

# Scientific Horizon Scanning at the HFEA

Annual Report 2009/10

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HUMAN  
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AUTHORITY

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Horizon scanning is an early-warning system which allows the Human Fertilisation and Embryology Authority (HFEA) to consider the legal, ethical, social and regulatory implications of any techniques that researchers or clinicians may wish to use in the future. By identifying developments early, we can think ahead about the licensing of new techniques, or have guidance in place to ensure that new treatments are carried out safely and appropriately. We can also ensure that patients and the wider public are suitably informed.

#### *Identifying new research*

Through our horizon scanning process, the HFEA Executive discovers relevant research throughout the year by reviewing journals and attending conferences. They then highlight and investigate common themes and seek views from experts, such as members of the HFEA's Horizon Scanning Panel, a panel of international experts with a range of research interests. The next step is to prioritise the different techniques and decide whether we need to carry out more work on them.

This report summarises work carried out in 2009 as well as setting out issues that have been identified for this year's work plan.

#### *Licensable activities*

In some cases the issues described in this report involve licensable activities. If centres wish to carry out licensable activities they would need to inform the Authority or apply for a licence. In some cases, further consultation will be carried out. A licence committee of the Authority considers the details of each individual application before the technique can be used.



# Work carried out during 2009 on issues identified previously

## 2.1 Health outcomes and birth defects following assisted reproductive techniques

### What is it?

Some research suggests that assisted reproduction techniques (ART) are associated with birth defects in infants, however, a direct link is yet to be established as it is possible that the link is due to other factors. For instance, the cause of an increase in reported ART births may be either related to the underlying subfertility in the patients or due to a bias because infants conceived as a result of ART tend to be more closely monitored.

### What impact could it have?

Research in this field needs to be taken into account when patients are informed about the risks of treatment. Significant findings regarding the health of ART-conceived children may influence whether or not patients decide to go ahead with treatment. In order to keep patients fully informed the HFEA must ensure that information which clinics give to patients and the information the HFEA makes available directly to patients via its website takes into account the latest research.

### What research has been carried out?

A population-based, multi-centre, case-control study of birth defects using data from the National Birth Defects Prevention Study in the US was released in late 2008. The study reported that some birth defects occur more frequently in infants conceived using ART (eg, cardiac defects, cleft lip and digestive tract defects)<sup>1</sup>.

These results were similar to those reported in a large study in Sweden<sup>2</sup>. A number of other studies have also reported an association with heart defects<sup>3,4,5,6</sup> cleft lip<sup>7</sup> and digestive tract defects<sup>7</sup>.

A study of the Western Australia register found a two-fold increase (from around 4% to around 8%) in the risk of major birth defects in *in vitro* fertilisation (IVF) and intra-cytoplasmic sperm injection (ICSI) conceived children<sup>8</sup>.

Other analyses of existing literature have reported around a 30% increase (from a risk of approximately 2% to 2.6%) in cases of major birth defects in children conceived as a result of IVF and ICSI<sup>9,10</sup>. One analysis also found there to be a possible increase in imprinting disorders (ie, Angelman syndrome and Beckwith-Weidemann syndrome) in pregnancies conceived using ART<sup>10</sup>.

There is weak evidence for an increased risk of major birth defects in ICSI compared to IVF conceived children<sup>11</sup>.

### The HFEA's views and outcomes

In January 2009, the HFEA's Scientific and Clinical Advances Advisory Committee (SCAAC) discussed a summary of research and reviews about health outcomes of ART-conceived children. The Committee felt that patients' assessment of these risks varied depending on whether they were undergoing ART for medical or social reasons. Another factor was patients' perception of risks associated with the stimulatory drugs necessary for ART.

As a result of the Committee's discussion the information the HFEA provides patients was reviewed. Information on birth defects and health outcomes in infants resulting from ICSI has been replaced with information on outcomes following ART in general (including the fact that some may be common to sub-fertile couples and therefore not directly associated with the ART technique).

HFEA guidance for licensed centres about what information they need to provide to patients, has also been updated in the Code of Practice. Centres now must provide patients with information about the nature and potential risks of treatment, including the risk of children conceived having developmental and birth defects.

## Work carried out during 2009 on issues identified previously

### 2.2 Intra-cytoplasmic morphologically selected sperm injection

#### What is it?

Conventional intracytoplasmic sperm injection (ICSI) involves selecting and injecting sperm into an egg *in vitro*. The embryologist selects one of the more normal-looking motile sperm using a normal microscope that magnifies the sample between 200 and 400 times.

Intra-cytoplasmic morphologically selected sperm injection (IMSI) is a variation of ICSI that uses a high power light microscope, enhanced by digital imaging, to magnify the sperm sample over 6000 times. This allows the embryologist to detect subtle structural alterations in sperm that a normal microscope could not detect. Sperm are selected that have the most morphologically normal nuclei.

#### What impact could it have?

Some studies suggest that using this technique selects better quality sperm and results in higher pregnancy rates and lower miscarriage rates compared to conventional ICSI.

#### What research has been carried out?

The technique for examining sperm to use in IMSI was developed by an Israeli team in 2002. They used a high-powered microscope to determine which morphological characteristics of sperm might affect the outcome of ICSI<sup>12</sup>.

They suggest that ICSI pregnancy rates may be affected by subtle morphological malformations of the sperm nucleus, which embryologists may not detect during routine ICSI sperm selection. The team found that IMSI improves the pregnancy rate in couples with repeated ICSI failures, when compared to conventional ICSI<sup>13</sup>.

Further studies have confirmed these findings<sup>14,15</sup> and one randomised controlled trial has shown that the use of IMSI for couples with severe male factor infertility (regardless of whether they had previous unsuccessful ICSI cycles or not) results in a significantly higher pregnancy rate than for ICSI.

#### The HFEA's views and outcomes

In May 2009 SCAAC discussed the use and safety of IMSI. The Committee had concerns about IMSI's efficiency and safety in relation to the exposure of sperm to light.

They felt that evidence of IMSI improving the success rate of ICSI was limited and that clinical evidence suggested the technique should remain in the research sector. The Committee felt, however, that the assessment of sperm for use in ICSI was of growing interest and that UK centres would be interested in using IMSI.

# Work carried out during 2009 on issues identified previously

## 2.3 Alternative methods of obtaining embryonic or embryonic-like stem cells

### What is it?

Embryonic stem cells (ES cells) are derived from an embryo's inner cell mass at the blastocyst stage and are pluripotent, ie, capable of forming many different cell types. There are a number of techniques being developed to derive ES cells or ES-like cells that do not rely on nuclear transfer into eggs (removing the DNA from an egg and injecting the nucleus with the DNA you want to clone) or destroying viable embryos.

These methods include:

- Induced pluripotent stem cells (iPS) – Adult cells, such as skin cells, can be directly reprogrammed into (changed into) pluripotent cells. These iPS cells have similar properties to ES cells.
- ES cells from blastomeres – ES cells can be derived from a single blastomere (a cell formed in the first stage of embryonic development) taken from an embryo. The remaining embryo can then potentially continue to develop normally.
- Parthenogenetic embryonic stem (pES) cells – Human pES cells can be generated through artificial activation of eggs to form diploid (two sets of chromosomes) parthenotes (eggs which have developed into an embryo without sperm). pES 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 a zygote (the cell formed as a result of fertilisation) – Zygotes, as opposed to eggs, 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 appear similar to ES cells, have been found in adult tissues and blood.

### What impact could it have?

The Human Fertilisation and Embryology Act 1990 (as amended) requires embryo research to be “necessary or desirable”. If practical alternative methods of deriving ES or ES-like cells are developed, our Research Licence Committee, which decides whether or not research can go ahead, may judge that it is not necessary for research groups to destroy viable embryos in order to derive ES cells.

### What research has been carried out?

#### *Induced pluripotent stem cells*

Techniques used to create iPS cells have traditionally involved the use of viruses (the specific types of viruses are called retroviruses and lentiviruses) to introduce genes into cells. However, these viruses can potentially activate oncogenes which, under certain circumstances, can cause cancer.

In 2009 it was demonstrated that proteins could be used to generate iPS cells, from human and mouse skin cells<sup>16,17</sup>. This method avoids the use of viruses or genetic alteration. It is also possible to create iPS cells using plasmids (DNA molecules separate from chromosomal DNA) to introduce the genes<sup>18</sup>. A number of other alternative techniques have also been developed<sup>19,20,21,22,23,24</sup>. The starting cell's stage of differentiation has been shown to influence the efficiency of reprogramming. Less differentiated cells are more efficiently reprogrammed.<sup>25</sup>

Research has been carried out into the degree of similarity between iPS cells and ES cells, comparing their telomere (sections of DNA at the end of chromosomes) length<sup>26</sup>, teratoma (a tumour containing different types of tissue) formation<sup>27</sup> and genomic methylation patterns (the way cells modify their DNA to alter gene expression)<sup>28</sup>. Research has also shown that additional reprogramming factors can increase the similarity of iPS cells to ES cells<sup>29</sup>.

## Work carried out during 2009 on issues identified previously

### *Embryonic stem cells from single blastomeres*

ES cell lines have been derived from single mouse blastomeres taken from 2, 4 and 8-cell embryos, which then go on to develop and produce offspring<sup>30,31</sup>. There have been a number of reports of the derivation of human embryonic stem (hES) cells lines from a single blastomere<sup>32,33,34,35</sup>.

The pluripotency of human blastomeres taken from developing embryos has been tested - single blastomeres of a 4-cell stage human embryo are able to develop into blastocysts with inner cell mass (cells which give rise to the fetus) and trophectoderm (cells which form the placenta)<sup>36</sup>.

### *Parthenogenetic embryonic stem cells*

Research groups continue to develop pES cell lines<sup>37,38,39,40</sup>. There have been no significant developments with this technique in the last year, although it has been shown that pES cell lines can also be derived from in vitro matured eggs<sup>41,42</sup>. One significant limitation of pES cells is that they only express genes from the mother's chromosomes. Therefore the paternal DNA may be abnormally expressed<sup>43</sup>. However, research shows that not all pES cell lines suffer from this<sup>44</sup>.

### *Embryonic stem cells from poor quality or non-viable embryos*

Poor quality or non-viable embryos can be used to derive ES cells<sup>45</sup>. However, the efficiency of derivation depends on the viability of the embryo, with early-arrested (embryos which stop growing or die) or highly fragmented embryos rarely yielding cell lines<sup>46</sup>.

