Scientific Horizon Scanning at the HFEA

Annual Report 2008/09

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

Contents

1	**Introduction**	**4**
2	**Outcomes of 2007/8 horizon scanning process**	**5**
2.1	Trophectoderm biopsy	5
2.2	Use of tripronucleated (3PN) embryos	6
2.3	Microcytoplast cryopreservation of oocytes	8
2.4	Gene transfer into embryos	9
2.5	Gene transfer into male germ lines	11
2.6	Cryopreservation of immature testicular tissue and spermatogonial cells	12
2.7	Alternative methods of obtaining embryonic or embryonic-like stem cells	13
2.8	*In vitro* derived gametes	16
3	**Issues identified in the 2008/9 horizon scanning process**	**18**
4	**References**	**29**

Section **1**

Introduction

Horizon scanning is an early-warning system which allows the HFEA to consider the legal, ethical and regulatory implications of any techniques that researchers or clinicians may wish to use in the future 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.

This year the horizon scanning process has also been used to gather information on techniques which the Human Fertilisation and Embryology Act 2008 will permit from October 2009 (eg, genetic modification of embryos for research).

Relevant research is identified throughout the year by reviewing journals and attending conferences. Common themes are then identified and researched further. Views are sought from experts, such as members of the HFEA's Horizon Scanning Panel.

This report summarises the HFEA's horizon scanning activity for 2008/9, and the outcomes from 2007/8, to inform interested researchers, clinicians, members of the public and other stakeholders by outlining the high priority issues considered throughout 2008, any resulting actions and the issues identified for consideration in 2009. If centres wish to carry out any of the techniques described in this report they would need to inform the Authority or apply for a licence and, where necessary, further consultation will be carried out. A licence committee of the Authority would consider the details of each individual application before the technique could be used.

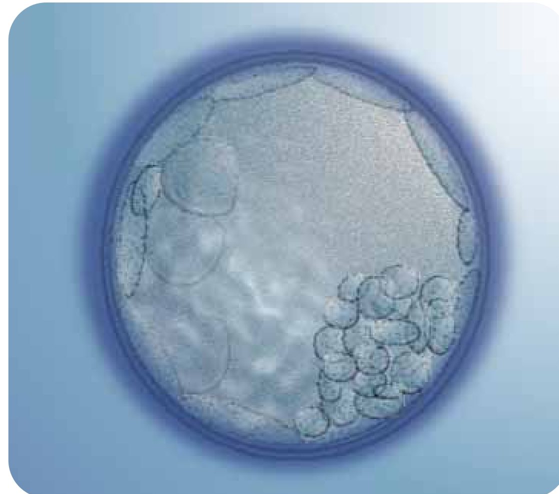
Outcomes of 2007/8 horizon scanning process

The following issues were identified in the 2007 horizon scanning process and prioritised for further consideration by the HFEA in 2008. Each high priority issue was considered in depth by the HFEA's Scientific and Clinical Advances Advisory Committee.

2.1 Trophectoderm biopsy

What is it?

Trophectoderm biopsy is a new technique for the genetic screening of embryos (for particular diseases or for an abnormal number of chromosomes ie, preimplantation genetic diagnosis or preimplantation genetic screening). The technique involves taking cells from the outer layer of an embryo (that will form the placenta not the fetus) at day 5 or 6 of development (blastocyst stage).



What impact could it have?

Trophectoderm biopsy at the blastocyst stage may be more successful in testing for monogenic disease than cleavage stage biopsy because:

- more cells are available to analyse, leading to a more reliable diagnosis
- only embryos competent to develop to blastocyst stage are biopsied
- the implantation potential of the blastocyst following trophectoderm biopsy is less likely to be compromised because the cells removed are non-embryonic.

Use of this technique may result in preimplantation genetic diagnosis (PGD) being more cost effective and less labour intensive, although there is less time available to conduct the genetic tests (24 hours). However, it is possible that use of this technique may result in an increase in PGD cycles which do not result in embryo transfer, if the number of embryos available to biopsy is reduced following culture to blastocyst stage.

This technique could also be used for preimplantation genetic screening for aneuploidy (PGS). It could be particularly useful in the move towards single embryo transfer, for patients who have blastocysts in storage and have an indication for preimplantation genetic screening for aneuploidy.

What research has been carried out?

Blastocyst trophectoderm biopsy using micromanipulation methods was first reported in 1990[1], although it was not originally used in the clinical context. Since then, some pregnancies have been reported following trophectoderm biopsy and PGD[2 3 4 5 6 7].

The outcome of a pilot study suggests that trophectoderm biopsy with day-6 blastocyst transfer may have better outcomes for PGD for monogenic diseases than cleavage stage biopsy with day-5 blastocyst transfer[4]. The removal of four to five trophectoderm cells from each blastocyst achieved a complete genotype in 94.3% of the biopsed blastocysts.

When could it be introduced in the UK?

This technique has been successfully used in PGD or PGS cycles by a number of groups in the US, Greece, Australia and Thailand. Some UK clinics are also beginning to use this technique. It is therefore likely that more PGD clinics in the UK will want to use the technique in the near future.

Section **2**

Outcomes of 2007/8 horizon scanning process

The HFEA's view and outcomes

The HFEA's Scientific and Clinical Advances Advisory Committee (SCAAC) felt that fewer embryos will be biopsied when trophectoderm biopsy is used because fewer embryos would develop to day 5. They felt that it would be fairly easy for clinics to carry out this technique if they are already carrying out PGD. Clinics would just need to notify the HFEA if they wished to carry out this technique.

The Committee thought that trophectoderm biopsy is safer than conventional embryo biopsy because taking cells from the trophectoderm is less likely to affect the development of the embryo. The Committee discussed whether information from trophectoderm cells will be the same as that from inner cell mass cells and whether the results will be affected by mosaicism. They decided that because a larger number of cells are extracted and analysed when trophectoderm biopsy is used, cell mosaicism was not an issue. It was not thought that the technique would affect placenta development.

The Committee recommended that there should be information for patients regarding trophectoderm biopsy on the HFEA website. Therefore patient information on preimplantation genetic diagnosis has been updated accordingly. The HFEA Executive and Licence Committees will refer to the information outlined in the paper and minutes of the SCAAC meeting held on 21 May 2008 when receiving notifications or licence applications from centres which wish to use this technique for treatment or research.

2.2 Use of tripronucleated (3PN) embryos

What is it?

A normally-fertilised egg (zygote) has one set of genetic material from the egg and one from the sperm. Sometimes during IVF two sperm can fertilise an egg at the same time. Also, sperm with two sets of chromosomes (diploid), which form when there is an error in the process of sperm formation (a meiotic error), can fertilise an egg. This leads to the zygote containing a nucleus from the egg and two nuclei from sperm. The nuclei at this stage are called pronuclei and therefore the zygote is said to be 'tripronucleated' (3PN). 3PN zygotes occur in about 4-7% of IVF cycles and are automatically discarded from IVF treatment, as they would not lead to a viable pregnancy. If the extra paternal pronucleus is identified and removed the embryos could potentially be used for research or treatment.

What impact could it have?

Tripronucleated embryos could potentially be corrected and used in IVF treatment cycles, instead of being discarded, thereby increasing the number of embryos available for treatment. Embryo biopsy would have to be carried out before embryo transfer to check whether the correct pronucleus (ie, a paternal pronucleus) had been removed.

The technique could also be used in research to generate embryonic stem cell lines from embryos that would otherwise have been discarded. 3PN zygotes could therefore potentially be an alternative source of embryos for human embryonic stem cell research.

What research has been carried out?

Altering the number of sets of chromosomes and parental constitution of zygotes has been carried out efficiently in several mammalian species. Early studies demonstrated that a pronucleus can be removed from human embryos to create bi-pronucleated diploid embryos, but the *in vitro* developmental ability of these embryos was restricted[8,9]. There was also uncertainty as to whether or not embryos inherited chromosomes from both parents (heteroparental).

Recently, however, a Spanish group developed a technique that improves the identification and removal of the extra paternal pronucleus[10]. The group microsurgically removed the pronucleus located furthest from the second polar body in 3PN human zygotes using cytoskeletal relaxing agents. The resulting embryos were diploid and developed to blastocyst stage, with the majority being heteroparental.

