could therefore be derived without destroying viable embryos.

- **Derivation of human embryonic stem cell lines from arrested embryos**

Embryonic stem cells are derived from the inner cell mass of arrested embryos. The technique allows stem cells to be derived without needing to create embryos specifically for that purpose.

The following ongoing issues were identified and considered by the HFEA in previous years. Updates on these techniques were considered during 2007-08:

- **Germinal vesicle transfer**

The germinal vesicle-stage nucleus from one oocyte is transferred into another oocyte that has had its nucleus removed. This could be used to treat mitochondrial disease if the nucleus from a patient with unhealthy mitochondria could be successfully transferred into an enucleated donor oocyte with healthy mitochondria.

- ***In vitro* derived (artificial) gametes**

Gametes are derived in the laboratory from alternate sources, such as embryonic stem cells. This technique could allow patients who are unable to produce gametes naturally to produce oocytes or sperm.

- **Dry storage of sperm**

Sperm samples are frozen to subzero temperatures and water removed. The technique could provide an alternative to cryopreservation for storing sperm.

- **Microarrays**

Microarrays are silicon or glass chips containing thousands of specific DNA sequences. They can detect specific gene variants in samples of DNA. Microarrays could be used to detect aneuploidy or identify specific mutations in embryos, or be used to identify the most viable embryos.

## Outcomes of 2006-07 horizon scanning process

### 4.2 Committee consideration and outputs

The HFEA's Scientific and Clinical Advances Group (SCAG) considered the following issues in 2007-08:

#### 4.2.1 *In vitro* growth of oocytes

Dr Helen Picton from Leeds Reproductive Medicine Unit gave a presentation to members of SCAG on *in vitro* growth of oocytes. The growth of human oocytes from primordial follicles and clinical use of this technique is thought to be 5 to 10 years away. The main call for this technique would be for cancer patients who have stored ovarian tissue prior to chemotherapy.

Members thought that the culture time needed for the technique was long and would therefore be expensive. They also thought that ovarian tissue freezing would have to be standard practice in order to use the *in vitro* growth technique. The process of freezing ovarian tissue would have to be improved and the risk of ovarian tissue damage following cancer treatment would need to be quantified.

April 2007

#### 4.2.2 Embryo selection using metabolomic techniques

Professor Henry Leese, from University of York, and Dr Daniel Brison, from University of Manchester, gave members of SCAG a presentation on the metabolomic assessment of embryos. Metabolomics aims to analyse metabolites within an embryo or embryo culture medium on a non-invasive biochemical basis. This provides diagnostic information additional to that based on morphology.

Initial studies suggest that this technique could be used as a predictive test to increase the implantation and pregnancy rates of embryos. Members thought that as there is no significant work in animal models, it would be at least 2 to 3 years before the technique could be used in a clinical setting. Members asked to be kept informed of developments in the field.

June 2007

#### 4.2.3 Derivation of human embryonic stem cells from single blastomeres

Members of SCAG thought that the potential of this technique to be used clinically was largely dependent on whether removing a blastomere from an embryo has a negative impact on the development of the remaining embryo. They thought that there was insufficient evidence to come to a conclusion on this. However the data available suggested there was not a harmful effect. Members thought that this technique was achievable but were unsure of who would want to use it.

SCAG concluded that it was likely that the HFEA would receive a research licence application for this technique in the near future. They also thought that the issue should be considered at the Horizon Scanning Panel's annual meeting.

April 2007

#### 4.2.4 *In vitro* derived (artificial) gametes

Members of SCAG discussed *in vitro* derived gametes (also known as artificial gametes) previously in September 2006. They had agreed to reconsider the issue in 2007.

Members thought that although there was progress in this area, there was a lack of high quality data published since they had considered the issue previously. They agreed to reconsider the issue in a year but asked to be kept informed of any important updates in the meantime.

June 2007

Further information was gathered from the Horizon Scanning Panel and scientists carrying out research in this area in January 2008. This indicated that work in this area had advanced and the timescale for deriving gametes from human embryonic stem (ES) cells may be relatively short. In particular one member thought that human sperm may be derived from male ES cells for research within 1-2 years. The timescale for deriving gametes suitable for treatment was thought to be 5-10 years. This has implications under the draft Human Fertilisation and Embryology Bill because sperm derived in this manner may not be permitted for treatment. SCAG members agreed that a meeting should be arranged to discuss the issue, involving researchers, the Department of Health and the HFEA.

February 2008

## horizon scanning process

**5.1 New issues identified**

This table presents all the issues that were identified through the 2007-08 horizon scanning process. It includes research published up to November 2007. Issues have been prioritised based on the potential of the technique to impact assisted reproduction and embryo research, the likely timescale of introduction and the potential ethical, legal or public interest in the technique. High priority issues will be considered in depth by the HFEA's Scientific and Clinical Advances Group (SCAG) in 2008-09. Low and medium priority issues will not be followed-up in any detail but have been provided for information.