### *Nuclear transfer into zygotes*

A group from the United States has reported successfully using mouse zygotes (formed immediately after a sperm and egg fuse) as recipients in nuclear transfer as opposed to using unfertilised eggs<sup>47</sup>. The group were then able to derive stem cells from the resulting embryos. They also successfully used trippronucleated zygotes (an abnormally fertilised egg which contains a nucleus from the egg and two nuclei from sperm) as recipients for nuclear transfer using the same technique. Trippronucleated zygotes are automatically discarded from IVF treatment. This technique could therefore potentially derive ES cells without destroying viable embryos.

The group has since investigated into whether cleavage-stage embryos also retain the reprogramming activities illustrated by fertilised zygotes<sup>48</sup>. Using chromosome transplantation techniques and blastomeres of 2-cell stage mouse embryos, the group illustrated that cleavage-stage mouse embryos do also retain reprogramming activities.



# Work carried out during 2009 on issues identified previously

## *Embryonic stem-like cells from other tissues*

Populations of very small ES-like cells have been derived from mouse bone marrow and adult tissues<sup>49</sup>. Some of the cells show characteristics of ES cells, an indication that they may differentiate into all three germ layers (the three layers of cells from which various organs and parts of the body develop)<sup>50</sup>. The cells have also been found to circulate at very low levels in peripheral blood and similar ES-like cells have been found in human umbilical cord blood<sup>50</sup>. Some researchers are transplanting these cells in mice<sup>51</sup>.

A group has derived human adult germline stem cells (the cells which develop into eggs or sperm) from spermatogonial cells of the adult human testis<sup>52</sup>.

## *Update on embryonic stem cells from cloned embryos*

ES cell lines have been produced following somatic cell nuclear transfer (SCNT) into primate eggs (removing the DNA from an egg and injecting the nucleus of a somatic cell)<sup>53</sup> and cloned human blastocysts have been produced following SCNT with adult skin cells<sup>54</sup>. A group has used SCNT to create ES cells to model Parkinson's disease<sup>55</sup>. A variation of the method, removing the nucleus of human eggs, has also been researched<sup>56</sup>.

Research has demonstrated similarities between ES cells derived from cloned and fertilised embryos<sup>57,58</sup>.

## *The HFEA's view and outcomes*

In September 2009 SCAAC reviewed advances in these techniques. They concluded that of all the potential sources of ES or embryonic-like stem cells, research into iPS cells is progressing the most rapidly. For example, there has been research into the similarity of iPS cells to ES cells. The current limitations of these cells include iPS cell differentiation and the risk of tumours developing.

The Committee concluded that existing ES cell lines are required as a comparison to iPS cells. Researchers will still need to develop new hES cells to aid their understanding. The Committee commented that research is being carried out into whether there is a more pluripotent cell state than that exhibited by ES cells.

The degree of similarity between iPS and ES cells was also discussed at the HFEA Horizon Scanning Panel meeting in June 2009. The Panel felt that more research was needed into the risk of tumours once iPS were differentiated, the quality of iPS cells and whether the cells retained any 'memory' of their origin (ie, characteristics relating to the cell type they used to be).

The HFEA's Research Licence Committee considers the latest developments in these techniques, along with the view of the above groups, when deciding whether the creation and use of embryos for proposed research is necessary or desirable.

## Work carried out during 2009 on issues identified previously

### 2.4 *In vitro* derived gametes

#### What is it?

Stem cells have the potential to form different cell types in the body, including sperm and egg cells. Gametes (sperm and eggs) formed in this way are known as *in vitro* derived gametes. These gametes can potentially be produced from a range of cells, including stem cells from bone marrow, embryos and from adult cells (such as skin cells) that have been reprogrammed to behave like ES cells.

#### What impact could it have?

*In vitro* derived gametes could be used in researching germ cell development (cells that give rise to gametes), cell differentiation, meiosis (cell division which reduces the number of chromosomes) and imprinting (a process by which gene expression varies according to maternal or paternal inheritance). *In vitro* derived gametes also have potential clinical applications, such as the treatment of infertility and germline gene therapy. Gene therapy introduces functional genes into germ cells in order to avoid heritable conditions in future generations.

Cloned sperm could potentially be used where a man produces low levels of sperm, or only a single sperm is extracted. This method could be used to propagate this sperm and provide an 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 1990 (as amended) prohibits the use of gametes for treatment that have not been produced or extracted from the ovaries of a woman or the testes of a man.

#### What research has been carried out?

##### *Pluripotent stem cells*

Researchers have demonstrated that it is possible to produce germ cells from ES cells in mice. Male germ cells have subsequently been shown to differentiate into male sperm, which are capable of producing live male offspring<sup>59,60</sup>. Maturing eggs remains more problematic. However, a recent study that transplanted ES cell-derived mouse eggs into an ovarian niche (area of an ovary which maintains stem cells) reported that these eggs then fully matured.<sup>61</sup>

Research in humans has demonstrated that hES cells can differentiate into germ cells. Research at the North-East England Stem Cell Institute in Newcastle, reported the *in vitro* derivation of mature human sperm from such germ cells<sup>62</sup>. However, this publication was subsequently retracted.

Similar work on deriving human gametes *in vitro* from pluripotent stem cells has been carried out by a group at Cambridge University<sup>63</sup>. This group have reported the generation of induced primordial germ cells (iPG cells) from embryonic germ cells and ES cells. These iPG cells entered meiosis and were shown to be similar to primordial germ cells created *in vivo*.

Research into *in vitro* derivation of gametes using pluripotent stem cells has investigated not just the use of ES cells, as described above, but also other stem cells, such as mesenchymal stem cells. Mesenchymal stem cells naturally differentiate into a variety of cell types eg, bone, muscle and fat cells. Mesenchymal stem cells have been re-programmed and differentiated into sperm and egg germ cells in both the mouse<sup>64,65</sup> and the human<sup>66</sup>. A recent report illustrates the potential of these cells to differentiate into human sperm-like cells<sup>67</sup>.

In 2009 the Hinxton Group published a review of research into *in vitro* derived gametes from pluripotent stem cells<sup>68</sup>. The Group estimated that it would be more than ten years before such gametes were likely to be developed. The group suggested that *in vitro* derived gametes would not be available for treatment purposes until several years later.

# Work carried out during 2009 on issues identified previously

## Somatic cells

Research into *in vitro* derived gametes has also looked at using somatic cells, but there is less research in this area compared to the use of pluripotent stem cells. One study has shown that fusing an ES cell with a somatic cell can reprogram the genome into one that allows differentiation into primordial germ cells *in vitro*<sup>69</sup>. These cells can then undergo further differentiation into germ-like cells and could provide personalised stem cells applicable to regenerative medicine and fertility treatment.

In reviewing the technique of somatic cell haploidisation (transformation of a diploid into a haploid cell) Nagy et al (2008)<sup>70</sup> concluded that there were difficulties in ensuring accurate chromosome segregation and preventing epigenetic defects (defects that affect how genes are expressed) in imprinted genes (genes which are expressed differently according to which parent they were inherited from) of the somatic cell nucleus. These need to be overcome before the technique provides a valid method for deriving gametes *in vitro*.



## The HFEA's view and outcomes

In September 2009 SCAAC considered a review of advances in this field and concluded that no published research had convincingly shown that hES cells could be differentiated *in vitro* into mature human sperm. The Committee thought it would take between 5 to 10 years for gametes to be derived entirely *in vitro* and that one of the main barriers was incorrect imprinting. It was suggested that transplanting gamete precursor cells to their normal environment for the later stages of gamete maturation could help, eg, putting sperm precursor cells into a Sertoli cell (cells in the testes) environment or egg precursors into a follicle cell environment.

The HFEA's Horizon Scanning Panel expressed a similar view in June 2009. They felt that there needed to be more animal models, investigations into the meiotic process and long term studies of offspring resulting from *in vitro* derived gametes before the technique could be considered successful or potentially used in treatment.

The HFEA Executive and Licence Committees will refer to relevant information and advice if they receive licence applications from centres wishing to create embryos using *in vitro* gametes, for research<sup>71</sup>.

## Work carried out during 2009 on issues identified previously

### 2.5 Genetic modification of embryos

#### What is it?

It is possible to introduce transgenes (genes transferred from one organism to another) into human embryos in order to create genetically modified embryos for research. This has been achieved using viral vectors which are the most common method of delivering genetic material into cells and genetic modification. Since October 2009 the Human Fertilisation and Embryology Act 1990 (as amended) has allowed the HFEA to issue licences permitting the creation of genetically modified embryos for research purposes. It is not permitted for treatment.

#### What impact could it have?

The technique could be used to generate genetically modified ES cells for studying human embryogenesis (embryo formation and development) and human disease. Investigating gene function in early

embryogenesis could lead to more objective criteria for selecting embryos for use in fertility treatment. The technique could also be used to increase the efficiency of stem cell derivation.

#### What research has been carried out?

No significant new research has been identified since it was considered by SCAAC and the Horizon Scanning Panel in 2008 (as summarised in the 2008/9 HFEA Horizon Scanning report).

#### The HFEA's views and outcomes

Current research into genetic modification of embryos and the views of HFEA committees have been summarised and provided to the HFEA's Research Licence Committee. This ensures the Committee is sufficiently informed ahead of any relevant licence applications. However, to date, the Authority has not received any applications for this type of research.



# Work carried out during 2009 on issues identified previously

## 2.6 Embryo culture media

### What is it?

Embryo culture medium is the solution used to support the development of embryos (in a petri dish) before they are transferred to the womb. In the past, UK centres made their own embryo culture media from basic components. Now the majority, if not all, centres use commercially manufactured culture media.

Many different culture media, with different formulations, have been used in the past and this has led some clinicians to believe that the choice of culture media for cleavage-stage embryos (early embryos – up to about 5 days) is not important. However, this is challenged by recent studies and increased knowledge about the biochemistry, genetics and epigenetic control of the human embryos.

### What impact could it have?

Components of commercially available culture media are not novel agents, therefore media are not generally patentable and manufacturers do not have to disclose the details of composition. There is therefore a possible impact on assessing the success and safety of using a certain medium.

### What research has been carried out?

#### *Evidence that the early embryo is affected by its environment*

There is significant evidence that early embryos are very sensitive to their environment, both *in vivo* and *in vitro*<sup>72</sup>. Animal models show that exposure of embryos to less than optimum culture conditions has significant effects on post implantation development and fetal growth following transfer<sup>73,74,75</sup>. An embryo's environment is also shown to effect gene expression in the blastocyst<sup>79</sup>.