Use for treatment

There has been one case report in Singapore of corrected 3PN human embryos being used for reproductive purposes, resulting in a live birth[11]. The child appeared developmentally normal.

Use for research

One US group recently used 3PN mice zygotes as recipients in nuclear transfer[12]. Zygotes fertilised with two spermatozoa were generated and inhibitors were used to temporarily block the cell during mitosis. All the chromosomes were then removed from the zygote and replaced with chromosomes from a donor embryonic stem cell. When the inhibitors were removed, development resumed and a blastocyst formed. These blastocysts could potentially be used to derive new embryonic stem cell lines.

When could it be introduced in the UK?

The technique has already been used for reproductive purposes in other countries and improvements in the technique have recently been shown in human embryos.

The potential for 3PN embryos to be used in stem cell derivation has been explored in a recent paper[12]. However the experiment described was conducted in mice and has not yet been transferred to humans.

3PN embryos have been used for other research purposes and for example in the UK, the HFEA has licensed one research project involving poly-pronucleated embryos (research project R0026 at www.hfea.gov.uk).

All UK groups wanting to use 3PN embryos for stem cell research will require a research licence from the HFEA.

The HFEA's view and outcomes

The HFEA's Scientific and Clinical Advances Advisory Committee (SCAAC) discussed the technique. The Committee felt that it was very unlikely that clinics in the UK would want to use corrected 3PN embryos for treatment purposes; one case study is not sufficient to demonstrate that the technique could be used for treatment. The Committee thought that researchers would want to use 3PN embryos for stem cell research and that there are other research applications for 3PN embryos, for example looking at the origin of hydatidiform moles.

The Department of Health advised the HFEA that they would need to consider whether removing a pronucleus from a zygote would be classed as altering the nuclear DNA of an embryo and therefore whether use of corrected 3PN embryos for treatment would be prohibited by the Human Fertilisation and Embryology Act 1990 (as amended).

Information about the use of 3PN embryos to create stem cells has been included in a literature review regarding alternative methods to derive stem cells (outlined in papers of 21 February 2008 SCAAC meeting), which the HFEA's Research Licence Committee refers to when deciding whether creation and use of embryos for proposed research is necessary or desirable.

Section **2**

Outcomes of 2007/8 horizon scanning process

2.3 Microcytoplast cryopreservation of oocytes

What is it?

Microcytoplast cryopreservation involves removing a segment of an egg (microcytoplast) and freezing it. These segments are then thawed and reconstructed into a complete egg.

What impact could it have?

This technique could impact upon assisted reproduction if it provides a more effective way to cryopreserve eggs than conventional methods. In theory, this technique could increase the survival rate of cryopreserved eggs as it increases the surface area to volume ratio of the egg.

Freezing egg segments would also allow ooplasm (cytoplasm) to be stored and potentially transferred to eggs or embryos in order to improve their developmental potential in assisted reproduction treatment. This would not be permitted under the Human Fertilisation and Embryology Act 1990 (as amended) because it would be classed as altering the mitochondrial DNA of the egg, which is prohibited. However, the Act contains a regulation making power to allow for eggs or embryos, which have had their nuclear or mitochondrial DNA altered, to be permitted for treatment designed to prevent the transmission of serious mitochondrial disease.

What research has been carried out?

The technique of microcytoplast cryopreservaton has been developed in mice by a group in the US[14]. Eggs were micromanipulated to remove ooplast fragments (microcytoplasts). The microcytoplasts were then injected into the perivitelline space and cryopreserved along with the eggs they were derived from (parent eggs) and control eggs using a conventional slow freezing technique. The microcytoplasts were thawed and used to reconstruct an egg by electrofusion, either with or without a zona pellucida.

Survival of the total fragments (parent segments and microcytoplasts) was significantly higher (75.5%) than the survival of control whole eggs (64.2%) after thawing. However the survival of parent egg segments only (66.9%) was not significantly higher than the control eggs.

Electrofusion of frozen-thawed microcytoplast segments with parent or recipient eggs was more successful when carried out within the zona pellucida (91.4%), compared to those fused without zona pellucida (56.2%). The spindle was intact following reconstruction of the segment and parent egg, but the group did not investigate if the egg would develop any further.

When could it be introduced in the UK?

The technique is at an early stage of development and has only been carried out in mouse eggs reported in one study. The technique has not yet been shown to be effective at producing eggs that can undergo fertilisation and develop.

The HFEA's view and outcomes

The HFEA's Scientific and Clinical Advances Advisory Committee (SCAAC) considered this technique. They advised that microcytoplast cryopreservation was not a viable technique for treatment, but groups may want to use it for research. They thought the mouse model was not relevant to human eggs and that the safety issues of this technique have not yet been addressed. They expressed concerns that the technique would disrupt the internal components of the egg and there is a risk that the egg would be destroyed. They pointed out that vitrification of eggs can offer survival rates of 96%, so there would not be a demand for this technique.

There are safety implications of using this technique as a means for ooplasm transfer. A recent study[15] found significant physiological differences between heteroplasmic and control mice. Problems in heteroplasmic mice included pulmonary hypertension, increased body mass and fat mass and abnormal electrolyte levels. The Committee suggested that, as ooplasm transfer appears to be significantly associated with the mitochondrial heteroplasmy, if children were to be conceived through ooplasm transfer would need to be closely monitored for health problems.

The HFEA Executive and Licence Committees will refer to the information outlined in the paper and minutes of the SCAAC meeting held on 21 February 2008 in the potential event of receiving notifications or licence applications from centres which wish to use this technique in research.

2.4 Gene transfer into embryos

What is it?

It is possible to introduce transgenes into human embryos in order to create genetically modified embryos for research. This has been achieved with the use of viral vectors, which is the most common method of genetic modification.

What impact could it have?

The technique could be used to generate genetically modified embryonic stem (ES) cells for studying human embryogenesis and modelling disease. Investigating gene function in early embryogenesis could lead to the development of more objective criteria for embryo selection. The technique could also be used to introduce a gene to increase the efficiency of stem cell derivation.

What research has been carried out?

One group has reported that transgenes can be introduced into mouse and human embryos by viral transgenesis (with lentiviral vectors), leading to the production of transgenic blastocysts. Stably transfected ES cell lines were derived from the mouse transgenic blastocysts[16].

There has been recent work on new efficient methods to create transgenic rats. The "sleeping beauty" transposon system has been found to be an efficient method to generate mutant rats *in vivo*. This method has been used to create bigenic males in which the DNA becomes incorporated in the germ cells[17].

There is evidence that lentiviral vectors are a promising new tool for gene transfer in mammals. These viruses have a long incubation period, they can efficiently incorporate a significant amount of genetic information into the DNA of the host cell and they can be used in different species[18,19,20,21,22]. In particular it has been suggested that they could be used to create genetic models in rats.

Section **2**

Outcomes of 2007/8 horizon scanning process

One group has demonstrated that the trophectoderm of mouse embryos can be infected with lentivirus without the inner cell mass becoming infected[20]. The trophectoderm acts as a barrier to the virus particles and shields the inner cell mass cells from viral infection (and therefore from genetic alteration). This suggests that lineage-specific genetic alteration of embryos is possible.

When could it be introduced in the UK?

It has already been demonstrated that it is technically possible to produce transgenic human blastocysts. The Human Fertilisation and Embryology Act 1990 (as amended) will allow the HFEA to issue licences for the creation of genetically altered embryos for research purposes from October 2009.

The HFEA's views and outcomes

The HFEA's Scientific and Clinical Advances Advisory Committee advised the Authority that it is likely they would receive applications for research projects involving genetic modification of embryos from October 2009.

The Committee considered that the reasons for conducting research into gene transfer into embryos are numerous. Examples of the kinds of research are:

- research into human embryo development, specifically the roles of genes and growth factors involved in early development
- the development of more objective criteria for embryo selection, by investigating gene function in early embryogenesis
- research into the genetic background of adverse medical conditions – genetically modified ES cells could be created to model medical conditions
- research into the fate of cells during embryo development
- introducing a gene to increase the efficiency of stem cell derivation.

The potential benefits of this research include the increased understanding of medical conditions, and more effective stem cell derivation techniques resulting in less wastage of embryos.