*ES cells refers to embryonic stem cells*

Name	Description	Use	Priority
<b>Gamete selection, storage and manipulation</b>			
<b>Cryopreservation of immature testicular tissue and spermatogonial stem cells</b>	Cryopreservation of human and primate immature testicular tissue maintains spermatogonial cell populations. Spermatogonial stem cells in adult testis display many properties in common with ES cells.	Fertility preservation of prepubertal boys.	High
<b>Microcytoplast cryopreservation</b>	Micromanipulated mouse ooplast segments (microcytoplasts) created from oocytes can be successfully cryopreserved, thawed and used to reconstruct oocytes with intact spindles.	Ooplasm banking and alternative to oocyte cryopreservation.	High
<b>Assessing chromosomal abnormalities in sperm</b>	Assessing chromosomal abnormalities in sperm, in particular by examining the relationship between chromosomal aberrations and sperm morphology.	Select sperm without chromosomal abnormalities based on their morphology.	Medium
<b>New flow cytometric method for sperm analysis</b>	New multiparameter flow cytometric method simultaneously assesses sperm concentration, viability, apoptosis and leukocyte concentration. Flow cytometry can be used to help evaluate the ploidy status of spermatozoa.	Selection of sperm that will improve assisted reproductive technology (ART) outcomes.	Medium
<b>Sperm biomarkers</b>	Development of new biomarkers that are associated with sperm function and fertilisation rate.	Selection of sperm that will improve ART outcomes.	Medium
<b>Sperm DNA fragmentation</b>	High sperm DNA fragmentation compromises embryo viability. Various methods have been developed to assess sperm DNA fragmentation and isolate sperm with low levels of DNA damage.	Selection of sperm that will improve ART outcomes.	Medium

<b>Gamete selection, storage and manipulation</b> (continued)			
<b>Cryopreservation of whole human ovary</b>	Freeze-thaw protocols of intact human ovary with its vascular pedicle.	Restore fertility in women through ovary transplants.	Medium
<b>Vitrification of preantral follicles</b>	Vitrification of isolated pre-antral follicles from the mouse ovary.	Alternative to oocyte or ovarian tissue cryopreservation.	Medium
<b>Birefringence imaging</b>	Polarisation microscopy was used to assess the birefringence in the sperm head. ICSI using sperm with a birefringent head resulted in higher ongoing pregnancy rate. Birefringence imaging also used to assess oocyte quality.	Additional assessment tool that does not rely on morphology (or motility in sperm).	Medium
<b>Micro-testicular sperm extraction</b>	Surgical sperm retrieval using microTESE technique is a viable option when PESE or TESE is unsuccessful, and can be used to extract sperm from cancerous testicles in azoospermic patients and used in subsequent ICSI cycles.	Allow ART treatment in azoospermic men, including cancer patients.	Low
<b>Sperm retrieval from frozen and crushed testicular tissue</b>	Freezing and crushing testicular tissue opens most of the seminiferous tubules revealing sperm that would not have been detected using the standard mechanical TESE technique.	Variation of standard mechanical TESE technique to retrieve sperm from azoospermic men.	Low
<b>Platelet activating factor</b>	Platelet activating factor (PAF) can influence sperm function by affecting the motility, capacitation, acrosome reaction and fertility of spermatozoa.	PAF could be used as a predictor of male fertility. It could also enhance sperm maturation, sperm motility, sperm capacitation and acrosome reaction and cryopreservation.	Low
<b>New sperm filtration system</b>	Molecular glass wool filtration system combined with annexin V binding selects sperm free of apoptosis markers.	Selection of sperm that will improve ART outcomes.	Low
<b>RNA profiling of sperm</b>	Molecular analysis of mRNA in bovine spermatozoa showed a natural segmentation of the mRNA population which indicated a wide array of cell functions.	Selection of sperm that will improve ART outcomes.	Low

## horizon scanning process

**Gamete selection, storage and manipulation** (continued)

<b>Sperm selection by hyaluronic acid (HA) binding</b>	The sperm HA-binding test provides a 15 minute microscopic assay for the assessment of the proportion of spermatozoa that would bind to the zona pellucida.	Selection of sperm that will improve ART outcomes.	Low
<b>Co-culture of oocytes with cumulus cells</b>	Co-culture of oocytes with attached cumulus cells may enhance preimplantation development.	Enhance embryo quality and blastocyst development prior to ICSI.	Low
<b>Preservation of sperm without freezing</b>	Human sperm can be stored in electrolyte free solution for prolonged time.	Alternative method of storage to cryopreservation, would simplify sperm preparation for ICSI in certain classes of patients.	Low