A simple way of demonstrating that the culture medium in which an embryo is grown can have dramatic effects on development is to observe the affect of adding a component to the medium. The addition of serum to culture media increases the speed of development – blastocysts appear earlier than normal<sup>78</sup>.

### *Possible negative effects of culture media components*

Glucose, amino acids, ammonium, chelators (molecules which can bind tightly with metal ions eg, EDTA), macromolecules and growth factors are possible components of embryo culture media.

Potential negative effects of these components are:

- A high level of glucose in media containing phosphate and lacking amino acids increases the risk of retardation or developmental arrest of cleavage-stage embryos<sup>79,80,81,82,83</sup>.
- Amino acids in culture media break down to produce ammonium. Animal model studies show that the presence of ammonium results in a significant reduction in the ability of embryos to implant and significant fetal loss once pregnancy is established. Moderate levels of ammonium are shown to affect fetal growth rates resulting in smaller fetuses. One study has found that development of human blastocysts is inhibited by the ammonium levels produced whilst culturing embryos in medium containing glutamine<sup>84</sup>.
- It is well established that EDTA has an important role in the development of cleavage-stage embryos<sup>85</sup>, but it has a negative effect on later stages as its presence inhibits the development of the inner cell mass.
- There is very little data on how growth factors and cytokines (small proteins secreted by cells of the immune system) affect human embryos and gene expression studies suggest that their effects are not necessarily as anticipated<sup>86</sup>.

Growth factors can affect embryo metabolism, cell differentiation and apoptosis (cell death). There is a risk that adding growth factors to culture media may lead to an increase in the number of abnormal cells and embryos, because damaged embryos, which would otherwise arrest, may be rescued<sup>87</sup>. It is possible that growth factors may block the action of p53, the tumour suppressor protein responsible for triggering DNA repair or apoptosis in the event of errors occurring during cell division.

## Work carried out during 2009 on issues identified previously



### *Potential negative effects of culture media on health outcomes*

There is evidence for an increased risk of imprinting disorders (disorders caused by incorrect gene expression) in children conceived by IVF/ICSI eg, Beckwith-Weidemann, Prader-Willi and Angelman syndromes. Also, domestic animal studies reveal that embryos created *in vitro* are at risk of large offspring syndrome. This involves abnormal embryo growth and development at fetal, neonatal and later stages after transfer of embryos, cultured *in vitro* for up to a week after fertilisation<sup>88</sup>. It is possible that embryo culture conditions may be responsible for the increased risk of these disorders and there is a small amount of evidence to suggest this.

For example, there is evidence that expression of imprinted genes (genes that are expressed differently according to whether they were inherited from the mother or father) is sensitive to embryo culture conditions. Genes involved in particular processes (eg, protein synthesis) have been shown to become down regulated after culture in particular media (eg, Whitten's medium)<sup>89</sup> or expressed when they naturally would not be<sup>90</sup>. Therefore, there is a risk that a component of culture media could be causing inappropriate expression or silencing of genes via some epigenetic mechanism eg, DNA methylation (modification of the structure of DNA, which affects gene expression).

# Work carried out during 2009 on issues identified previously

The postnatal growth and physiological activity of mouse embryos, which were cultured *in vitro*, have been compared to embryos developing *in vivo*. Mouse embryo culture was found to induce physical postnatal changes including raised blood pressure<sup>91</sup>. Mouse embryos which developed *in vitro* had a reduced number of cells in the trophectoderm (the outer layer of cells in an embryo which result in the placenta) and inner cell mass (cells which give rise to the fetus) compared to embryos *in vivo*. Embryo culture was shown to have minimal effects on postnatal growth. However, mouse embryo culture was found to lead to enhanced systolic blood pressure at 21 weeks, plus other changes linked to cardiovascular and metabolic physiology.

A Dutch group investigated several cardiometabolic measures in 255 children born after IVF compared to a control group of spontaneously conceived children from subfertile parents (matched for age and sex)<sup>92</sup>. Blood pressure, skin-fold thickness (used to determine amount of body fat) and fasting glucose levels were all significantly higher in children conceived following IVF than in controls. The authors concluded that the periconceptual period of IVF-conceived children might contain a critical time window during which the cardiometabolic function could be perturbed. These findings highlight the importance of continued monitoring of the postnatal development of children born after IVF.

### The HFEA's view and outcomes

During meetings in 2008 and 2009 SCAAC and the Horizon Scanning Panel expressed concerns over the lack of published information regarding culture media constituents and the unknown long term effects of culture media manipulation on developing embryos.

In September 2009 SCAAC discussed research regarding culture media components and their potential effects. It agreed that research suggested culture media components might affect the safety and development potential of embryos and possibly the long term health of the children. In particular, the Committee expressed concern about the addition of growth factors to embryo culture media.

SCAAC concluded that the long-term effects of *in vitro* embryo culture and media components on children born as a result of ART should be studied.

The HFEA Executive have clarified that, following implementation of the European Cosmetics Directive (76/768/EEC), culture media is now classed as a Class III Medical Device under the remit of the Medicine and Healthcare products Regulatory Agency (MHRA). Therefore, commercially available media should be CE marked and produced to Good Manufacturing Practice standards.

The HFEA Executive has raised the concerns expressed by SCAAC and the Horizon Scanning Panel with the Department of Health and the MHRA. The HFEA Executive continues to be in discussion with these groups and to explore effective methods of ensuring the safety of culture media used in IVF.

## Issues identified in the 2009/10 horizon scanning process

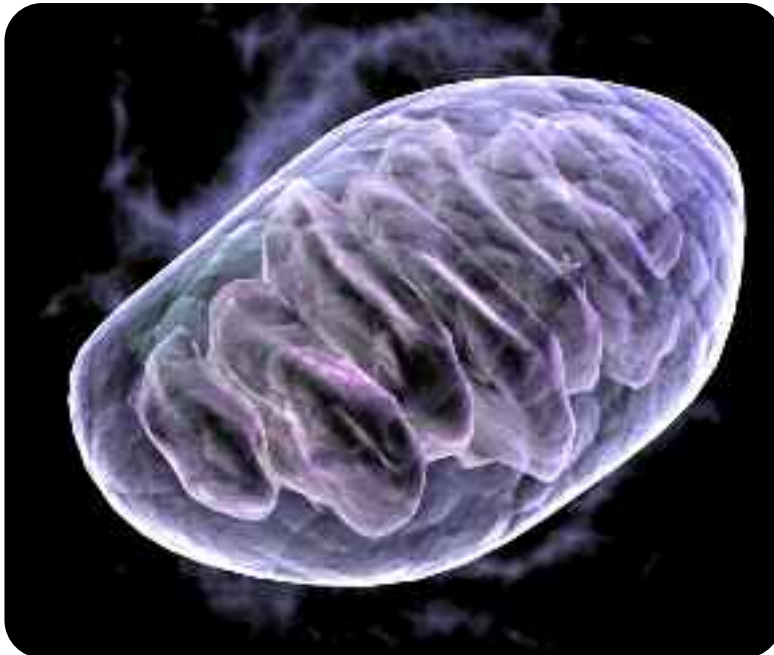
The following table presents issues identified through the 2009/10 horizon scanning process, including research published up to December 2009. Issues are prioritised using a systematic approach which looks at whether:

- 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.

SCAAC will consider the following high priority issues in depth throughout 2010:

- safety of embryo biopsy (particularly polar body and blastomere biopsy),
- the effectiveness and safety of different methods for fertility preservation (eg, freezing and vitrification of gametes or ovarian and testicular tissue),
- *in vitro* derived gametes,
- alternative methods of obtaining embryonic or embryonic-like stem cells,
- regulation of embryo culture media,
- methods to avoid mitochondrial disease transmission, and
- health outcomes of children conceived using ART.

Lower priority issues will not be followed up in detail but have been provided in the table for information.



# Issues identified in the 2009/10 horizon scanning process

Cryopreservation		
Use of aim	Description	Reference
<b>Embryo cryopreservation protocols (relevant to single embryo transfer)</b>	<p>The effects of multiple rounds of embryo cryopreservation on subsequent pregnancy and implantation rates.</p> <p>The comparison of treatment outcomes using vitrified and fresh embryos eg, monozygotic twinning, congenital defects and neonatal complications.</p> <p>The comparison of fresh and vitrified embryo recovery and survival.</p>	<p>Kumasako Y et al. (2009) The efficacy of the transfer of twice frozen-thawed embryos with the vitrification method. <i>Fertility and Sterility</i> 91(2):383-386.</p> <p>Mukaida T et al. (2009) Perinatal outcome of vitrified human blastocysts in 9 year experience (3601 attempted cycles) including the incidence rate of monozygotic twinning. <i>Hum Rep</i> 24 suppl 1:i28-i31.</p> <p>Nagy Z et al. (2009) Obstetrical and prenatal outcome of pregnancies following vitrification in comparison to slow freezing of human embryos. <i>Hum Rep</i> 24 suppl 1:i74-i76.</p> <p>Ciotti P et al. (2009) Meiotic spindle recovery is faster in vitrification of human oocytes compared to slow freezing. <i>Fertility and Sterility</i> 91(6):2399-2400.</p> <p>Lin PY et al. (2009) Comparison of the offspring sex ratio between fresh and vitrification-thawed blastocyst transfer. <i>Fertility and Sterility</i> 92(5):1764-1766.</p>
<b>Cryopreservation of eggs</b>	<p>The effect of time between egg retrieval and cryopreservation on embryo quality, pregnancy and implantation rates.</p> <p>Use of vitrification to improve egg survival rate, fertilisation, and embryo development.</p> <p>The effect of short versus long term storage of eggs in relation to egg survival post thawing, fertilisation, cleavage, embryo quality and development, implantation and birth.</p> <p>Evidence-based clinical and laboratory guidelines on the effectiveness and safety of egg cryopreservation by slow freezing and vitrification.</p> <p>Collection and cryopreservation of eggs from young patients (5-10 years).</p>	<p>Parmegiani F et al. (2009) Efficiency of human oocyte slow freezing: results from five assisted reproduction centers. <i>RBMO</i> 18(3):352-358.</p> <p>Cao YX et al. (2009) Comparison of survival and embryonic development in human oocytes cryopreserved by slow-freezing and vitrification. <i>Fertility and Sterility</i> 92(4):1306-1311.</p> <p>Keskintepe L et al. (2009) High survival rate of metaphase II human oocytes after first polar body biopsy and vitrification: determining the effect of pre-vitrification conditions. <i>Fertility and Sterility</i> 92(5):1706-1715.</p> <p>Son WY et al. (2009) Comparison of survival rate of cleavage stage embryos produced from in vitro maturation cycles after slow freezing and after vitrification. <i>Fertility and Sterility</i> 92(3):956-958.</p> <p>Parmegiani L et al. (2009) Long-term cryostorage does not adversely affect the outcome of oocyte thawing cycles. <i>RBMO</i> 19(3):374-379.</p> <p>Cutting R et al. (2009) Human oocyte cryopreservation: Evidence for practice. <i>Human Fertility</i> 3:125-126.</p> <p>Revel A et al. (2009) At what age can human oocytes be obtained? <i>Fertility and Sterility</i> 92(2):458-463.</p>