The Committee highlighted the possibility of mutations arising as a result of some of the techniques, but felt that this would be less of an issue in research than treatment. They felt that the most efficient method for gene transfer was the "sleeping beauty" vector, which had been used in chickens and rats. This is integrated largely at random so there is a risk of inactivating genes, leading to mutations.

The HFEA's Ethics and Law Advisory Committee considered the ethical and public interest issues associated with the genetic modification of embryos for research. The Committee agreed that the public would benefit from an increased understanding of the kinds of research projects which might involve the use of genetically modified embryos.

The HFEA's Horizon Scanning Panel discussed the potential use of genetic modification at its meetings in July and November 2008. The Panel thought that the technique could be used to answer basic biological questions about early human development and could be used in comparative studies of early development. For example, it could be used to compare human and mouse embryo development, such as why there are abnormal nuclei in human blastomeres.

The Panel discussed how inserting genes instead of RNA would genetically alter the embryos for a longer period. This would be useful if, for example, the embryos were going to be used to derive stem cells.

The Panel felt that consideration would need to be given to whether the research could be done on embryos of other species before it is applied to human embryo research.

A paper outlining current research in the field of genetic alteration of embryos, the potential methods researchers may wish to use for genetically altering embryos and the views of the HFEA committees on this topic is being produced. This will ensure the HFEA's Research Licence Committee is sufficiently informed ahead of licence applications for this research which the Authority could potentially receive from October 2009.

2.5 Gene transfer into male germ lines

What is it?

It is possible to transfer genes into stem cells that will form sperm (spermatogonial cells) by adenovirus vectors and transplant them into seminiferous tubules.

What impact could it have?

This technique could be used to introduce a germ cell growth factor into Sertoli cells, thereby restoring spermatogenesis in infertile males. Resulting offspring would not show viral integration. It is thought that this is currently the only method to restore spermatogenesis because of Sertoli cell defects and further studies are necessary to test whether such stable infection occurs in the male germ line[23].

What research has been carried out?

Genes have been successfully introduced into the chicken male germ line by transplanting retrovirus-infected spermatogonia into the testes of sterilised recipient cockerels[24]. The retrovirus-infected cells restored spermatogenesis within 9 weeks with approximately the same efficiency as non-infected cells.

In addition, adenoviruses can be used to integrate genes into the male germ cell line, a technique which was successful with mouse spermatogonial cells[23]. The infected spermatogonial cells were transplanted into the seminiferous tubules of infertile mice where they reinitiated spermatogenesis. However, the virus gene was not present in the offspring, which suggests it was not stably integrated into the germ line. This technique was also recently used in goats, where genes were stably integrated into the germ cell lines which led to the production of transgenic offspring[25].

When could it be introduced in the UK?

To date the transplantation of transgenic spermatogonia to restore spermatogenesis has been successful in the mouse and a group recently achieved success in the goat (the first large mammalian species). To date there is no evidence of this technique being used in humans.

The creation and transplantation of transgenic human sperm to the testes of a man falls outside the remit of the HFEA. However, legislation prohibits sperm (which includes early sperm cells) being used in HFEA-licensed treatment if its nuclear or mitochondrial DNA has been altered. Sertoli cells do not fall within the definition of ‘sperm’ in the Human Fertilisation and Embryology Act 1990 (as amended) therefore the legislation does not prevent the genetic alteration of Sertoli cells.

The HFEA’s view and outcomes

The HFEA’s Scientific and Clinical Advances Advisory Committee considered this technique and thought that there were significant safety issues for gene transfer into male germ lines. It is likely to be a long time before this technique is transferred to humans.

2.6 Cryopreservation of immature testicular tissue and spermatogonial cells

What is it?

Cancer treatment can often be toxic to the gonads. Men and boys producing mature sperm can preserve their fertility by freezing sperm samples. However this is not an option for pre-pubescent boys who do not produce mature sperm. Recent studies have looked into freezing samples of pre-pubescent boys’ immature testicular tissue containing stem cells that will form sperm (spermatogonial stem cells) and early sperm cells (spermatogonia and spermatocytes).

What impact could it have?

The technique of cryopreserving immature testicular tissue and spermatogonial cells could be used to preserve fertility in pre-pubescent boys undergoing cancer treatment.

Clinicians may be able to transplant this tissue back into the patient when they are older in the hope that this will allow them to produce sperm naturally. Alternatively, researchers may be able to mature sperm from this tissue in the laboratory, an approach which would benefit patients suffering from haematological malignancies that may be re-transmitted by transplantation. The sperm could then fertilise an egg *in vitro* and be used in fertility treatment.

What research has been carried out?

An effective method for cryopreserving immature human testicular sperm has been developed[26]. The method maintains spermatogonia, Sertoli cells and the tissue structure during freezing, thawing and tissue culture. Spermatogonial stem cells, from cryopreserved immature non-human primate testicular tissue, have been shown to be capable of beginning spermatogenesis[27].

Research has also investigated methods of testicular tissue transplantation. Freeze-thawed human cryptorchid testicular tissue has been grafted into mice for 21 days[28]. 14.5% of spermatogonia survived, with 32% of those showing proliferative activity.

Cryopreserved immature human testicular tissue has also been transplanted into mice for six months. Spermatogonia were able to survive and proliferate and a few spermatocytes and spermatid-like cells were seen. However, normal spermatogenesis was not observed and there was an increasing loss of spermatogonia over time[29].

In comparison, restoration of normal spermatogenesis after transplantation of mouse immature testicular tissue has been achieved in mice, demonstrated by the production of mature sperm and offspring[30].

When could it be introduced in the UK?

Though groups have developed effective cryopreservation protocols, they have not managed to restore normal spermatogenesis following transplantation.

To date there is no evidence of *in vitro* spermatogenesis being carried out using immature human testicular tissue. Research into the use of human immature testicular tissue is still at a very early stage.

The HFEA's view and outcomes

The HFEA's Scientific and Clinical Advances Advisory Committee discussed this technique and concluded that transplanting tissue back into the patient would have a higher chance of success than *in vitro* spermatogenesis. The Committee thought that more extensive studies in animals were needed to look into the genetic and epigenetic issues raised by the technique. The Commitee also thought that the timescale for *in vitro* spermatogenesis was likely to be quite long, maybe around 10 years, but that clinics were likely to be interested in the technique.

The HFEA and Human Tissue Authority have begun discussions to ensure that this technique will be appropriately regulated after October 2009 when the HFE Act 1990 (as amended) extends the HFEA's remit to include immature gametes.

2.7 Alternative methods of obtaining embryonic or embryonic-like stem cells

What is it?

There are a number of techniques being developed to derive embryonic stem (ES) cells or ES-like cells that do not rely on nuclear transfer into oocytes or destroying viable embryos. These methods include:

- Induced pluripotent stem cells (iPS) – Adult cells, such as skin cells, can be directly reprogrammed into pluripotent cells. These iPS cells have similar properties to ES cells.
- Embryonic stem cells from blastomeres – ES cells can be derived from a single blastomere taken from an embryo. The remaining embryo can then potentially continue to develop as normal.
- Parthenogenetic embryonic stem (pES) cells – Human pES cells can be generated through artificial activation of oocytes to form diploid parthenotes (a process by which an egg can develop into an embryo in the absence of sperm). Parthenogenetic stem cell lines can then be derived from these parthenotes.
- ES cells from poor/non-viable embryos – Cells can be taken from poor quality or non-viable embryos and used to create ES cell lines.
- Nuclear transfer into zygotes – Zygotes, as opposed to oocytes, can be used as recipients of nuclear transfer. Stem cells can then be derived from the resulting embryos.
- ES-like cells from other tissues – Populations of stem cells, which express markers characteristic of ES cells populations, have been found in adult tissues and blood.

What impact could it have?

Paragraph 3(2) of Schedule 2 of Human Fertilisation and Embryology Act 1990 requires embryo research to be "necessary or desirable" for defined purposes. If viable alternative methods of deriving ES or ES-like cells are developed a licence committee of the Authority may judge that it is not "necessary" for research groups to destroy viable embryos.

Section **2**

Outcomes of 2007/8 horizon scanning process

It is important for the Authority to keep up to date with developments regarding these alternative methods so the Research Licence Committee of the Authority can bear them in mind when considering licence applications.