**In vitro derived (artificial) gametes**

<b>Production of haploid androgenotes</b>	Duplication of the sperm genome by generating haploid androgenotes (by injection into ooplasts) which form diploid constructs when transferred into parthenotes.	Create multiple copies of the male genome through which to gain genetic information on a particular gamete or to propagate it when scarce.	High
<b>Oocyte-like cells from pancreatic stem cells</b>	Oocyte-like cells can be derived from clonal pancreatic stem cell lines in rats.	Derive oocytes from female patients unable to produce them.	Low
<b>In vitro differentiation of spermatogenic cells in 3D culture</b>	3D culture in a collagen gel matrix is suitable for inducing spermatocytes to differentiate into spermatids.	Obtain sperm from azoospermic patients.	Low

<b>Embryo selection</b>			
<b>Assessment of factors and proteins in follicular fluid</b>	Levels of proteins, growth factors and cytokines in follicular fluid can predict oocyte and embryo quality.	Select embryos that will improve ART outcomes.	Medium
<b>Pronucleus morphology</b>	Pronuclear morphology is associated with euploidy and implantation. Germinal vesicle positioning indicates mouse oocyte quality.	Select oocytes and embryos likely to develop and implant.	Medium
<b>Association between embryo morphology and chromosome abnormalities</b>	Describes current methods of aneuploidy detection and explores the association between morphology of embryos and chromosomal constitution.	Morphological markers associated with aneuploidy may improve selection of chromosomally normal embryos for transfer.	Medium
<b>PolScope imaging of the meiotic spindle and zona pellucida</b>	PolScope imaging can assess characteristics of the meiotic spindle and zona pellucida, and these characteristics associated with embryonic development potential.	Select embryos likely to develop to blastocyst stage.	Medium

<b>Embryo manipulation and culture</b>			
<b>Gene transfer into embryos and male germ line cells</b>	Transplantation of retrovirus-infected spermatogonia is efficient at introducing genes into the chicken male germ line. Transgenes can also be introduced into embryos by viral transgenesis and stably transfected ES cells can be produced.	Introducing desirable genes into the male germ line. Generating genetically modified ES cells for studying human embryogenesis and disease.	High
<b>Use of tripronucleated (3PN) embryos</b>	3PN embryos are corrected by microsurgical removal of the pronucleus furthest from the second polar body. Heteroparental blastocysts can be derived. 3PN mouse zygotes can be used as recipients for nuclear transfer.	Enable 3PN embryos (e.g. eggs fertilised with 2 sperm), usually discarded from IVF cycles, to be used as alternative source of chromosomally normal embryos for stem cell research or reproductive purposes.	High

## horizon scanning process

<b>Embryo manipulation and culture</b> (continued)			
<b>Blastomere fragment removal</b>	Removal of fragments from blastomeres to improve their quality. The percentage of blastocyst formation can be increased and the quality of blastocysts can be improved.	Improve development of fragmented embryos, increase number of embryos available for transfer and improve success rates.	Medium
<b>Embryo splitting</b>	Half the blastomeres are removed from 4 and 8 cell mouse embryos and 2-4 and 6-8 cell human embryos. They are placed in empty zona pellucida and cultured as separate embryos. This yields twin embryos of equal developmental potential as the donor embryos.	Increase the number of embryos for patients with a limited number of embryos to transfer. Standard licence conditions state that clinics cannot use embryo splitting for treatment purposes.	Medium
<b>Extended embryo culture</b>	Sequential culture media has greatly increased the ability to extend the duration of embryo culture to 5 days.	Extended culture of embryos could improve embryo selection.	Low
<b>Zona pellucida removal</b>	Total removal of the zona pellucida using a laser and mechanical pipetting might be associated with higher implantation and live birth rates.	Improve ART outcomes.	Low
<b>Embryo collapse prior to vitrification</b>	Artificial collapse, by mechanical puncture or laser, of blastocysts prior to vitrification.	May improve post thaw outcomes.	Low
<b>PPAR-delta activation</b>	Implantation of cultured embryos is enhanced by PPAR-delta activation.	Enhance implantation rates and improve ART outcome.	Low

**Genetic screening**

<b>Trophectoderm biopsy</b>	Trophectoderm biopsy at the blastocyst stage may be more advantageous than the more commonly cleavage stage biopsy with respect to outcome of PGD for $\beta$ thalassaemia.	More effective PGD techniques for monogenic diseases.	High
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**IVF/ICSI technologies**