# Issues identified in the 2009/10 horizon scanning process

Use of aim	Description	Reference
<b>Cryopreservation of sperm</b>	Freezing sperm in small numbers.  The effect of cryopreservation of sperm within an empty zona pellucida on sperm recovery rate and the post-thaw sperm functions.	Hafez F et al. (2009) Techniques for cryopreservation of individual or small numbers of human spermatozoa: a systematic review. <i>Hum Rep Update</i> 15(2):153-164. Yinghui Y (2009) Evaluation of human sperm function after being cryopreserved within the zona pellucida. <i>Fertility and Sterility</i> 92(3):1002-1008.
<b>Cryopreservation of ovarian tissue</b>	A comparison of conventional freezing and vitrification of human ovarian tissue.	Isachenko V et al. (2009) Human ovarian tissue vitrification versus conventional freezing: morphological, endocrinological, and molecular biological evaluation. <i>Reproduction</i> 138:319-327.
<b>Optimising embryo culture medium to improve live birth rates</b>	The effects of embryo culture environment manipulation including: - free oxygen radicals and incubator oxygen levels - serum substitute supplement added to commercial human serum albumin-supplemented embryo culture media - follicular fluid supplements	Meintjes M et al. (2009) A controlled randomised trial evaluating the effect of lowered incubator oxygen tension on live births in a predominately blastocyst transfer program. <i>Hum Rep</i> 24(2):300-307. Meintjes M et al. (2009) A randomised controlled study of human serum albumin and serum substitute supplement as protein supplements for IVF culture and the effect on live birth rates. <i>Hum Rep</i> 24(4):782-789. Otsuki J et al. (2009) Redox state of albumin and patient follicular fluid used as an alternative culture supplement to replace commercial human serum albumin. <i>Hum Rep</i> 24 suppl 1:i105-i107. Otsuki J et al. (2009) Damage of embryo development caused by peroxidised mineral oil and its association with albumin in culture. <i>Fertility and Sterility</i> 91(5):1745-1749.
<b>In vivo culture system for embryos</b>	Novel in utero culture system for embryo development.	Blockeel C et al. (2009) An in vivo culture system for human embryos using an encapsulation technology: a pilot study. <i>Hum Rep</i> 24(4):790-796.
<b>Increasing the number of transferable embryos</b>	Removal of a pronucleus from tripronuclear human eggs to restore diploidy.	Rosenbusch B (2009). Selective microsurgical removal of a pronucleus from tripronuclear human oocytes to restore diploidy: disregarded but valuable? <i>Fertility and Sterility</i> 92(3):897-903.
<b>Blastocyst transfer</b>	Blastocyst transfer and the incidence of monozygotic twinning.	Goncalves S et al. (2009) Blastocyst transfer may increase the incidence of monozygotic twinning. <i>Fertility and Sterility</i> 92(3) suppl:S23.

# Issues identified in the 2009/10 horizon scanning process

## Embryo culture, manipulation and selection (continued)

Use of aim	Description	Reference
<b>Blastomere biopsy</b>	A number of studies on the impact of blastomere biopsy. For example, a mouse model studying risk of neurodegenerative disorders, live birth rates following 1-cell biopsy compared to 2-cell biopsy of human embryos and another mouse study of time needed to develop following blastomere biopsy.	<p>Desmyttere S et al. (2009) Two year auxological and medical outcome of singletons born after embryo biopsy applied in preimplantation genetic diagnosis or preimplantation genetic screening. <i>Hum Rep</i> 24(2):470-476.</p> <p>De Vos A et al. (2009) Impact of cleavage-stage embryo biopsy in view of PGD on human blastocyst implantation: a prospective cohort of single embryo transfers. <i>Hum Rep</i> 24(12):2988-2996.</p> <p>Terada Y et al. (2009) Different embryonic development after blastomere biopsy for preimplantation genetic diagnosis, observed by time-lapse imaging. <i>Fertility and Sterility</i> 92(4):1470-1471.</p> <p>Wang WH et al. (2009) Comparison of development and implantation of human embryos biopsied with two different methods: aspiration and displacement. <i>Fertility and Sterility</i> 92(2):536-540.</p> <p>Yu Y et al. (2009) Evaluation of blastomere biopsy using a mouse model indicates the potential high risk of neurodegenerative disorders in the offspring. <i>Mol and Cell Prot</i> 8(7):1490-1500.</p>
<b>Metabolomics (non-invasive embryo selection)</b>	A number of reported developments in the field of metabolomics. For example, metabolomics has been used to predict the implantation potential of embryos (leading to the development of a viability score, which is useful when used in conjunction with morphological assessment), amino acid profiling of single embryos can be used as a non-invasive marker of DNA damage at blastocyst stage and profiling of used egg culture media can predict embryo development.	<p>Gardner D et al. (2009) Viable human blastocysts exhibit a different amino acid utilisation profile than those which fail to implant. <i>Hum Rep</i> 24 suppl 1:i46.</p> <p>Gluschenko K et al. (2009) Evaluation of metabolomic-profiling as a tool for embryo selection in single embryo transfer (SET). <i>Hum Rep</i> 24 suppl 1:i17-i18.</p> <p>Hardarson T et al. (2009) Non-invasive metabolomic profiling of day 5 embryo culture media: blinded cross clinic prediction of embryo viability using near infrared spectroscopy. <i>Hum Rep</i> 24 suppl 1:i46-i48.</p> <p>Nagy Z et al. (2009) Metabolic assessment of oocyte viability. <i>RBMO</i> 18(2):219-255.</p> <p>Sturmey R et al. (2009) DNA damage and metabolomic activity in the preimplantation embryo. <i>Hum Rep</i> 24(1):81-91.</p>
<b>Assessment of developmental capacity</b>	Use of live-cell imaging technology to determine the developmental capacity of mouse embryos by analysing the chromosomal dynamics during the first meiotic division.	<p>Yamagata K et al. (2009) Assessment of chromosomal integrity using a novel live-cell imaging technique in mouse embryos produced by intracytoplasmic sperm injection. <i>Hum Rep</i> 24(10):2490-2499.</p>

# Issues identified in the 2009/10 horizon scanning process

Gamete selection and manipulation		
Use of aim	Description	Reference
<b>Sperm quality assessment</b>	Methods for assessing sperm quality including sperm morphology assessment and automated sperm scoring (identifying sperm aneuploidy).	<p>Cassuto N et al. (2009) A new real-time morphology classification for human spermatozoa: a link for fertilization and improved embryo quality. <i>Fertility and Sterility</i> 92(5):1616-1625.</p> <p>Cohen-Bacrie P et al. (2009) Correlation between DNA damage and sperm parameters: a prospective study of 1633 patients. <i>Fertility and Sterility</i> 91(5):1801-1805.</p> <p>Enciso M et al. (2009) A two-tailed Comet assay for assessing DNA damage in spermatozoa. <i>RBMO</i> 18(5):609-616.</p> <p>Kheirollahi-Kouhestani M et al. (2009) Selection of sperm based on combined density gradient and Zeta method may improve ICSI outcome. <i>Hum Rep</i> 24(10):2409-2416.</p> <p>Tunc O et al. (2009) Improvement in sperm DNA quality using an oral antioxidant therapy. <i>RBMO</i> 18(6):761-786.</p> <p>Tempest HG et al. (2009) Should we routinely test for sperm aneuploidy prior to ICSI? <i>Hum Rep</i> 24 suppl 1:i236-i256.</p>
<b>Non-invasive egg selection</b>	Use of non-invasive markers in human eggs (eg, cumulus genes expression and follicular fluid) to predict the likelihood of embryos implanting and pregnancy outcome.	<p>Anderson RA et al. (2009) Cumulus gene expression as a predictor of human oocyte fertilisation, embryo development and competence to establish a pregnancy. <i>Reproduction</i> 138:629-637.</p> <p>Fragouli E (2009) Novel targets for oocyte quality assessment revealed by transcriptome profiling. <i>Hum Rep</i> 24 suppl 1:i236-i256.</p> <p>Kilani S (2009) Are there non-invasive markers in human oocytes that can predict pregnancy outcome? <i>RBMO</i> 18(5):674-680.</p> <p>Osipova A et al. (2009) The value of follicular fluid G-CSF as a biomarker of embryo implantation potential in monofollicular IVF cycle. <i>Hum Rep</i> 24 suppl 1:i236-i256.</p> <p>Piccinni MP et al. (2009) Appropriate methodology for the measurement of follicular fluid G-CSF, which is a non invasive and prospective marker for successful implantation. <i>Hum Rep</i> 24 suppl 1:i236-i256.</p>