What research has been carried out?

Induced pluripotent stem cells

A combination of four factors, Oct3/4 and Sox2 with either Klf4 and c-Myc or Nanog and Lin28, have been shown to directly reprogram a range of adult human, mouse and monkey cells to form iPS cells[31 32 33].

The techniques used to introduce these factors into cells have traditionally involved the use of retroviruses or lentiviruses. These can be mutagenic and potentially activate oncogenes. Drug-inducible transgenic systems have been developed to reprogram somatic cells into iPS cells in the mouse and human[34 35]. Mouse iPS cells have been generated without viral vectors using repeated transfection of two expression plasmids[36].

The potential use of iPS cells in gene or cell therapy has been demonstrated[37 38].

Embryonic stem cells from blastomeres

Embryonic stem cell lines have been derived from single mouse blastomeres, taken from 2, 4 and 8-cell embryos[39]. Human embryonic stem cell lines have been derived from a single blastomere taken from a 4-cell stage embryo[40] and blastomeres removed from 8-cell stage human embryos have been shown to form multi-cell aggregates[41].

There have also been reports of single embryos producing both ES cell lines and offspring. An ES cell line from a blastomere removed from a mouse embryo differentiated into some types of tissue and contributed to a chimera mouse, whilst the remaining embryo continued to develop and produce offspring[42]. Another group reported the derivation of 5 human ES cell lines from individual blastomeres without destroying the embryo. The blastomeres were removed using a technique similar to preimplantation genetic diagnosis[43].

A laser system has been developed to isolate the inner cell mass as an alternative to the usual immunosurgery that utilises animal products[44].

Parthenogenetic embryonic stem (pES) cells

Parthenogenetic embryonic stem (pES) cell lines continue to be developed[45 46 47 48] and it has been shown that pES cell lines can be derived from *in vitro* matured oocytes grown in preantral follicles[49 50].

Parthenogenetic embryonic stem cells, derived from eggs, have been shown to exhibit ES cell pluripotency but have poor differentiation potential *in vivo*[51]. The differentiation potential of pES cells has been improved by using a nuclear transfer technique whereby the original pES cell nucleus is transferred into the enucleated oocytes and the resulting embryos establish new nuclear transfer-pES cell lines[52]. It is suggested that this improvement could be due to epigenetic changes brought about by nuclear transfer[53].

The pES cell genome carries entirely maternal imprinting. DNA that is normally paternally imprinted may therefore be abnormally expressed[54]. Although there is research that suggests not all pES cell lines suffer from this[55], it is a significant limitation of pES cell application[48].

ES cells from poor/non-viable embryos

Work has been published on a correlation between the developmental stage of the embryo used and creation of ES cell lines. Early-arrested or highly fragmented embryos only rarely yield cell lines, whereas those that have achieved blastocyst stage are a robust source of normal hES cells[56]. A human ES cell line has also been generated from a single blastomere of an arrested four cell stage embryo[57].

Nuclear transfer into zygotes

A US group has reported the derivation of stem cells from such embryos. It also successfully used tripronucleated (3PN) zygotes as recipients for nuclear transfer using the same technique, though did not attempt to derive stem cells from the resulting blastocysts[58].

Outcomes of 2007/8 horizon scanning process

Tripronucleated zygotes are automatically discarded from IVF treatment. This technique could therefore potentially derive ES cells without destroying viable embryos.

ES-like cells from other tissues

Populations of very small embryonic-like stem cells have been derived from murine bone marrow and adult tissues[59]. Some of these cells express markers characteristic of ES cells and indications that they may differentiate into all three germ layers[60]. There is evidence that the cells circulate at very low levels in peripheral blood and that similar ES-like cells exist in human umbilical cord blood[61]. Some work has been carried out looking at transplantation in mice[62].

Human adult germline stem cells have been derived from spermatogonial cells of the adult human testis[63]. The human adult germline stem cells produced teratomas and differentiated into all three germ layers when grown under conditions used to induce the differentiation of human ES cells.

ES cells from cloned embryos

ES cell lines have been produced from primate somatic cell nuclear transfer (SCNT) embryos[64] and cloned human blastocysts have been produced following SCNT with adult fibroblasts[65]. DNA fingerprinting analyses of some of the cloned blastocysts showed that the genomic DNA matched the donor fibroblast and was not of the oocyte or of parthenogenetic origin. No attempt was made to derive ES cell lines from the cloned embryos.

When could it be introduced in the UK?

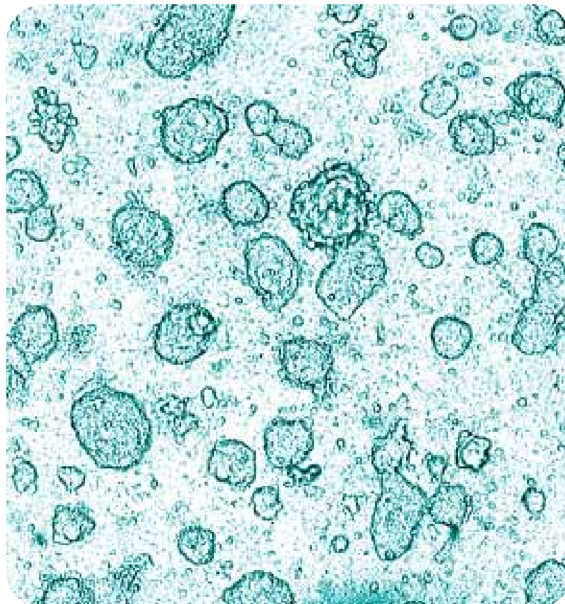
Members of the HFEA's Scientific and Clinical Advances Advisory Committee (SCAAC) expressed the view in 2008 that research into iPS cells and stem cells derived from single blastomeres was progressing very rapidly. Both sources of ES or ES-like cells were thought to be more useful in research than therapy and given the legal prohibition, in the UK, of using the same embryo for both treatment and research, iPS cells appeared to be the most promising alternative to ES cells.

HFEA Horizon Scanning Panel members, at a meeting in November 2008, thought that more research was required into the degree of similarity between ES cells and iPS cells.

The HFEA's view and outcomes

The HFEA's SCAAC considered a review of advances in these techniques and concluded that whilst research into iPS cells and stem cells derived from single blastomeres was progressing well, currently the only feasible way to derive ES cells for therapy may be from viable embryos.

Information about the latest developments regarding these alternative methods and the view of the HFEA groups on this topic has been outlined in a paper which the HFEA's Research Licence Committee refers to when deciding whether creation and use of embryos for proposed research is necessary or desirable (outlined in papers of 21 February 2008 SCAAC meeting).



Section **2**

Outcomes of 2007/8 horizon scanning process

2.8 *In vitro* derived gametes

What is it?

Stem cells have the potential to form different cell types in the body. Potentially they can therefore be used to form sperm and egg cells. Gametes formed in this way have been termed *in vitro* derived gametes.

In vitro derived gametes can potentially be produced from a range of cells, including stem cells from bone marrow, from embryos and from adult cells (such as skin cells) that have been reprogrammed to behave like embryonic-like stem cells.

What impact could it have?

In vitro derived gametes could be used for researching germ cell development, differentiation, meiosis and imprinting. They also have potential clinical applications, such as treatment of infertility and germline gene therapy.

Haploid androgenotes (cloned sperm) could potentially be used where a man is producing low levels of sperm, or only a single sperm is extracted. This method could be used to propagate this sperm and provide the opportunity to use replicates of the sperm nucleus for diagnostic purposes.

In theory *in vitro* derived gametes could be used in treatment to allow men and women otherwise unable to produce gametes to have children genetically related to them. However, the Human Fertilisation and Embryology Act 2008, when it comes into force in October 2009, will prohibit the use of gametes for treatment which have not been produced or extracted from the ovaries of a woman or the testes of a man.

What research has been carried out?

Bone marrow cells

Sperm and oocyte germ cells have been derived from bone marrow derived mesenchymal stem cells (MSC) in the mouse[66 67]. Bone marrow stem cells transplanted into the testes of mice can differentiate into germ cells lineages[68]. Early human sperm germ cells can also be derived from bone marrow MSC, but these sperm cells do not undergo meiosis[69].