<b>IVF with microfluidic channels</b>	'Lab on a chip' integrates multiple steps of different ART procedures. Blastocysts derived following use in humans. Isolation of motile sperm using a microfluidic device without centrifugation. Dielectrophoresis can predict blastocyst development and could be used in conjunction with a microfluidic device.	May help to improve efficiency of human ART.	High
<b>Elongated spermatid sorting</b>	Flow-cytometric cell sorting can purify elongated spermatids with normal developmental ability, for use in ICSI.	Increase the spermatid retrieval rate and improve ICSI outcome in men with azoospermia.	Medium
<b>ICSI on immature oocytes</b>	Immature oocytes can be normally fertilised but pregnancy rate is still low.	Increase the number of embryos available for transfer.	Medium
<b>Endometrial scratching</b>	Carrying out endometrial biopsy prior to ICSI can improve pregnancy rates.	Improve pregnancy rates in patients with high-order implantation failure.	Low

## horizon scanning process

Embryonic stem cell derivation and cloned embryos			
<b>Alternative methods of obtaining ES-like cells</b>	Range of techniques including direct reprogramming of somatic cells (induced pluripotent stem cells), deriving ES cells from a single blastomere and nuclear transfer into non-viable embryos.	Create ES cells without destroying viable embryos. Patient specific ES cell lines can be stored and potentially used for targeted cell therapy with reduced risk of immunorejection and genetic alterations.	High
<b>Improvements in somatic cell nuclear transfer (SCNT)</b>	Improved methods of SCNT techniques and deriving ES cells from SCNT embryos in primates.	Improve methods of differentiating human ES cells.	High
<b>Derivation and banking of personal embryonic stem cells</b>	Derivation of ES cells from the inner cell mass of blastocysts from patient's embryos not used in an IVF treatment cycle. ES cell lines will then be banked for the patient's own future use.	Create personalised stem cell lines for future therapeutic use.	High but for referral to Ethics and Law Advisory Group (ELAG)
<b>SCNT using <i>in vitro</i> matured (IVM) oocytes</b>	IVM germinal vesicle oocytes can be used as hosts for nuclear transfer. Embryo development reached morula stage.	Improve methods of nuclear transfer and deriving ES cells in humans.	Medium
<b>Pluripotent epiblast stem cells from mammalian embryos</b>	Post-implantation epiblast-derived stem cells (EpiSCs) can be derived from the epiblast layer of mouse (and rat) embryos. They are distinct from mouse ES cells and are more similar to human ES cells in their epigenetic state and the signals controlling their differentiation.	Mouse epiblast stem cells share patterns of gene expression and signalling pathways with human ES cells and could provide a modelling system.	Medium

Transplantation			
<b>Transplanting ovarian follicles</b>	Isolated human follicles transplanted into mice are able to survive and grow, and showed <i>in vivo</i> follicular activation.	Transplant ovarian follicles in women whose ovaries do not produce follicles.	Medium
<b>Ovarian tissue transplantation</b>	Transplantation of fresh or cryopreserved ovarian tissue is feasible in mammals. Ovarian transplants in monozygotic twins discordant for ovarian failure. In sheep ovarian function continues for 6 years after whole organ cryopreservation.	Restore fertility in women made infertile by some medical treatment.	Medium
<b>Transplanting spermatogonia</b>	Spermatogonia from rainbow trout were intraperitoneally transplanted into newly hatched sterile salmon. Spermatogonia underwent spermatogenesis and oogenesis and 2 years after transplantation the salmon recipients only produced trout sperm and eggs.	Transplant spermatogonia into sterile men so they can produce sperm.	Medium
<b>Cryopreservation of immature ovaries</b>	Ultrarapid cryopreservation of newborn rat ovaries preserves developmental potential of immature oocytes in ovariectomized adult recipients.	Preserve fertility in female patients by transplanting ovaries.	Low
<b>Transplanting uteri</b>	Primate uterus can be perfused, preserved and circulated with blood post transplantation. Other large animal pregnancies following womb transplantation have been achieved.	A model for uterine transplantation in humans.	Low



## horizon scanning process

**5.2 Ongoing issues identified**

The following ongoing issues have already been considered by the HFEA in previous years. However new developments within these issues have been identified through the 2007-08 horizon scanning process. Therefore the issues may need to be reconsidered in 2008-09.