# Issues identified in the 2009/10 horizon scanning process

## Gamete selection and manipulation (continued)

Use of aim	Description	Reference
<b>Techniques to avoid the transmission of mitochondrial disease</b>	<p>Mitochondrial genome replacement in eggs – studies in non-primates using a technique called spindle- chromosomal complex transfer.</p> <p>Metaphase II karyotype transfer.</p> <p>Ooplasm donation – studies using ICSI and its effect on the function of the paternal genome.</p>	<p>Tachibana M et al. (2009) Mitochondrial gene replacement in primate offspring and embryonic stem cells. <i>Nature</i> 461:367-372.</p> <p>Tanaka A et al. (2009) Metaphase II karyoplast transfer from human in-vitro mature oocytes to enucleated mature oocytes. <i>RBMO</i> 19(4):514-520.</p> <p>Liang CG et al. (2009) Effects of ooplasm transfer on paternal genome function in mice. <i>Hum Rep</i> 24(11):2718-2728.</p>
<b>Egg selection</b>	Factors determining the size and quality of ovarian reserve.	Hartshorne GM et al. (2009) Oogenesis and cell death in human prenatal ovaries: what are the criteria for oocyte selection? <i>Molecular Hum Rep</i> 2009 15(12):805-819.
<b>Sperm quality</b>	The effect of sperm incubation and freezing on genome integrity.	Feliciano M et al. (2009) Environmental factors on sperm genomic integrity. <i>Hum Rep</i> 24 suppl 1:i236-i256.
<b>In vitro maturation (IVM) of eggs</b>	The effectiveness of and advances in the technique and application of IVM. For example, the use of IVM following retrieval of eggs from patients about to undergo chemotherapy and use of artificial constructs to aid maturation.	<p>Albuz FK et al. (2009) Substantial improvements in embryos yield using a novel system of induced IVM by exploiting cAMP modulators in pre-IVM and IVM. <i>Hum Rep</i> 24 suppl 1:i236-i256.</p> <p>Amorim C et al. (2009) Survival of human pre-antral follicle after cryopreservation of ovarian tissue, follicular isolation and in vitro culture in calcium alginate matrix. <i>Hum Rep</i> 24(1):92-99.</p> <p>Cao Y et al. (2009) Cryopreservation of immature and in-vitro matured human oocytes by vitrification. <i>RBMO</i> 19(3):369-373.</p> <p>Chian RC et al. (2009) Obstetric outcomes following vitrification of in vitro and in vivo matured oocytes. <i>Fertility and Sterility</i> 91(6):2391-2398.</p> <p>Chian RC et al. (2009) Live birth after vitrification of in vitro matured human oocytes. <i>Fertility and Sterility</i> 91(2):372-376.</p> <p>Epic J et al. (2009) Effect of in vitro maturation of mouse oocytes on the health and lifespan of adult offspring. <i>Hum Rep</i> 24(4):922-928.</p> <p>Kim IW et al. (2009) Derivation of developmentally competent oocytes by the culture of preantral follicles retrieved from adult ovaries: maturation, blastocyst formation, and embryonic stem cell transformation. <i>Fertility and Sterility</i> 92(5):1716-1724.</p>

## Issues identified in the 2009/10 horizon scanning process

### Gamete selection and manipulation (continued)

Use of aim	Description	Reference
In vitro maturation (IVM) of eggs (continued)		<p>Lim J-H et al. (2009) Selection of patients for natural cycle in vitro fertilization combined with in vitro maturation of immature oocytes. <i>Fertility and Sterility</i> 91(4):1050-1055.</p> <p>Lin YH et al. (2009) Effects of growth factors and granulosa cell co-culture on in vitro maturation of oocytes. <i>RBMO</i> 19(2):165-170.</p> <p>Maman E et al. (2009) Oocyte retrieval and in vitro maturation during the leuteal phase is an optional procedure for fertility preservation. <i>Hum Rep</i> 24 suppl 1:i236-i256.</p> <p>Motluk A (2009) Artificial ovary matures human eggs. <i>New Scientist</i> 2707:8.</p> <p>Requena A. et al. (2009) The impact of in-vitro maturation of oocytes on aneuploidy rate. <i>RMBO</i> 18(6):777-783.</p> <p>Xu M. et al. (2009) In vitro grown human ovarian follicles from cancer patients support oocyte growth. <i>Hum Rep</i> 24(10):2531-2540.</p> <p>Zech N et al. (2009) 166 In vitro maturation (IVM) of oocytes: replacement of developing embryos in a fresh or vitrified cycle? <i>Hum Rep</i> 24 suppl 1:i66-i68.</p>

### Gene transfer

Use of aim	Description	Reference
Gene transfer to increase the quality of sperm	Gene transfer into mouse epithelial cells and its effect on the fertilising capacity of sperm.	Esponda P and Carballada R (2009) In vivo gene transfer induces transgene expressing cells and secretions of the mouse cauda epididymis. <i>Molecular Hum Rep</i> 15(6):355-361.

# Issues identified in the 2009/10 horizon scanning process

## Genetic screening

Use of aim	Description	Reference
<b>Prenatal testing following preimplantation genetic diagnosis (PGD)</b>	Testing of cell-free fetal nucleic acids in maternal blood is a non-invasive method for prenatal diagnosis, which can be used after PGD.	Li Y et al. (2009) Non-invasive prenatal diagnosis using cell-free fetal DNA in maternal plasma from PGD pregnancies. <i>RBMO</i> 19(5):714-720. Wright C and Burton H (2009) the use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis. <i>Hum Rep Update</i> 15(1):139-15.
<b>Preimplantation genetic screening (PGS)</b>	<p>A number of studies on PGS have focused on the following:</p> <ul style="list-style-type: none"> <li>the genome of a single blastomere is not representative of the genome of the other cells of the embryo,</li> <li>comparative genome hybridisation increases implantation and birth rate and decreases rate of miscarriage,</li> <li>24-chromosome aneuploidy screening,</li> <li>self-correction of aneuploid and mosaic embryos during development toward the blastocyst stage,</li> <li>the effect of PGS on pregnancy outcome for women with recurrent pregnancy loss,</li> <li>the effect of PGS on the outcome for patients of advanced maternal age,</li> <li>embryo implantation, live birth and multiple rates in women undergoing PGS,</li> <li>PGS when recurrent pregnancy loss is associated with miscarriages, and</li> <li>the effect of changing the method for embryo biopsy and the culture system on clinical outcome.</li> </ul>	<p>Barbash-Hazan S et al. (2009) Preimplantation aneuploid embryos undergo self-correction in correlation with their developmental potential. <i>Fertility and Sterility</i> 92(3):890-896.</p> <p>Beyer C et al. (2009) Preimplantation genetic screening outcomes are associated with culture conditions. <i>Hum Rep</i> 24(5):1212-1220.</p> <p>Fragouli E et al. (2009) Comparative genomic hybridisation of oocytes and first polar bodies from young donors. <i>RBMO</i> 19(2):28-237.</p> <p>Garrisi J et al. (2009) Effect of infertility, maternal age, and number of previous miscarriages on the outcome of preimplantation genetic diagnosis for idiopathic recurrent pregnancy loss. <i>Fertility and Sterility</i> 92(1):288-295.</p> <p>Meyer L et al. (2009) A prospective randomised controlled trial of preimplantation genetic screening in the 'good prognosis' patient. <i>Fertility and Sterility</i> 91(5):1731-1738.</p> <p>Rubio C (2009) Prognostic factors for preimplantation genetic screening in repeated pregnancy loss. <i>RBMO</i> 18(5):687-693.</p> <p>Schoolcraft W et al. (2009) Preimplantation aneuploidy testing for infertile patients of advanced maternal age: a randomized prospective trial. <i>Fertility and Sterility</i> 92(1):157-162.</p> <p>Sher G et al. (2009) Genetic Analysis of human embryos by metaphase comparative genomic hybridisation (mCGH) improves rate and reducing multiple pregnancies and spontaneous miscarriages. <i>Fertility and Sterility</i> 92(6):1886-1894.</p> <p>Treff N et al. (2009) First IVF babies born after rapid 24 chromosome embryo aneuploidy screening and fresh embryo transfer. <i>Fertility and Sterility</i> 92(3) suppl:S49.</p> <p>Treff N et al. (2009) Four hour chromosome aneuploidy screening using high throughput PCR SNP allele ratio analyses. <i>Fertility and Sterility</i> 92(3) suppl:S49.</p> <p>Vanneste E et al. (2009) What next for preimplantation genetic screening? High mitotic chromosome instability rate provides the biological basis for the low success rate. <i>Hum Rep</i> 24(11):2679-2682.</p>

## Issues identified in the 2009/10 horizon scanning process

Genetic screening (continued)		
Use of aim	Description	Reference
<b>Assessing PGD outcomes</b>	The incidence of monozygotic twinning following PGD compared with regular ICSI and blastocyst transfer.	Verpoest W et al. (2009) The incidence of monozygotic twinning following PGD is not increased. <i>Hum Rep</i> 24(11):2945-2950.
<b>Chromosomal abnormalities and mosaicism in embryos – explanation for some cases of infertility</b>	The cause of uniform aneuploidies and mosaic aneuploidies.	Chatzimeletiou K et al. (2009) The mechanism leading to chromosomal abnormalities and mosaicism in the human embryo as revealed by cytoskeletal and molecular cytogenetic analysis. <i>Hum Rep</i> 24 suppl 1:i5.
<b>New method for PGD</b>	<p>Karyomapping identifies the parental and grandparental origin of each chromosome or chromosome segment present which means, for PGD, there is no requirement to develop a patient or disease specific test for any single gene defect within the regions of the genome covered by the single nucleotide polymorphisms used.</p> <p>Karyomapping can provide accurate linkage analysis for cystic fibrosis combined with detection of aneuploidy. Embryos could be vitrified at the blastocyst stage, after biopsy, and the embryos could be analysed for virtually any genetic disease and screened for aneuploidy of all 24 chromosomes simultaneously.</p>	Harton G et al. (2009) Genome-wide karyomapping for PGD of cystic fibrosis combines accurate linkage based testing with 24 chromosome aneuploidy screening. <i>Hum Rep</i> 24 suppl 1:i52.