Embryonic stem cells

ES cells have been used to derive sperm germ cells in both mice and humans[70 71 72 73]. In mice, the resulting sperm can give rise to offspring when injected into unfertilised eggs[74]. However the offspring were abnormal due to defective imprinting and died prematurely. Early oocyte-like germ cells have also been derived from ES cells in mice[75 76]. One study has suggested that both presumptive sperm and oocytes could be produced from XY ES cells in mice[77]. In 2008 a group reported what they believed to be the first research showing evidence for *in vitro* production of male gametes from ES cells without genetic manipulation or pre-selection[78].

The fusion of an embryonic stem cell with a somatic cell has been shown to reprogram the hybrid cell genome into one that allows differentiation into primordial germ cells *in vitro*[79]. These cells can then undergo further differentiation into germ-like cells and could provide personalised stem cells applicable to regenerative medicine and assisted reproductive technologies.

Somatic cells

Somatic cells can be reprogrammed to behave like ES cells. These induced pluripotent stem cells (iPS cells) could potentially be used to derive human sperm and eggs. In reviewing somatic cell haploidisation, however, this technique presents difficulties in ensuring accurate chromosome segregation and prevention of epigenetic defects in imprinted genes of the somatic cell nucleus[80]. These limitations need to be overcome before the technique provides a valid method for deriving gametes *in vitro*.

Haploid androgenotes

A group from the US have reported that it is possible to replicate a haploid male genome by injecting enucleated mouse eggs with a single healthy mouse sperm. The sperm genome was found to be chromosomally identical to its originator in over 80% of the clones analysed. The resulting cells were fused with an egg that had previously been chemically activated. The embryos derived had chromosomes from both parents, some of which resulted in offspring. All of the offspring were phenotypically normal, some grew normally and some died soon after birth[81,82]. This demonstrates that it is possible to replicate the male genome and that such a cloned genome has the ability to develop to term.

When could it be introduced in the UK?

So far researchers have not been able to derive mature human gametes from stem cells. However, in mice, *in vitro* derived sperm have been produced from stem cells capable of fertilising eggs and producing offspring, although the offspring died shortly after birth.

Members of the Scientific and Clinical Advances Advisory Committee (SCAAC) thought, in May 2008, that the timescale for deriving gametes for treatment was between 5-10 years. The timescale for deriving sperm from stem cells would be shorter (1-2 years) than that for deriving eggs.

One member of the Horizon Scanning Panel thought, in November 2008, that within 12 months researchers would wish to test the competency of male germ cells derived from human ES cells by injecting them into human oocytes. Members believed that such research must be at an appropriate stage for using human material and reviewers must consider whether the aim of the study could be achieved by alternative means.

The one research group investigating haploid androgenotes considers that the technique will not be used in humans in the near future. Further work needs to be done to understand why impaired development and abnormalities in the embryo occur, and to take steps to avoid that occurrence.

The HFEA's view and outcomes

The HFEA Executive and Licence Committees will refer to information and advice (outlined in the paper and minutes of the 21 May 2008 SCAAC meeting and the 7July and 6 November 2008 Horizon Scanning Panel meetings) if they receive licence applications from centres wishing to use this technique for research.

Section **3**

Issues identified in the 2008/9 horizon scanning process

This table presents all the issues that were identified through the 2008/9 horizon scanning process. It includes researched published up to November 2008. Issues were prioritised using a systematic approach which 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, and
- the technique is within the remit of the HFEA.

High priority issues will be considered in depth by the HFEA's Scientific and Clinical Advances Advisory Committee throughout 2009. Low and medium priority issues will not be followed up in any detail but have been provided for information.

Embryo selection

Issue	Priority	Use or aim	Description	Research identified
Assessment of embryo morphology	**High**	To select embryos that will improve ART outcomes.	Morphology of the early embryo can act as a marker of embryo quality. Various methods of assessing morphology have been investigated including cleavage rate and persistence of pronuclei.	**Hammoud I *et al*. (2008)** *Fertility and Sterility* 90(3):551-556. **Hesters L *et al*. (2008)** *Fertility and Sterility* 89(6):1677-1684. **Moayeri S *et al.* (2008)** *Fertility and Sterility* 89(1):118-123. **Muna M *et al*. (2008)** *Fertility and Sterility* 89(2):358-363.
Metabolomic profiling of embryo culture media	**Medium**	To select embryos that will improve ART outcomes.	The study of embryo culture media using techniques such as Raman or near-infrared spectroscopy reveals a relationship between the metabolic profile of an embryo and its quality.	**Blow N. (2008)** *Nature* 455:698. **Scott R *et al*. (2008)** *Fertility and Sterility* 90(1):77-83. **Sel E *et al*. (2007)** *Fertility and Sterility* 88(5):1350-1357. **Sturmey R *et al*. (2009)** *Human Reproduction* 24:81-91. **Vergouw C *et al*. (2008)** *Human Reproduction* 23(7):1499-1504. **Warner C *et al*. (2008)** *Reproductive BioMedicine Online* 17(4):470-485.
A combination of invasive selection strategies	**Medium**	To select embryos that will improve ART outcomes.	Research into blastocyst biopsy, microarray gene expression profiling and DNA finger printing as methods of invasively assessing embryo quality.	**Goossens V *et al*. (2008)** *Human Reproduction* 23(3):481-492. **Jones G *et al*. (2008)** *Human Reproduction* 23(8):1748-1759. **Wang W-H *et al*. (2008)** *Fertility and Sterility* 90(2):438-442.

Gamete selection, storage and manipulation

Issue	Priority	Use or aim	Description	Research identified
Interspecies fertilisation	**High**	To select gametes that will improve ART outcomes.	Interspecies fertilisation used to analyse sperm epigenetic status and normality.	**Fulka H. (2008)** *Human Reproduction* 23(3):627-634.
Gene expression	**Medium**	To select gametes that will improve ART outcomes.	Identification, using microarrays, of gene expression patterns in single embryo cells, polar bodies and cumulus cells can predict gamete quality.	**Aafke P. *et al*. (2008)** *Molecular Human Reproduction* 14(3):157-168. **Cill F *et al*. (2007)** *Reproduction* 134:645-650. **Feuerstein P *et al*. (2007)** *Human Reproduction* 22(12):3069-3077. **Hamel M *et al*. (2008)** *Human Reproduction* 23(5):1118-1127. **Jones G *et al*. (2008)** *Human Reproduction* 23(5):1138-1144. **Kearns W *et al*. (2008)** *Human Reproduction* 23, Suppl:O-164, i66.
Assessment of factors and proteins in follicular fluid	**Medium**	To select gametes that will improve ART outcomes.	Levels of proteins, growth factors and cytokines in follicular fluid can predict oocyte and embryo quality.	**D'Aniello G *et al*. (2007)** *Human Reproduction* 22(12):3178-3183. **Fujwara H and Matsubayashi H *et al* (2007)** *Human Reproduction* 22(11):3042-3045. **Ledee N *et al*. (2008).** *Human Reproduction* 23, Suppl 1:O-063, i27. **Ledee N *et al*. (2008)** *Human Reproduction* 23(9):2001-2009. **Lee J *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-0179, i73. **Sinclair K *et al*. (2008)** *Reproductive Biomedicine Online* 16(6):859–868. **Takahashi C *et al*. (2008)** *Fertility and Sterility* 89(3):586-591.
Metabolomic profiling of oocyte culture media	**Medium**	To select gametes that will improve ART outcomes.	Metabolomic profiling of oocyte culture media to predict embryo development and quality.	**Behr B *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-205, i83. **Picton H *et al*. (2008)** *Human Reproduction* 23, Suppl 1:P-286.