Name	Description of developments	Use	Last considered
<b>Microarray analysis for oocytes</b>	Microarray screening has identified markers in oocytes that relate to oocyte quality. Karyotyping to identify euploidy oocytes and embryos to transfer markedly improves IVF outcome. Selecting euploid oocytes by removing and analysing first polar body.	Selection of oocytes and embryos that will improve ART outcomes.	June 2007
<b>Freeze drying sperm</b>	Long term storage of partially evaporatively dried mouse spermatozoa at -20 and -80°C and the influence of primary drying pressure.	Alternative methods to cryopreservation for long term storage of sperm.	April 2007
<b><i>In vitro</i> derived (artificial) gametes from stem cells</b>	Immature gametes can be derived from ES cells. Ovary like structures have been derived following transplantation of ES cells into host mouse ovaries.	Fertility treatment for patients who cannot produce gametes.	June 2007
<b><i>In vitro</i> growth (IVG) of oocytes</b>	Mature oocytes were obtained from mouse fetal germ cells <i>in vitro</i> . Live births from IVG mouse oocytes. Early mouse preantral follicles can be isolated and cultured <i>in vitro</i> , with oocytes development similar to <i>in vivo</i> development. Metabolic markers could assess follicle development.	Derive oocytes from female patients unable to produce them.	April 2007
<b><i>In vitro</i> maturation (IVM) of oocytes</b>	Pregnancy rates from IVM oocytes were comparable to IVF in some groups of women. Development of more effective techniques of IVM.	Alternative to conventional IVF for women at high risk from OHSS.	June 2006
<b>Metabolomic assessment of embryos (including the use of microfluidics)</b>	The oxidative metabolism and protein turnover of oocytes and embryos can predict their developmental competence. Near infrared spectroscopy can detect oxidative metabolism biomarkers that may predict embryo viability. Microfluidic systems may be able to improve accuracy and throughput of metabolomic analysis of embryos. Assessment of follicular fluid can predict oocyte quality.	Select oocytes and embryos that will improve ART outcomes.	June 2007

Name	Description of developments	Use	Last considered
<b>Single blastomere whole genome DNA fingerprinting using microarrays</b>	Whole genome DNA fingerprinting of a single blastomere using microarrays. Single fibroblast cells have also been screened for copy number aberrations using this technique.	Allows parallel analysis of whole genome aneuploidy and single gene disorders for PGD. Can also be used to determine which embryos healthy children have developed from (therefore assessing the power of new diagnostics and interventions that improve IVF outcome).	June 2007
<b>ES cells derived from single blastomeres</b>	Simple and efficient method of establishing mouse ES cell lines from single blastomeres, by planting blastomeres onto a feeder layer of mouse embryonic fibroblasts with modified ES cell medium.	Create human ES stem cell lines without destroying embryos and allow the generation of patient matched tissue.	April 2007

### 5.3 Issues prioritised for further consideration in 2008-09

These new and ongoing issues were presented to the HFEA's Scientific and Clinical Advances Group (SCAG) along with recommendations for further work and prioritisation. SCAG identified the following issues as high priority, to be considered in depth during 2008-09. The Human Fertilisation and Embryology Bill, currently passing through Parliament, has implications for some of these techniques. These are outlined in the relevant sections below.

#### New issues:

- **Gene transfer into embryos and male germ lines**

Genes can be introduced into embryos by various methods, including using viruses. The Human Fertilisation and Embryology (HFE) Bill will remove restrictions on genetically altering embryos for research. It is anticipated that the HFEA will receive licence applications for this technique after the HFE Bill receives Royal Assent.

Gene transfer into male germ cell lines involves transplanting spermatogonial cells that have been infected with retroviruses. This could restore spermatogenesis, allowing infertile men to produce sperm. However, it is questionable whether this technique will be permitted under the HFE Bill.

- **Alternative methods for obtaining embryonic stem (ES) cells or ES-like cells**

The Authority is legally obliged to consider whether human embryo research proposed in licence applications is 'necessary' or 'desirable'.<sup>1</sup> Therefore it is important for HFEA licence committees to be aware of any alternative methods to creating and using human embryos in research.

<sup>1</sup>Schedule 2 S.3(2) Human Fertilisation and Embryology Act 1990 requires the Authority to only issue licences for research that is 'necessary' or 'desirable' for the stated purposes.

## horizon scanning process

There are a range of techniques that can be used to derive ES or ES-like cells without having to destroy viable embryos. These include direct reprogramming of somatic cells (induced pluripotent stem cells), deriving ES cells from a single blastomere and nuclear transfer into embryos that would otherwise have been discarded from IVF treatment.

- **Trophectoderm biopsy for preimplantation genetic diagnosis (PGD)**

PGD can be carried out on cells that are biopsied from the trophoctoderm of a blastocyst. This technique may be less damaging to the embryo and allows more cells to be tested, potentially increasing accuracy of PGD.

- **Use of trippronucleated embryos**

Following fertilisation some embryos contain three pronuclei (3PN), for example those that have been fertilised by two sperm. These are automatically discarded from treatment. 3PN embryos can be manipulated to remove one pronucleus, potentially allowing the embryo to be used in treatment. They could also be used for research.

- **Immature testicular tissue and spermatogonial cell cryopreservation**

The Human Fertilisation and Embryology Bill will extend the definition of gamete to include all cells in the germ line. This will bring in some additional assisted reproductive techniques under the remit of the HFEA. Immature testicular tissue can be cryopreserved to maintain spermatogonial cell populations. This could potentially be used to preserve the fertility of prepubescent boys undergoing cancer treatment.