# Issues identified in the 2009/10 horizon scanning process

## Health outcomes for ART children

Use of aim	Description	Reference
Follow up studies	<p>Studies of the possible association between ART and an increased risk of birth defects, low birth weight and imprinting disorders.</p> <p>Studies of the neuromotor development and mental health of children born as a result of IVF.</p>	<p>Bonduelle M (2009) 205 Long-term development of children born after ART. <i>Hum Rep</i> 24:suppl 1:i83.</p> <p>Ceelan M et al. (2009) Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8-18 years of IVF children and spontaneously conceived controls born to sub-fertile patients. <i>Hum Rep</i> 24(11):2788-2795.</p> <p>El-Chaar D et al. (2009) Risk of birth defects increased in pregnancies conceived by assisted human reproduction. <i>Fertility and Sterility</i> 92(5):1557-1561.</p> <p>Epic J et al. (2009) Effect of in vitro maturation of mouse oocytes on the health and lifespan of adult offspring. <i>Hum Rep</i> 24(4):922-928.</p> <p>Desmyttere S et al. (2009) Two year auxological and medical outcome of singletons born after embryo biopsy applied in preimplantation genetic diagnosis or preimplantation genetic screening. <i>Hum Rep</i> 24(2):470-476.</p> <p>Golombok S et al. (2009) Parent-child relationships and the psychological well-being of 18-year-old adolescents conceived by in vitro fertilisation. <i>Human Fertility</i> 12(2):63-72.</p> <p>Hansen M et al. (2009) Twins born following assisted reproductive technology: perinatal outcome and admission to hospital. <i>Hum Rep</i> 24(9):2321-2331.</p> <p>Jungheim E et al. (2009) Fetal size and assisted reproduction: is the problem really with the technology? <i>Fertility and Sterility</i> 92(3) suppl:S7.</p> <p>Katari S et al. (2009) DNA methylation and gene expression differences in children conceived in vitro or in vivo. <i>Hum Mol Gen</i> 18(20):3769-3778.</p> <p>Lim D et al. (2009) Clinical and molecular genetic features of Beckwith-Weidemann syndrome associated with assisted reproductive technologies. <i>Hum Rep</i> 24(3):741-747.</p> <p>Ludwig A et al. (2009) Can we sense ART? The blinded examiner is not blind—a problem with follow-up studies on children born after assisted reproduction. <i>Fertility and Sterility</i> 92(3):950-952.</p> <p>Ludwig A et al. (2009) Physical health at 5.5 years of age of term-born singletons after intracytoplasmic sperm injection: results of a prospective, controlled, single-blinded study. <i>Fertility and Sterility</i> 91(1):115-124.</p> <p>Ludwig A et al. (2009). Neuromotor development and mental health at 5.5 years of age of singletons born at term after intracytoplasmic sperm injection ICSI: results of a prospective controlled single-blinded study in German. <i>Fertility and Sterility</i> 91(1):125-132.</p>

## Issues identified in the 2009/10 horizon scanning process

Health outcomes for ART children (continued)		
Use of aim	Description	Reference
Follow up studies (continued)		<p>Macaluso M et al. (2009) 172 The increased risk of low birth weight among infants conceived using ART in the United States is largely associated with multiple embryo transfer. <i>Hum Rep 24</i>: suppl 1:i69.</p> <p>Manipalviratn S et al. (2009) Imprinting disorders and assisted reproductive technology. <i>Fertility and Sterility</i> 91(2):305-315.</p> <p>Marees T et al. (2009) Incidence of retinoblastoma in Dutch children conceived by IVF: an expanded study. <i>Hum Rep 24</i>(12):3220-3224.</p> <p>Neri Q et al. (2009) Neonatal outcome of ICSI offspring generated through embryo cryopreservation. <i>Hum Rep 24</i>: suppl 1:i24.</p> <p>Noyes N (2009) Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. <i>RBMO</i> 18(6):769-776.</p> <p>Raju G et al. (2009) Neonatal outcome after vitrified day 3 embryo transfers: a preliminary study. <i>Fertility and Sterility</i> 92(1):143-148.</p> <p>Reefuis J et al. (2009) Assisted reproductive technology and major structural birth defects in the United States. <i>Hum Rep 24</i>(2):360-366.</p> <p>Sazhenova E and Lebedev I (2009) Loss of methylation of imprinted genes in first trimester spontaneous abortions: implication of estimation of epigenetic risks related to ART. <i>Hum Rep 24</i> suppl 1:i6.</p> <p>Steel A and Sutcliffe A (2009) Long-term health implications for children conceived by IVF/ICSI. <i>Human Fertility</i> 12(1):21-27.</p> <p>Stomberg B et al. (2009) Neurological sequel in children born after in-vitro fertilisation: a population-based study. <i>The Lancet</i> 359(9305):461-465.</p> <p>Wagenaar K et al. (2009) Behavior and socio-emotional functioning in 9-18 year old children born after in vitro fertilisation. <i>Fertility and Sterility</i> 92(6):1907-1914.</p> <p>Wennerholm UB et al. (2009) Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. <i>Hum Rep 24</i>(9):2158-2172.</p> <p>Wikland M et al. (2009) Perinatal outcome of children born after transfer of vitrified blastocysts. <i>Hum Rep 24</i>: suppl 1:i24-i25.</p> <p>Wisborg K et al. (2009) IVF and stillbirth: a prospective follow-up study. <i>Hum Rep 25</i>(5):1312-1316.</p> <p>Woldringh G et al. (2009) Constitutional DNA copy number changes in ICSI children. <i>Hum Rep 24</i>(1):233-240.</p> <p>Wright K et al. (2009) Widespread genomic DNA methylation changes are not apparent at the blastocyst stage in ART versus normally conceived mice. <i>Fertility and Sterility</i> 92(3) suppl:S25-S26.</p>

# Issues identified in the 2009/10 horizon scanning process

IVF and ICSI		
Use of aim	Description	Reference
<b>Improving success of ICSI</b>	The effect of egg electroactivation after ICSI on the fertilisation rate in cases of severe oligoasthenoteratospermia and nonobstructive azoospermia.	Mansour R et al. (2009) Electrical activation of oocytes after intracytoplasmic sperm injection: a controlled randomized study. <i>Fertility and Sterility</i> 91(1):133-139. Montag M et al. (2009) ART, laboratory: ICSI, MESA, TESE. <i>Hum Rep</i> 24 suppl 1:i70.
<b>Predicting likelihood of pregnancy</b>	Analysis of gene expression in women before undergoing IVF as a method of determining the likelihood of pregnancy.	Allen et al. (2009) Gene expression profiles during in vitro fertilization treatment describe the functional transcriptome of the peri-conceptual period. <i>Hum Rep</i> 24 suppl 1: i108-109.
<b>Mild-stimulation in IVF treatment cycles</b>	<p>Mild-stimulation protocols use smaller doses or alternative hormones to those used in standard IVF to stimulate a lower rate of follicle development. They may reduce the incidence of OHSS, treatment cost and drop-out rate, potentially provide a better rate of good quality embryos for transfer and help reduce multiple births.</p> <p>Clinical trials using mild-stimulation protocols study the potential benefit to patients and effect on pregnancy rate per cycle.</p>	<p>Altmae S et al. (2009) Aromatase gene (CYP19A1) variants, female infertility and ovarian stimulation outcome: a preliminary report <i>RBMO</i> 18(5):651-657.</p> <p>Brodin T et al. (2009) High basal LH levels in combination with low basal FSH levels are associated with high success rates at assisted reproduction. <i>Hum rep</i> 24(11):2755-2759.</p> <p>Collins J (2009) Mild stimulation of IVF: making progress downward. <i>Hum Rep Update</i> 15(1):1-3.</p> <p>Dickey R (2009) Strategies to reduce multiple pregnancies due to ovulation stimulation. <i>Fertility and sterility</i> 91(1):1-17.</p> <p>Feliciani E et al. (2009) IVF light versus conventional ART: cumulative ongoing pregnancy rate over a given period of time. <i>Hum Rep</i> 24 suppl 1:i65.</p> <p>Givens C et al. (2009) Outcomes of natural cycles versus programmed cycles for 1677 frozen thawed embryo transfers. <i>RBMO</i> 19(3):380-384.</p> <p>Muasher S et al. (2009) Fewer medications for in vitro fertilisation can be better: thinking outside the box. <i>Fertility and Sterility</i> 92(4):1187-1189.</p> <p>Schimberni M et al. (2009) Natural-cycle in vitro fertilisation in poor responder patients: a survey of 500 consecutive cycles. <i>Fertility and Sterility</i> 92(4):1297-130.</p> <p>Verberg MF et al. (2009) Mild ovarian stimulation for IVF. <i>Hum Rep Update</i> 15(1):13-29.</p> <p>Weghofer A et al. (2009) The impact of LH-containing gonadotrophin stimulation on euploidy rates in preimplantation embryos: antagonist cycles. <i>Fertility and Sterility</i> 92(3):937-942.</p> <p>Weissmann A et al. (2009) Timing natural cycle frozen/thawed embryo transfer by HCG triggering: a randomised prospective trial. <i>Fertility and Sterility</i> 92(3) suppl:S24.</p>

# Issues identified in the 2009/10 horizon scanning process

IVF and ICSI		
Use of aim	Description	Reference
<b>Assessing impact of lifestyle on ART success</b>	The impact of diet on sperm DNA damage and sperm concentration, the effects of reducing calorie intake on quality of eggs produced and how ambient fine particulate matter may affect female reproductive health.	Geddes L (2009) Fasting extends female fertility. <i>New Scientist</i> 2724:14. Maluf M et al. (2009) In vitro fertilization, embryo development, and cell lineage segregation after pre- and/or postnatal exposure of female mice to ambient fine particulate matter. <i>Fertility and Sterility</i> 92(5):1725-1735. Vujkovic M et al. (2009) Associations between dietary patterns and semen quality in men undergoing IVF/ICSI treatment. <i>Hum Rep</i> 24(6):1304-1323.
<b>Long-term health effects of fertility drugs</b>	The risk of ovarian cancer after use of fertility drugs.	Jensen A (2009) Use of fertility drugs and risk of ovarian cancer: Danish population based cohort study. <i>BMJ</i> 338:7694. Punt-van-der Zalm A et al. (2009) Toxicity testing of human assisted reproduction devices using the mouse embryo assay. <i>RBMO</i> 18(4):529-535. Webb P (2009) Fertility drugs and ovarian cancer. <i>BMJ</i> 338:7694.
<b>Sex selection (only permitted for medical reasons in the UK) and factors which affect the sex ratio</b>	The use of ICSI, particularly with blastocyst-stage embryos, and the sex ratio of infants.  Assessment of the reliability of methods for the separation of X and Y bearing spermatozoa for clinical purposes.  Investigation of Anti-Müllerian hormone in pregnant women and fetal sex.	Aleahmad F et al. (2009) Separation of X and Y bearing human spermatozoa by sperm isolation medium gradient evaluated by FISH. <i>RBMO</i> 18(4):475-478. Luke B et al. (2009) The sex ratio of singleton offspring in assisted-conception pregnancies. <i>Fertility and Sterility</i> 92(5):1579-1585. Lutterodt M (2009) Anti-Müllerian hormone in pregnant women in relation to other hormones, fetal sex and in circulation of second trimester fetuses. <i>RBMO</i> 18(5):694-699.
<b>Sperm selection for ICSI</b>	Spermatozoa -zona pellucida binding test to select sperm for ICSI.	Borges E et al. (2009) Outcome of ICSI using zona pellucida - bound spermatozoa and conventionally selected spermatozoa. <i>RBMO</i> (19)6:802-807.