Section **3**

Issues identified in the 2008/9 horizon scanning process

Gamete selection, storage and manipulation (continued)

Issue	Priority	Use or aim	Description	Research identified
Respiration rate as a marker of gamete quality	**Medium**	To select gametes that will improve ART outcomes.	Novel non-invasive method of assessing ability of oocytes to respire at a rate that can sustain fertilisation and development.	**Scott L *et al*. (2008)** *Reproductive BioMedicine Online* 17(4):461-469.
Cumulus oophorous cell selection	**Medium**	To select gametes that will improve ART outcomes.	Mixing sperm with cumulus oophorous cells can help physiologically select sperm to be used in ICSI.	**Rijsdijk M & Franken D R (2007)** *Fertility and Sterility* 88(6):1595-1602.
Assessment of gamete morphology	**Medium**	To select gametes that will improve ART outcomes.	Assessment of gamete morphological characteristics, such as smooth endoplasmic reticulum aggregations in oocytes, to assess quality eg, birefringence.	**Ebner T *et al*. (2008)** *Reproductive Biomedicine Online* 16(1):113-118. **Gianaroli L *et al*. (2008)** *Fertility and Sterility* 90(1):104-112. **Montag M *et al*. (2008)** *Reproductive BioMedicine Online* 17(4):454-460.
Assessment of gamete morphology	**Low**	To select gametes that will improve ART outcomes.	Spindle visualisation in eggs predicts embryo development; sperm morphology influences rate of euploidy, implantation and clinical pregnancy.	**Dubey A *et al* (2008)** *Fertility and Sterility* 89(6):1665-1669. **Madaschi C et al. (2008)** *Fertility and Sterility* 90(1):194-198.
DNA fragmentation	**Low**	To select gametes that will improve ART outcomes.	The level of DNA fragmentation can predict sperm and embryo quality.	**Collins J *et al*. (2008)** *Fertility and Sterility* 89(4):823-831. **Vagnin L *et al*. (2007)** *Reproductive Biomedicine Online* 15(5):514-519.
Automation of sperm selection	**Low**	To select gametes that will improve ART outcomes.	Automation of aneuploidy/ euploidy sperm sorting has similar accuracy to manual sorting.	**Carrell D *et al*. (2008)** *Fertility and Sterility* 90(2):434-437.
G6PDH selection marker	**Low**	To select gametes that will improve ART outcomes.	G6PDH as a marker of bovine egg competency and underlying molecular and subcellular differences.	**Torner H *et al*. (2008)** *Reproduction* 135(2):197-212.

***In vitro* derived gametes**				
Issue	**Priority**	**Use or aim**	**Description**	**Research identified**
Gamete derivation	**High**	Research into the germ line.	*In vitro* production of germ-like cells and spermatogenesis.	**Kerkis I *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-228, i92. **Lavagnolli T *et al*. (2008).** *Human Reproduction* 23, Suppl 1:O-231, i94. **Scholer H. (2008)** Derivation of gametes from embryonic stem cells. Society for Reproduction and Fertility conference. **West F *et al*. (2008)** *Stem cells* 26(11):2768-2776.
Mature oocyte derivation	**Medium**	Research into the germ line.	Production of mature oocytes from purified mouse fetal germ cells.	**Qing T *et al*. (2008)** *Human Reproduction* 23(1):54-61.
Oocyte Maturation	**Low**	Improve outcomes of oocyte *in vitro* maturation.	Gonadotrophins, steroids and oocyte secreted factor GDF9 shown to increase embryo development and fetal viability.	**Yeo C *et al*. (2008)** *Human Reproduction* 23(1):67-73. **Murray A *et al*. (2008)** *Molecular Human Reproduction* 14(2):75-83.
Germ line stem cells	**Low**	Research into the germ line.	Discovered to be two distinct populations of germ line stem cells, one gives rise to spermatogonial stem cells and the other multipotent cell lines with some pluripotent characteristics.	**Izadyar F *et al*. (2008)** *Reproduction* 135(7):177-784.
Isolation of primordial germ cells from genital ridges	**Low**	Research into the germ line.	Comparison of two gradient methods of isolation shows similar isolation rate but more germ cells with higher viability using Percoll.	**Saiti D *et al*. (2008)** *Reproductive BioMedicine Online* 16(5):730-740.
Review of technology	**Low**	Research into the germ line.	Mouse ES cells derived from cloned and fertilised blastocysts are transcriptionally and functionally indistinguishable.	**Wkayama T *et al*. (2008)** *Reproductive BioMedicine Online* 16(4):545-552.

Issues identified in the 2008/9 horizon scanning process

Stem cell derivation and cloned embryos

Issue	Priority	Use or aim	Description	Research identified
Human admixed embryos	**High**	Reduce need for human oocytes for training purposes and improve methods of nuclear transfer.	Pig ovaries may be a useful tool for oocyte retrieval and intracytoplasmic sperm injection.	**Braga D *et al*. (2007)** *Fertility and Sterility* 88(5):1408-1412. **Beyhan Z *et al* (2007)** *Cell Stem Cell* 1(5):502-512.
Alternative methods of obtaining embryonic stem cells or stem cell-like cells	**High**	Alternative methods of deriving embryonic stem cells or stem cell-like cells without destroying viable embryos.	Induced pluripotent stem cells from a variety of cell types, work on increasing efficiency of re-programming, development of a drug inducible system and comparison of cell lines.	**Aoi T *et al*. (2008)** *Science* 321(5889):699-702. **Costello J (2008)** *Nature* 454:45-46. **Cyranoski D *et al*. (2008)** *Nature* 452:132. **Dimos J *et al*. (2008)** *Science* 321(5893):1218-1221. **Hanna J *et al* (2007)** *Science* 318:1920-1923. **Hockemeyer D *et al*. (2008)** *Cell stem cell* 3(3):346-353. **Holden C *et al*. (2008)** *Science* 319:560-563. **Kim J *et al*. (2008)** *Nature* 454:646-650. **Maherali N *et al*. (2008)** *Cell Stem Cell* 3(3):340-345. **Mali P *et al*. (2008)** *Stem Cells* 26(8):1998-2005. **Park I-H *et al*. (2008)** *Nature* 451(7174):141-146. **Tada T *et al*. (2008)** *Cell stem cell* 3(2):121-122. **Takahashi *et al* (2007)** *Cell* 131(5):861-872. **Yu J *et al*. (2008)** *Genes and Development* 22(15): 1987-1997. **Yu J *et al* (2007)** *Science* 318:1917-1920.

Transplantation

Issue	Priority	Use or aim	Description	Research identified
Human testicular tissue grafts into SCID mice maintain functionality	High	Reinstating fertility post testicular failure.	Evidence of nude mice being good hosts to testicular grafts.	**Lo K *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-203, i82.
Human ovarian tissue grafts maintain functionality in humans and SCID mice	High	Reinstating fertility post ovarian failure.	Fresh and cryopreserved ovarian tissue and follicles can maintain functionality after being grafted orthotopically into humans and in various locations in severe combined immunodeficient mice.	**Dolmans M *et al*. (2007)** *Reproduction* 134(2):253-62. **Dolmans M *et al* (2008)** *Reproductive BioMedicine Online* 16(5):705-711. **Nottola S *et al*. (2008)** *Fertility and Sterility* 90(1):23-32. **Paris M *et al*. (2007)** *Reproduction, Fertility and Development* 19(6):771-8. **Schubert B *et al*. (2008)** *Fertility and Sterility* 89(6):1787-1794. **Soleimani R *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-172, i69. **Van Eyck A *et al*. (2008)** *Fertility and Sterility* 2008 Aug 8. [Epub ahead of print].

Embryo manipulation and culture

Issue	Priority	Use or aim	Description	Research identified
Comparison of vitrification and slow freezing methods	Low	Efficient embryo freezing.	Evidence of a higher post-thawing survival rate of human embryos and successful pregnancy following vitrification compared to slow freezing.	**Balaban B *et al*. (2008)** *Human Reproduction* 23(9):1976-1982. **Chang C-C *et al*. (2008)** *Reproductive BioMedicine Online* 16(4):346-349. **El-Toukhy T *et al*. (2008)** *Human Fertility* 11(2), Suppl 1:OC6. **Lappi M *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-173, i70. **Loutradi K *et al*. (2008)** *Fertility and Sterility* 90(1):186-193. **Pinborg A *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-122, i51. **Youssry M *et al* (2008)** *Reproductive BioMedicine Online* 16(2):311-320.