### Ongoing issues:

- **Treatments for mitochondrial disease**

Under the Human Fertilisation and Embryology Bill, an egg or embryo permitted for treatment cannot have its mitochondrial or nuclear DNA altered. However there is a regulation-making power to override this prohibition for the treatment of serious mitochondrial disease.

Three techniques have been prioritised as potential treatments for mitochondrial disease. Germinal vesicle transfer and pronuclei transfer involve removing the nuclei from a patient's oocyte/zygote that has affected mitochondria and transferring it into an enucleated donor egg/zygote that has unaffected mitochondria. Microcytoplasm cryopreservation involves removing a segment of an oocyte and cryopreserving it separately from the remaining oocyte. The segment can then be used to reconstruct a recipient oocyte.

- **Metabolomics and the use of microfluidics**

Metabolomics analyses the embryo culture medium non-invasively to provide information on embryo quality additional to that based on morphology. Microfluidic systems may improve the accuracy of metabolomic analysis of embryos. Assessment of follicular fluid may also predict oocyte quality.

- **In vitro derived (artificial) gametes**

Gametes are derived in the laboratory from alternate sources, such as embryonic stem cells. The Human Fertilisation and Embryology Bill as it stands, permits this for research but not for use in treatment.

- **Microarray analysis of oocytes and embryos**

Microarray screening can identify DNA markers in oocytes and embryos that relate to quality. Whole genome DNA microarray screening of blastomeres will allow parallel analysis of whole genome aneuploidy and single gene disorders for PGD.

- **Freeze drying sperm**

Freeze drying is an alternative method to cryopreservation for storage of sperm.

- **In vitro growth and maturation of oocytes**

Oocytes from immature follicles are developed and matured in the laboratory. This technique allows oocytes to be collected without using fertility drugs.

All outputs following consideration of these issues will be available in the horizon scanning report for 2008-09.

# 2007 Horizon Scanning Panel meeting

## Minutes of the fourth annual meeting of the HFEA Horizon Scanning Panel held on 2 July 2007 in Lyon

### Present:

Members	Authority	Executive
Keith Campbell	Chris Barratt (Chair)	Hannah Darby
John Carroll	Shirley Harrison	Juliet Tizzard
Jacques Cohen	Maybeth Jamieson	
Alan Decherney		
Chris De Jonge		
Paul Devroey		
Bart Fauser		
Joyce Harper		
Outi Hovatta		
Mark Hughes		
Gab Kovacs		
Alan Trounson		
André Van Steirteghem		
Stéphane Viville		

### 1. Introduction – the horizon scanning process

1.1 Hannah Darby gave a general overview of the horizon scanning process and how the HFEA takes forward issues identified by panel members. Issues are identified through the panel, publications, conference attendance and conversations with researchers and scientists. High priority issues are considered further 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 an output is produced.

1.2 Examples of some of the issues identified and taken forward for 2006 were outlined, these included sperm dessication, microarray to analyse gene expression of oocytes, testicular stem cells and *in vitro* growth. This was then followed with the list of issues which had been prioritised for 2007. These were *in vitro* growth of oocytes, embryo selection using metabolomic techniques and derivation of human embryonic stem cells from single blastomeres.

1.3 Members were presented with the first annual horizon scanning report which outlines activities of the panel and the horizon scanning function of the HFEA to date.

### 2. Discussion on hybrids and chimeras

2.1 Alan Trounson introduced the discussion on this subject. Alan gave an introduction on the progress so far regarding derivation of pluripotent stem cells and the strategies for producing patient/disease specific stem cells from somatic cell nuclear transfer (SCNT). It was suggested that deriving stem cells by parthenogenesis is more efficient than SCNT.

2.2 The results for the study which derived embryonic stem cells from human-rabbit cytoplasmic hybrid embryos were outlined. It was noted that no sustainable cell lines have been derived from interspecies cytoplasmic hybrids and not a lot of work has been done in this area.

# 2007 Horizon Scanning Panel meeting

2.3 One panel member was of the view that limited work had been carried out on animal-animal interspecies cytoplasmic hybrid embryos. The creation of horse-cow cytoplasmic hybrid embryos by Alan Trounson's group suggested that stem cell lines derived from these entities show slow cell replication and no connection between the mitochondrial and nuclear components of the embryo. The panel member thought that the method is currently inefficient. This was not the general view of the group.

2.4 Members discussed alternative sources of embryonic stem cells. These sources/methods included:

- Mitotically arrested zygotes
- Triploid embryos
- Tri or mono pronuclear eggs
- Reprogramming somatic cells

2.5 It was noted that studies which have shown success of deriving stem cells following SCNT on mitotically arrested pronuclear zygotes are encouraging. In this method the chromosomes of the zygote are removed, not the whole nucleus, leaving the transcription factors. Timing and skill is crucial for success of this technique.