# Issues identified in the 2009/10 horizon scanning process

## In vitro derived gametes

Use of aim	Description	Reference
<b>Production of gametes to treat male infertility, female infertility and same sex parents (prohibited by HFE Act 1990, as amended)</b>	Possible differentiation of human bone marrow stem cells into male germ cells.	Hua J et al. (2009) Derivation of male germ cell-like lineage from human fetal bone marrow stem cells. <i>RBMO</i> 19(1):99-105.
<b>As above</b>	The capacity of epiblast stem cells to generate primordial germ cells, under conditions that sustain their pluripotency and self-renewal.	Hayashi K and Surani MA (2009) Self-renewing epiblast stem cells exhibit continual delineation of germ cells with epigenetic reprogramming in vitro. <i>Development</i> 136:3549-3556.
<b>As above</b>	The creation of mouse pups from parthenogenetic mouse ES cells and a method of overcoming imprinting.	Keefe D et al. (2009) Parthenogenetic babies from IVM eggs and tetraploid complementation. <i>Fertility and Sterility</i> 92(3) suppl:S24.
<b>As above</b>	Strategies for establishing germline stem cells from human embryonic stem cells.	Aflatoonian B et al. (2009) In vitro post-meiotic germ cell development from human embryonic stem cells. <i>Hum Rep</i> 24(12):3150-3159. Bucay N et al. (2009) A Novel Approach for the Derivation of Putative Primordial Germ Cells and Sertoli Cells from Human Embryonic Stem Cells. <i>Stem cells</i> 27(1):68-77. Kee K et al. (2009) Human DAZL, DAZ and BOULE genes modulate primordial germ-cell and haploid gamete formation. <i>Nature</i> 462(7270):222-225. Marques-Mari AI et al. (2009) Differentiation of germ cells and gametes from stem cells. <i>Hum Rep Update</i> 15(3):379-390. Mathews D et al. (2009) Pluripotent Stem Cell-Derived Gametes: Truth and (Potential) Consequences. <i>Cell Stem Cell</i> 5(1):11-14. Park T et al. (2009) Derivation of primordial germ cells from human embryonic and induced pluripotent stem cells is significantly improved by coculture with human fetal gonadal cells. <i>Stem Cells</i> 27(4):783-795.
<b>As above</b>	Identification of a signaling principle in germ cell specification to establish a strategy for reconstituting the mammalian germ cell lineage <i>in vitro</i> .	Ohinata Y et al. (2009) A signaling principle for the specification of the germ cell lineage in mice. <i>Cell</i> 137(3):571-84.

# Issues identified in the 2009/10 horizon scanning process

Multiple births		
Use of aim	Description	Reference
Reducing multiple birth rates	<p>A number of studies regarding the following areas:</p> <ul style="list-style-type: none"> <li>• Cost effectiveness of elective single embryo transfer (eSET)</li> <li>• Use of eSET and PGD</li> <li>• Risks of multiple births</li> <li>• Embryo storage and eSET</li> <li>• Comparison of single and double embryo transfer</li> <li>• Monozygotic twinning</li> </ul>	<p>Bechoua S et al. (2009) How to demonstrate that eSET does not compromise the likelihood of having a baby? <i>Hum Rep</i> 24(12):3073-3081.</p> <p>Brison D (2008) Controversies in clinical embryology: embryo selection towards single embryo transfer - 16 July 2008, University of Manchester. <i>Human Fertility</i> 11(4):263-264.</p> <p>Chang H et al. (2009) Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: a systematic review and meta-analysis. <i>Fertility and Sterility</i> 91(6):2381-2390.</p> <p>El-Toukhy T et al. (2009) Reduction of the multiple pregnancy rate in preimplantation genetic diagnosis program after introduction of single blastocyst transfer and cryopreservation of blastocysts biopsied on Day 3. <i>Hum Rep</i> 24(10):2642-2648.</p> <p>Fiddlers A et al. (2009) Cost-effectiveness of seven IVF strategies: results of a Markov decision-analysis model <i>Hum Rep</i> 24(7):1648-55.</p> <p>Harrid K et al. (2009) Clinical effectiveness of elective single versus double embryo transfer: results from an individual patient data meta-analysis of randomised trials. <i>Hum Rep</i> 24 suppl 1:i77.</p> <p>Hydanen-Granskog C et al. (2009) The possibility of a second child after delivery from eSET combined with cryopreservation. <i>Hum Rep</i> 24 suppl 1:i77-i78.</p> <p>Motluk A. (2009) When eight children is seven too many. <i>New Scientist</i> 2695:24.</p> <p>Pharoah P et al. (2009) Congenital anomalies in multiple births after early loss of a conceptus. <i>Hum Rep</i> 24(3):726-731.</p> <p>Prades M et al. (2009) Can cumulative pregnancy rates be increased by freezing and thawing single embryos? <i>Fertility and Sterility</i> 91(2):395-400.</p> <p>Roberts et al. (2009) Modeling the impact of single embryo transfer in a national health service IVF program. <i>Hum Rep</i> 24(1):122-131.</p> <p>Thurin-Kjellberg A et al. (2009) Cumulative live birth rates after single and double embryo transfer: follow up from the Scandinavian randomised controlled trial. <i>Hum Rep</i> 24 suppl 1:i76-i77.</p> <p>Veleva Z et al. (2009) Elective single embryos transfer with cryopreservation improves the outcome and diminishes the costs of IVF/ICSI. <i>Hum Rep</i> 24(7):1632-1639.</p> <p>Vitthala S et al. (2009) The risk of monozygotic twins after assisted reproductive technology: a systematic review and meta analysis. <i>Hum Rep Update</i> 15(1):45-55.</p>

# Issues identified in the 2009/10 horizon scanning process

## Stem cell derivation

Use of aim	Description	Reference
<p><b>Induced pluripotent stem (iPS) cells as a possible alternative to ES cells</b></p>	<p>Various developments with iPS cells:</p> <ul style="list-style-type: none"> <li>• Generation of iPS cells from human cord blood (avoids somatic cell mutations being passed to the iPS cells)</li> <li>• Methods of inducing pluripotent stem cells: direct delivery of reprogramming proteins, single lentiviral vector expressing a stem cell cassette, piggyBac transposition, and adenoviral vectors</li> <li>• Epigenetics and imprinting</li> <li>• Pluripotency and p53</li> <li>• Reprogramming efficiency and enhancement</li> <li>• iPS cell stability</li> <li>• Characterisation of pluripotency and stem-ness markers</li> </ul>	<p>Banito A et al. (2009) Senescence impairs successful reprogramming to pluripotent stem cells. <i>Genes and Dev.</i> 2009. 23:2134-2139.</p> <p>Bao S et al. (2009) Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells. <i>Nature</i> 461(7268):1292.</p> <p>Chambers I and Tomlinson S (2009) The transcriptional foundation of pluripotency. <i>Development</i> 136:2311-2322.</p> <p>Chang C-W et al. (2009) Polycistronic Lentiviral Vector for "Hit and Run" Reprogramming of Adult Skin Fibroblasts to Induced Pluripotent Stem. <i>Cells Stem Cells</i> 27(5):1042-1049.</p> <p>Colman A and Dreesen O. (2009) Induced pluripotent stem cells and the stability of the differentiated state. <i>EMBO Rep.</i> 10(7):714-21.</p> <p>Daley G et al. (2009) Broader Implications of Defining Standards for the Pluripotency of iPSCs. <i>Cell Stem Cell</i> 4(3):200.</p> <p>Ellis J et al. (2009) Alternative Induced Pluripotent Stem Cell Characterization Criteria for In Vitro Applications. <i>Cell Stem Cell</i> 4(3):198.</p> <p>Ensenat-Waser R et al. (2009) Reprogrammed induced pluripotent stem cells: how suitable could they be in reproductive medicine? <i>Fertility and Sterility</i> 91(4):971-974.</p> <p>Feng B et al. (2009) Molecules that Promote or Enhance Reprogramming of Somatic Cells to Induced Pluripotent Stem Cells. <i>Cell Stem Cell</i> 4(4):301-312.</p> <p>Gaspar-Maia A et al. (2009) Chd1 regulates open chromatin and pluripotency of embryonic stem cells. <i>Nature</i> 460(7257):863.</p> <p>Hasse A et al. (2009) Generation of Induced Pluripotent Stem Cells from Human Cord Blood. <i>Cell Stem Cell</i> 5(4):434-441.</p> <p>Hayashi K and Surani MA (2009) Resetting the Epigenome beyond Pluripotency in the Germline. <i>Cell Stem Cell</i> 4(6):493-498.</p> <p>Hochedlinger K and Plath K (2009) Epigenetic reprogramming and induced pluripotency. <i>Development</i> 136:509-523.</p> <p>Hong H et al. (2009) Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. <i>Nature</i> 460(7259):1132.</p> <p>Kang L et al. (2009) iPS Cells Can Support Full-Term Development of Tetraploid Blastocyst-Complemented Embryos. <i>Cell Stem Cell</i> 5(2):135-138.</p> <p>Kim D et al. (2009) Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins. <i>Cell Stem Cell</i> 4(6):472-476.</p>

# Issues identified in the 2009/10 horizon scanning process

Stem cell derivation (continued)		
Use of aim	Description	Reference
<p><b>Induced pluripotent stem (iPS) cells as a possible alternative to ES cells (continued)</b></p>		<p>Kim JB et al. (2009) Direct reprogramming of human neural stem cells by OCT4. <i>Nature</i> 461(7264):649.</p> <p>Kosak N et al. (2009) Isolation and Characterization of Pluripotent Human Spermatogonial Stem Cell-Derived Cells. <i>Stem cells</i> 27(1):138-149.</p> <p>Krizhanovsky and Lowe (2009) Stem cells: The promises and perils of p53 p, <i>Nature</i> 460(7259):1085.</p> <p>Latos PA et al. (2009) An in vitro ES cell imprinting model shows that imprinted expression of the Igf2r gene arises from an allele-specific expression bias. <i>Development</i> 136:437-448.</p> <p>Marion RS et al. (2009) Telomeres Acquire Embryonic Stem Cell Characteristics in Induced Pluripotent Stem Cells. <i>Cell Stem Cell</i>. 4(2):141-154.</p> <p>Papamichos S et al. (2009) OCT4B1 isoform: the novel OCT4 alternative spliced variant as a putative marker of stemness. <i>Mol Hum Rep</i> 15(5):269-270.</p> <p>Pick M et al. (2009) Clone- and Gene-Specific Aberrations of Parental Imprinting in Human Induced Pluripotent Stem Cells. <i>Stem Cells</i> 27(11):2686-2690.</p> <p>Somer C et al. (2009) Induced Pluripotent Stem Cell Generation Using a Single Lentiviral Stem Cell Cassette. <i>Stem Cells</i> 27(3):543-549.</p> <p>Sparman et al. (2009) Epigenetic Reprogramming by Somatic Cell Nuclear Transfer in Primates. <i>Stem Cells</i> 27(6):1255-1264.</p> <p>Woltjen K et al. (2009) piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. <i>Nature</i> 458(7239):766.</p> <p>Yoshida Y et al. (2009) Hypoxia Enhances the Generation of Induced Pluripotent Stem Cells. <i>Cell Stem Cell</i> 5(3):237-241.</p> <p>Zechner U et al. (2009) Comparative methylation profiles and telomerase biology of mouse multipotent adult germline stem cells and embryonic stem cells. <i>Mol Hum Rep</i> 15(6):345-353.</p> <p>Zhou H et al. (2009) Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins. <i>Cell Stem Cell</i> 4(5):381-384.</p> <p>Zhou W and Freed C (2009) Adenoviral Gene Delivery Can Reprogram Human Fibroblasts to Induced Pluripotent Stem Cells. <i>Stem Cells</i> 27(11):2667-2674.</p>