Section **3**

Issues identified in the 2008/9 horizon scanning process

Embryo manipulation and culture (continued)

Issue	Priority	Use or aim	Description	Research identified
Totipotent blastomeres	**Low**	Source of blastocysts for research.	Blastomeres taken from a 4-cell stage human embryo can develop into blastocysts.	**Van de Velde H *et al*. (2008)** *Human Reproduction* 23(8):1742-1747.
Embryo culture environment	**Low**	Efficient embryo culture.	An efficient culture environment for preimplantation embryos.	**Vajta G *et al*. (2008)** *Reproductive BioMedicine Online* 17(1):73-81.

Genetic screening

Issue	Priority	Use or aim	Description	Research identified
RhD screening	**Medium**	Predicting fetal RhD phenotype from fetal DNA in the plasma of pregnant women.	Support for universal screening to reduce necessary anti-Rhesus u antigenDprophylaxis. May be transferable to preimplantation genetic screening.	**Finning K *et al*. (2008)** *BMJ* 336:816-818. **Kumar S (2008)** *BMJ* 336:783-784.
Pre-implantation genetic diagnosis (PGD)	**Low**	More effective PGD techniques.	Novel, more efficient and sensitive methods of PGD, evidence of no major ill effect on resulting child and report of successful aneuploid and achondroplasia detection.	**Altarescu G *et al*. (2008).** *Reproductive BioMedicine Online* 16(2):276-282. **Gabriel A *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-165, i67. **Landwehr C *et al*. (2008)** *Fertility and Sterility* 90(3):488-496. **Lathi R *et al*. (2008)** *Fertility and Sterility* 89(2):353-357. **Lledo B *et al*. (2007)** *Fertility and Sterility* 88(5):1327-1333. **Sutcliffe A *et al*. (2008)** *Reproductive BioMedicine Online* 16(4):376-381.

IVF/ICSI technologies

Issue	Priority	Use or aim	Description	Research identified
Defective mtDNA replacement	**Medium**	Mitochondrial disease treatment.	Suggestion that the ART techniques of cytoplasmic and pronuclear transplantation are effective means to replace defective mitochondria in maternal lineages.	**Smith L. (2008)** Mitochondrial DNA in the oocyte and embryo: consequences of ART. Society for Reproduction and Fertility conference.
Ultrasound guided embryo transfer	**Low**	Improve ART outcomes.	Evidence for and against ultrasound guided embryo transfer improving pregnancy rate.	**Ali R *et al*. (2008).** *Reproductive BioMedicine Online* 17(1):88-93. **Almodovar M *et al*. (2008).** *Human Reproduction 23,* Suppl 1:O-55, i23. **Drakeley A. *et al*. (2008).** *Human Reproduction* 23(5):1101-1106. **Zakova J *et al*. (2008)** *Human Reproduction* 23, Suppl 1:P-333.
Reproductive immunology	**Low**	Improve ART outcomes.	Research into embryo implantation and the function of immune system factors during pregnancy.	**Casals G *et al*. (2008)** *Reproductive Biomedicine Online* 16(6):808–816. **Fukui A. *et al*. (2008)** *Fertility and Sterility* 89(1):157-165. **Grewal S *et al*. (2008)** *Proc Natl Acad Sci USA* 105: 16189-16194. **Gremlich S. *et al*. (2008)** *Human Reproduction* 23(5):1200-1206. **Hapangama D *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-207, i84. **Hiby S. *et al*. (2008)** *Human Reproduction* 23(4):972-976. **Peng S. *et al*. (2008)** *Reproduction* 135(3):367-375. **Stoikos *et al*. (2008)** *Human Reproduction* 23(6):1447-1456. **Xu B *et al*. (2008)** *Human Reproduction* 23(6):1394-1406. **Yang H *et al*. (2008)** *Fertility and Sterility* 89(3):656-661. **Zhou W-H *et al*. (2008)** *Reproduction* 135(3):385-395.

IVF/ICSI technologies (continued)

Issue	Priority	Use or aim	Description	Research identified
Controlled Ovarian Stimulation (COH)	Low	Refining the COH protocol and prevention of ovarian hyper-stimulation syndrome (OHSS).	Research into improving and refining the COH protocol, identifying markers of ovarian response and methods of predicting and reducing the risk of OHSS.	**Andersen *et al*. (2008)** *Human Reproduction* 23(6):1424-1430. **Binder H *et al*. (2008)** *Reproductive BioMedicine Online* 17(2):185-189. **Busso C *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-149, i60. **Engmann L *et al* (2008)** *Fertility and Sterility* 89(1):84-91. **Fong S *et al*. (2008)** *Reproductive BioMedicine Online* 16(5):664-670. **Gome-Palomares J *et al* (2008)** *Fertility and Sterility* 89(3):620-624. **Homburg R (2008)** *Human Fertility* 11(1):17-22. **Schoolcraft W *et al* (2008)** *Fertility and Sterility* 89(1):151-156. **Soares S *et al*. (2008)** *Human Reproduction Update* 14(4):321-333. **Tsung-Hsien Lee *et al*. (2008)** *Human Reproduction* 23(1):160-167. **The Thessaloniki ESHRE/ ASRM-sponsored PCOS consensus workshop group. (2008)** *Human Reproduction* 23(3):462-477. **Weghofer A. (2008)** *Human Reproduction* 23(3):499-503.
Miscarriage indicator	Low	Reduce miscarriage rate.	Identification of indicators of miscarriage – elevated free androgen index and levels of cytokine thrombopoietin circulating in maternal blood.	**Cocksedge K. (2008)** *Human Reproduction* 23(4):797-802. **Whitcomb B *et al*. (2008)** *Fertility and Sterility* 89(6):1795-1802.
Sibling embryo behaviour	Low	Improve ART outcomes.	Sibling embryos from the same cycle have similar developmental potential. Development *in vitro* of a blastocyst can predict pregnancy outcome of sibling embryo.	**Yilmaz S *et al*. (2008)** *Reproductive Biomedicine Online* 16(1): 124-128.

IVF/ICSI technologies (continued)

Issue	Priority	Use or aim	Description	Research identified
ICSI	Low	Improve ICSI technique and outcomes.	Outline of risks and technical difficulties and indicators of success.	**ESHRE Capri Workshop Group (2007)** *Human Reproduction Update* 113(6):515-526. **Nicopoullos J *et al*. (2008)** *Human Reproduction* 23(2):240-250. **Varghese A C *et al*. (2007)** *Reproductive Biomedicine Online* 15(6):719-727.
Novel transfer methods	Low	Improve ART outcomes.	Novel methods of embryo transfer to increase pregnancy rate.	**Goto S *et al*. (2007)** *Fertility and Sterility* 88(5):1339-1343. **Korosec S *et al*. (2007)** *Reproductive Biomedicine Online* 15(6):701-707.
Markers of IVF success	Low	Predicting IVF success.	High sIL2R and low LIF maternal plasma levels on day of embryo transfer associated with rapid pregnancy loss after IVF.	**Gremlich S *et al*. (2008)** *Human Reproduction* 23, Suppl 1:P-345.
Marker of male infertility	Low	Improvement of male infertility diagnosis.	Novel biomarker for male infertility found in testicular spermatids and ejaculated spermatozoa.	**Steger K *et al*. (2008)** *Human Reproduction* 23(1):11-16.
Chromosome mutation after ART	Low	Identify risks associated with ART.	Indication of a higher than normal rate of de novo mutations in the Y chromosome of children created via ART.	**Feng C *et al*. (2008)** *Fertility and Sterility* 90(1):92-96.

Section **3**

Issues identified in the 2008/9 horizon scanning process

Other

Issue	Priority	Use or aim	Description	Research identified
Gene therapy	High	Treatment of infertility and research into human embryonic stem cells	Research into gene therapy to treat infertility and stable gene transfer in human embryonic stem cells.	**Ghadami M *et al*. (2008)** *Molecular Human Reproduction* 14(1):9-15. **Shimizu T *et al*. (2007)** *Reproduction* 134:677-682. **Kojima Y *et al*. (2008)** *Fertility and Sterility* 89, Suppl 3:1448-1454. **Wilber A *et al* (2007)** *Stem Cell* 25(11):2919-2927.
Fetal cells in maternal blood	**Medium**	Source of primitive stem cells	Fetal cells in the maternal blood are primitive stem cells with the potential for wide differentiation.	**Mikhail M *et al*. (2008)** *Human Reproduction* 23(4):928-933.
Epigenetic in ART and infertility	**Medium**	Information on safety of ART	Review of evidence suggests that ARTs and/or infertility may affect epigenetic processes leading to epigenetic diseases.	**Feliciano M *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-27, i12. **Huntriss J *et al*. (2008)** *Human Fertility* 11(2):85-94. **Marques C *et al*. (2008)** *Human Reproduction* 23, Suppl 1:O-72, i30.
Xeno-free cryo-preservation of human embryonic stem cells	**Low**	Human embryonic stem cell storage	Xeno-free cryopreservation of human embryonic stem cells, a slow freeze-rapid thawing technique.	**Valbuena D *et al*. (2008).** *Reproductive BioMedicine Online* 17(1):127-135.