2.6 There have also been a number of studies which have successfully demonstrated that mouse somatic cells can be reprogrammed, by changing their methylation patterns, to form pluripotent stem cells.

2.7 One member questioned why creating human-animal hybrids is being suggested as there is little evidence that it is a viable technique, it raises ethical issues and is a sensitive topic for the public. Another panel member was of the view that scientists in the UK should be allowed to create interspecies cytoplasmic hybrid embryos in order to demonstrate whether or not it is possible to repeat the results of the groups who have reported the creation of human-rabbit and human-cow entities.

This panel member felt that every avenue of research should be explored and that the creation of hybrids should be permitted as they will never be transferred to a woman and allowed to implant, as there is regulation in place to prevent this happening. It was

thought that after a number of cell divisions, cells within human-animal cytoplasmic hybrids will become human.

2.8 It was suggested that the term 'xeno transfer' should be used instead of 'interspecies cytoplasmic hybrids'.

### 3. Discussion on metabolomics

3.1 Jacques Cohen introduced discussion on the use of metabolomics for embryo selection and presented some slides on behalf of Henry Leese.

3.2 It was noted that currently the embryo selection criteria is based on morphology and this is subjective. Other methods used are observing development rates, preimplantation genetic screening (PGS), which causes possible embryo damage, and viewing the spindle with a pol-scope. There is a need for a rigorous, non-invasive, simple method for embryo selection.

3.3 One member was of the view that the use of metabolomics is a strictly controlled system and questioned how it could be applied to a clinic (i.e. certain medium and certain time) and it should be thought about in the context of nano-technology for culture (microfluidics).

3.4 It was noted that a spectrophotometer is commercially available which analyses spent culture media and it does not matter what culture media is used.

3.5 One member was of the view that if ~50% of eggs are chromosomally abnormal and discarded and success rates are still ~30% then it is likely that metabolomics will only make a small difference.

## 2007 Horizon Scanning Panel meeting

### 4. Discussion on derivation of stem cell lines from blastomeres and arrested embryos

4.1 The derivation of stem cells from blastomeres and arrested embryos were two methods discussed which have been suggested as alternative sources of embryonic stem cells. These methods would avoid destroying an embryo in the process of deriving stem cell lines.

4.2 Klimanskaya et al (2006) derived two human embryonic stem cells lines from individual human blastomeres. However, this study was considered to be misleading because blastomeres were derived from disaggregated embryos not from biopsy. No further studies have demonstrated success of the technique.

4.3 Some members of the panel were of the view that this technique is likely to be used to derive stem cell lines in the future but it is questionable as to whether patients would consent to this technique being carried out on embryos used for treatment.

4.4 The group discussed whether or not embryo biopsy is damaging. The general consensus of the panel was that removing a blastomere will have a negative effect on an embryo. It was noted that in the process of biopsy one eighth of the cells are lost, which equates to 12.5% of the implantation potential. One member mentioned a study for single embryo transfer in women under 35, by Katherine Stacey et al., that found PGS did not improve success rates but was not damaging.

4.5 It was noted that although cryopreserved embryos, used for treatment, often lose blastomeres in the freeze-thaw process this is not comparable to blastomere loss through biopsy as it does not involve zona drilling. Evidence from preimplantation genetic diagnosis (PGD) shows that embryos which have undergone biopsy are almost impossible to freeze/thaw successfully.

4.6 The group discussed the derivation of stem cells from embryos that have arrested before they reach blastocyst stage. This is another suggested method of obtaining human stem cell lines without destroying viable developing embryos. It was noted that Zhang et al (2006) demonstrated that human embryonic stem cell lines can be derived from arrested embryos from IVF treatment. The group waited 24-48 hours to check that the embryos had stopped dividing at the blastocyst stage.

4.7 One member noted that the study used a vague definition of 'no cleavage' in late arrested embryos with 16-24 cells and stem cells were only derived from late arrested embryos. Also, the question was raised as to how proliferation would be measured in intact morulae.

4.8 The general view of the panel was that the study on arrested embryos was not properly peer reviewed. It was noted that one panel member has established two stem cell lines from arrested embryos.

4.9 The general view of the panel was that arrested embryos could not be classed as dead because they may continue to develop.

# Horizon Scanning Panel questionnaire response summaries

## Question – July 2007

In relation to the creation of interspecies cytoplasmic hybrids for research:

1. Are you aware of any evidence that an embryo created through SCNT of a human somatic cell nucleus into an enucleated animal (e.g. rabbit or cow) oocyte would not be able to hatch from the zona pellucida in the normal way?
2. What are the mechanisms for embryo hatching? Is this process controlled by molecular signals dependent on the genome or is it mainly time dependent?

### Summary of responses

Panel members were unaware of any evidence that would suggest an interspecies cytoplasmic hybrid embryo would not hatch. It was pointed out that in order to create the cytoplasmic hybrid embryo a hole would have to be made in the zona pellucida, which may facilitate a pseudo-hatching process.

Members thought that the mechanisms of embryo hatching differed between species. Both molecular signals and timing were thought to be important. In some cases hatching may be assisted by secretion by the epithelial cells which line the endometrium. In the mouse the uterus secretes a zona lysine, but embryos can hatch later without it. One member thought that the factors required for hatching come from the embryo, because an uncleaved zygote or a blocked embryo remains encapsulated in the zona pellucida *in vitro*.

## Question – November 2007

In relation to reproductive immunology and natural killer (NK) cells:

1. Are you aware of any evidence of a correlation between peripheral blood and uterine NK cell populations?
2. Do you think the measurement of peripheral NK cells in women with reproductive failure, and subsequent treatment with drugs or intravenous immunoglobulin can be beneficial?

### Summary of responses

The majority of members felt they did not have expertise in this area. They recommended two scientists in the field who were contacted for more information. They did not think that there was a correlation between peripheral blood and uterine NK cells. They thought that there is insufficient data on reproductive immunology and that studies did not support its use.



# Horizon Scanning Panel questionnaire response summaries

## Question – January 2008

In relation to *in vitro* derived gametes from stem cells:

1. What are the different timescales for deriving eggs and deriving sperm from stem cells?
2. What is the feasibility and relative timescales of producing eggs from XX stem cells and sperm from XY stem cells, compared to eggs from XY stem cells and sperm from XX stem cells (if possible)?
3. What is the relative safety of the above techniques?

## Summary of responses

Members thought that the timescale for deriving sperm was likely to be very close. There have already been several reports of deriving sperm from mouse stem cells. A group in Newcastle is attempting this in humans. Current work on deriving oocytes for research was not thought to be as advanced and the timescale for this is likely to be longer (one member thought 1-2 years, others thought longer). There is also an issue with meiosis in oocytes. The timescale for deriving gametes for treatment was thought to be 5-10 years by some members. However several experimental and preclinical studies would be needed before any technique could be used for treating infertility.

Members thought that it would not be possible to derive sperm from XX stem cells because factors on the Y chromosome needed for spermatogenesis would not be present. Adding in genes from the Y chromosome would have many safety implications. Members thought in theory it would be possible to derive oocytes from XY stem cells, but it would not be an easy process. If researchers could identify the genes involved, they could possibly use a process similar to creating induced pluripotent stem cells. However there would be serious safety implications and any potential offspring may have abnormal sex chromosomes.

In general members thought that the process is very complex and that the safety issues are largely unknown. Researchers' knowledge of gametogenesis, in particular in terms of epigenesis, is insufficient to determine the safety of techniques. One key issue was normal imprinting. It was pointed out that mouse offspring from *in vitro* derived gametes died shortly after birth. *In vitro* derived oocytes would have to undergo *in vitro* growth before they could be used in treatment.

# Horizon Scanning Panel questionnaire response summaries

## Question – April 2008

In relation to preimplantation genetic screening (PGS):

1. The randomised controlled trials by Staessen et al (2004), Stevens et al (2004) and Mastenbroek et al (2007) all concluded that PGS has no beneficial effect for women with advanced maternal age. Are you aware of any other recent randomised controlled trials in this area?
2. What effective uses do you think PGS has in the treatment of patients with advanced maternal age, recurrent miscarriage, recurrent implantation failure and male factor infertility?

## Summary of responses

Members gave details of an international randomised trial for the use of PGS in infertile patients with advanced maternal age, which had just been started by Reprogenetics. More randomised controlled trials will also be published soon from Brussels. Members were aware that the Mastenbroek et al (2007) study had been criticised. A critical review of PGS had also been published in the British Medical Journal. One member was dubious about the prognostic value of PGS and its invasive nature.

One member thought that the use of PGS by fluorescent *in situ* hybridisation (FISH) for advanced maternal age is still debated, hence the need for more trials. They thought that the use of comparative genomic hybridisation (CGH) or microarrays will probably have advantages. However, error rate and sampling issues need to be considered as this is still a new area.

The member also thought that the use of PGS for patients with repeated pregnancy loss (RPL) should be examined carefully, since the majority of losses are correlated with chromosomal anomalies. CGH or micro-arrays may be a better option than FISH in these cases too. The data from preliminary sets and longer serial retrospective studies regarding repeated implantation failure (RIF) and PGS was not thought to be very encouraging. CGH/micro-arrays may improve this as it is feasible that failure of PGS in this group is caused by examining the wrong chromosomes with FISH. The data regarding mosaicism and certain forms of male factor infertility is also of interest. Members thought that more research was needed to better understand the mechanisms involved.





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