References

[1] Dokras A *et al*. (1990)
Human Reproduction 5(7):821-5.

[2] Anderson AR *et al*. (2007)
Fertility and Sterility 88, Suppl 1:S235.

[3] Katz-Jaffe MG *et al*. (2007)
Fertility and Sterility 88, Suppl 1:S266.

[4] Kokkali G *et al*. (2007)
Human Reproduction 22:1443-1449.

[5] Kokkali G *et al*. (2005)
Human Reproduction 20,1855–1859.

[6] McArthur SJ *et al*. (2005)
Fertility and Sterility 84,1628–1636.

[7] Tongyai T *et al*. (2004)
Human Reproduction 19, Suppl 1:i184.

[8] Gordon JW *et al*. (1989)
Fertility and Sterility 52:367-72.

[9] Malter HE & Cohen J (1989)
Fertility and Sterility 52:373-380.

[10] Escribá M *et al*. (2006)
Fertility and Sterility 86:1601-1607.

[11] Kattera S & Chen C (2003)
Human Reproduction 18(6):1319-1322.

[12] Egli D *et al*. (2007)
Nature 447:679-686.

[14] Goud A P *et al*. (2007)
Fertility and Sterility 87:923-933.

[15] Acton B M *et al*. (2007)
Biol Reprod. 77(3):569-576.

[16] Zaninovic N *et al*. (2007)
Fertility and Sterility 88, Suppl1:S310.

[17] Lu B *et al*. (2007)
Mamm Genome 18, 338-46.

[18] Cockrell AS, Kafri T. (2007)
Mol Biotechnol. 36, 184-204. (Review.)

[19] Dann CT. (2007)
Transgenic Res. 16, 571-80. (Review.)

[20] Malashicheva A *et al*. (2007)
Genesis 45, 456-9.

[21] Park F. (2007)
Physiol Genomics 31, 159-73. (Review.)

[22] Yang SH *et al*.(2007).
Genesis 45, 177-83.

[23] Takehashi *et al*. (2007)
Proc Natl Acad Sci USA 20; 104(8): 2596–2601.

[24] Kalina J *et al*. (2007)
Reproduction 134(3):445-53.

[25] Honaramooz A *et al*. (2008)
FASEB J 22(2):374-82.

[26] Keros V *et al*. (2007)
Human Reproduction 22(5):1384-1395.

[27] Jahnukainen K *et al* (2007)
Human Reproduction 22(4):1060-1067.

[28] Wyns C *et al*. (2007)
Human Reproduction 22(6):1603-1611.

[29] Wyns C *et al*. (2008)
Human Reproduction July 28 2008 advanced online publication.

[30] Shinohara T *et al* (2002)
Human Reproduction 17(2):3039-3045.

[31] Marson A. *et al*. (2008)
Cell stem cell 3(2):132.

[32] Takahashi *et al*. (2007)
Cell 131(5):861-72.

[33] Yu *et al*. (2007)
Science 318(5858):1917-20.

[34] Hockemeyer D *et al*. (2008)
Cell stem cell 3(3):346-353.

[35] Wernig *et al*. (2008)
Nat Biotechnol. 26(8):916-24.

[36] Okita *et al*. (2008)
Science 7:322(5903):949-53.

[37] Dimos *et al*. (2008)
Science 321(5893):1218-1221.

[38] Hanna *et al*. (2008)
Science 318(5858):1920-3.

[39] Wakayama *et al*. (2007)
Stem Cells 25(4):986-93.

[40] Van de Velde *et al*. (2008)
Human Reproduction 23(8):1742-1747.

[41] Klimanskaya *et al*. (2007)
Nat Protoc 2(8):1963-72.

[42] Teramura T *et al*. (2007)
Cloning Stem Cells 9(4):485-94.

[43] Chung *et al*. (2008)
Nature 2(2):113-117.

[44] Turetsky T *et al*. (2008)
Human Reproduction 23(1):46-53.

[45] Kim *et al*. (2007)
Cell Stem Cell 1:346-352.

[46] Revazova *et al*. (2007)
Cloning and Stem Cells 9(3):432-449.

[47] Mai *et al*. (2007)
Cell Res. 17(12):1008-19.

[48] Brevini & Gandolfi (2008)
Cell Prolif. 41 Suppl 1:20-30.

[49] Gong SP *et al*. (2008)
Stem Cells Dev. 17(4):695-712.

[50] Lee ST *et al*. (2008)
Fertility and Sterility 90(5):1910-20.

[51] Xing F *et al*. (2008)
Fertility and Sterility Mar 17 2008 advanced online publication.

[52] Hikichi *et al*. (2007)
Stem Cells 25(1):46-53.

[53] Hikichi *et al*. (2008)
Stem Cells 26(3):783-8.

[54] Lin *et al*. (2007)
Cell Res. 17(12):999-1007.

[55] Dighe V *et al*. (2008)
Stem Cells 26(3):756-766.

[56] Lerou *et al*. (2008)
Nat Niotechnol. 26(2):212-4.

[57] Feki *et al*. (2008)
Swiss Med Wkly 138(37-38):540-550.

[58] Egli *et al*. (2007)
Nature 447(7145):679-85.

[59] Ratajczak (2008a)
Micron Oct 19 2008 advanced online publication.

[60] Ratajczak (2008b)
Stem Cell Rev. 2008 Summer;4(2):89-99.

[61] Ratajczak (2008c)
Stem Cells 26(8):2083-92.

[62] Dawn *et al* (2008)
Stem Cells 26(6):1646-55.

[63] Conrad *et al*. (2008)
Nature 20;456(7220):344-9.

[64] Byrne *et al*. (2007)
Nature 450(7169):497-502.

[65] French *et al*. (2008)
Stem Cells Stem Cells, 26(2):485-493.

[66] Nayernia *et al*. (2006a)
Lab Invest 86(7):654-63.

[67] Bukovsky *et al*. (2007)
Cell cycle 6(18):2306-9.

[68] Lue *et al*. (2007)
Am J Pathol 170(3):899-908.

[69] Drusenheimer *et al*. (2007)
Soc Reprod Fertil Suppl 63:69-76.

[70] Aflatoonian & Moore (2006)
Reproduction 132(5):699-707.

[71] Clark *et al*. (2004)
Human Molecular Genetics 13:727-39.

[72] Geijsen *et al*. (2004)
Nature 427:148-154.

[73] Toyooka *et al*. (2003)
Proceedings of the National Academy of Sciences of the USA 100:11457-62.

[74] Nayernia *et al*. (2006b)
Developmental Cell 11:125-132.

[75] Hubner *et al*. (2003)
Science 300:1251-1256.

[76] Lacham-Kaplan *et al*. (2006)
Stem cells 24:266-273.

[77] Kerkis *et al*. (2007)
Cloning Stem Cells 9(4):535-48.

[78] Kerkis *et al*. (2008)
Human Reproduction 23, Suppl 1:i92.

[79] Lavagnolli *et al*. (2008)
Human Reproduction 23, Suppl 1:i94.

[80] Nagy Z *et al*. (2008)
Reproductive BioMedicine Online 16(4): 539-544.

[81] Takeuchi T *et al*. (2007)
Fertility and Sterility 88, Suppl 1:S75.

[82] Takeuchi T *et al*. (2007)
Human Reproduction 22, Suppl 1:i62.

Human Fertilisation
and Embryology Authority
21 Bloomsbury Street London WC1B 3HF
Telephone: **020 7291 8200**
Email: **hannah.darby@hfea.gov.uk**