# Issues identified in the 2009/10 horizon scanning process

## Stem cell derivation (continued)

Use of aim	Description	Reference
<b>Parthenogenesis-derived pluripotent cells</b>	The clinical feasibility of parthenogenesis-derived pluripotent cells and their degree of pluripotency.	<p>Do J et al. (2009) Generation of Parthenogenetic Induced Pluripotent Stem Cells from Parthenogenetic Neural Stem Cells. <i>Stem Cells</i> 27(12):2962-2968.</p> <p>Gong S et al. (2009) Change in gene expression of mouse embryonic stem cells derived from parthenogenetic activation. <i>Hum Rep</i> 24(4):805-814.</p> <p>Xing F et al. (2009) Parthenogenetic embryonic stem cells derived from cryopreserved newborn mouse ovaries: a new approach to autologous stem cell therapy. <i>Fertility and Sterility</i> 91(4):1238-1244.</p>
<b>Derivation of embryonic stem cells from embryos</b>	Poor/non-viable embryos as a source of ES cells.	<p>Chen A et al. (2009). Optimal Timing of Inner Cell Mass Isolation Increases the Efficiency of Human Embryonic Stem Cell Derivation and Allows Generation of Sibling Cell Lines. <i>Cell Stem Cell</i> 4(2):103.</p> <p>Cortes J et al. (2009) Mesenchymal stem cells facilitate the derivation of human embryonic stem cells from cryopreserved poor-quality embryos. <i>Hum Rep</i> 24(8):1844-1851.</p> <p>Gavrilov S et al. (2009) Non-viable human embryos as a source of viable cells for embryonic stem cell derivation. <i>RBMO</i> 18(2):301-308.</p> <p>Geens M et al. (2009) Human embryonic stem cell lines derived from single blastomeres of two 4-cell stage embryos. <i>Hum Rep</i> 24(11):2709-2717.</p> <p>Muller T (2009) A novel embryonic stem cell line derived from the common marmoset monkey exhibiting germ cell-like characteristics. <i>Hum Rep</i> 24(6):1359-1372.</p>
<b>ES or ES-like cell application</b>	ES cell differentiation, disease specific ES cells and human application of ES cells.	<p>Bongso A et al. (2009) Taking stem cells to the clinic: Major challenges. <i>J Cell Biochem</i> 15;105(6):1352-60.</p> <p>Borowiak M. et al. (2009) Small Molecules Efficiently Direct Endodermal Differentiation of Mouse and Human Embryonic Stem Cells. <i>Cell Stem Cell</i> 4(4):348-358.</p> <p>Deleu S. (2009) Human cystic fibrosis embryonic stem cell lines derived on placental mesenchymal stromal cells <i>RMBO</i> 18(5):704-716.</p> <p>Guenou H et al. (2009) Human embryonic stem-cell derivatives for full reconstruction of the pluristratified epidermis: a preclinical study. <i>Lancet</i> 374(9703):1745.</p> <p>Kiuru M et al. (2009) Genetic Control of Wayward Pluripotent Stem Cells and Their Progeny after Transplantation. <i>Cell Stem Cell</i> 4(4):289-300.</p> <p>Li X et al. (2009) ROCK inhibitor improves survival of cryopreserved serum/feeder-free single human embryonic stem cells. <i>Hum Rep</i> 24(3):580-589.</p>

# Issues identified in the 2009/10 horizon scanning process

Stem cell derivation (continued)		
Use of aim	Description	Reference
ES or ES-like cell application (continued)		<p>Saha K et al. (2009) Technical Challenges in Using Human Induced Pluripotent Stem Cells to Model Disease. <i>Cell Stem Cell</i> 5(6):584-595.</p> <p>Senjua S. et al. (2009) Characterization of Dendritic Cells and Macrophages Generated by Directed Differentiation from Mouse Induced Pluripotent Stem. <i>Cell Stem Cell</i> 27(5):1021-1031.</p> <p>Sermon K et al. (2009) Creation of a registry of human embryonic stem cells carrying an inherited defect: joint collaboration between ESHRE and hESCrg. <i>Hum Rep</i> 24(7):1556-1560.</p> <p>Vallier et al. (2009) Activin/Nodal signaling maintains pluripotency by controlling Nanog expression. <i>Development</i> 136:1339-1349.</p> <p>Zaret KS (2009) Using Small Molecules to Great Effect in Stem Cell Differentiation. <i>Cell Stem Cell</i> 4(5):373-374.</p> <p>Zheng Y-M et al. (2009) Development of cloned embryos from porcine neural stem cells and amniotic fluid-derived stem cells transfected with enhanced green fluorescence protein gene. <i>Reproduction</i> 137:793-801.</p> <p>Zhu S. et al. (2009) A Small Molecule Primes Embryonic Stem Cells for Differentiation. <i>Cell Stem Cell</i> 4(5):416-426.</p>

Transplantation		
Use of aim	Description	Reference
Storage of ovarian tissue to restore fertility	<p>Ovarian tissue cryopreservation.</p> <p>Comparison of fresh versus frozen ovarian tissue transplantation.</p> <p>Comparison of vitrification vs slow freezing of ovarian tissue.</p>	<p>Agca C (2009) Gene expression profile of rat ovarian tissue following xenotransplantation into immune-deficient mice. <i>Reproduction</i> 137:957-967.</p> <p>Demeestere I et al. (2009) Orthotopic and heterotopic ovarian tissue transplantation. <i>Hum Rep Update</i> 15(6):649-665.</p> <p>Dolmans M-M et al. (2009) IVF outcome in patients with orthotopically transplanted ovarian tissue. <i>Hum Rep</i> 24(11):2778-2787.</p> <p>Fabbri R et al. (2009) Culture of cryopreserved ovarian tissue: state of the art in 2008. <i>Fertility and Sterility</i> 91(5):1619-1629.</p> <p>Fain-Kahn V et al. (2009) Feasibility of ovarian cryopreservation in borderline ovarian tumors. <i>Hum Rep</i> 24(4):850-855.</p> <p>Hickey M (2009) Breast cancer in young women and its impact on reproductive function. <i>Hum Rep Update</i> 15(3):323-339.</p> <p>Isachenko V et al. (2009) Vitrification and conventional freezing of human ovarian tissue: follicle formation, hormone production and gene expression. <i>Hum Rep</i> 24 suppl 1:i16.</p>

# Issues identified in the 2009/10 horizon scanning process

## Transplantation (continued)

Use of aim	Description	Reference
Storage of ovarian tissue to restore fertility (continued)		<p>Kagawa N et al. (2009) Successful vitrification of bovine and human ovarian tissue. <i>RBMO</i> 18(4):586-577.</p> <p>Keros V et al. (2009) Vitrification versus controlled-rate freezing in cryopreservation of human ovarian tissue. <i>Hum Rep</i> 24(7):1670-1683.</p> <p>Oktem O et al. (2009) Vitrified human ovaries harbor less primordial follicles and produce less AMH than slow frozen ovaries. <i>Fertility and Sterility</i> 92(3) suppl:S66.</p>
Transplantation of testicular tissue to restore fertility	Transplantation of testicular tissue leading to spermatogenesis.	<p>Rodriguez-Sosa J and Dobrinski I (2009) Recent developments in testis tissue xenografting. <i>Reproduction</i> 138:187-194.</p> <p>Van Saen D et al. (2009) Regeneration of spermatogenesis by grafting testicular tissue or injecting testicular cells into the testis of sterile mice: a comparative study. <i>Fertility and Sterility</i> 91(5) suppl:2264-2272.</p>
Transplantation of ovarian tissue to restore fertility	Reports of fertility success rate following ovarian tissue transplantation.	<p>Deng X et al. (2009) Cryopreserved ovarian tissues can maintain a long-term function after heterotopic autotransplantation in rat. <i>Reproduction</i> 138:519-525.</p> <p>Eimani H et al. (2009) Comparative study between intact and non-intact intramuscular auto-grafted mouse ovaries. <i>RBMO</i> 18(1):53-60.</p> <p>Kim S et al. (2009) Long-term ovarian function and fertility after heterotopic autotransplantation of cryobanked human ovarian tissue: 8-year experience in cancer patients. <i>Fertility and Sterility</i> 91(6):2349-2354.</p> <p>Onions V et al. (2009) Ovarian endocrine profile and long-term vascular patency following heterotopic autotransplantation of cryopreserved whole ovine ovaries. <i>Hum Rep</i> 24(11):2845-2855.</p> <p>Piver P et al. (2009) Two pregnancies obtained after a new technique of autotransplantation of cryopreservation ovarian tissue. <i>Hum Rep</i> 24 suppl 1:i15.</p> <p>Silber et al. (2009) Duration of function of fresh and frozen human ovarian grafts. <i>Hum Rep</i> 24 suppl 1:i15-i16.</p> <p>Wallin A et al. (2009) Viability and function of the cryopreserved whole ovary: in vitro studies in the sheep. <i>Hum Rep</i> 24(7):1684-1694.</p> <p>Wang X et al. (2009) Live offspring from vitrified blastocysts derived from fresh and cryopreserved ovarian tissue grafts of adult mice. <i>Reproduction</i> 138:527-535.</p>

# Issues identified in the 2009/10 horizon scanning process

Reproductive immunology		
Use of aim	Description	Reference
<p><b>Reproductive immunology tests and treatments, which aim to improve ART outcome</b></p>	<p>Reports of new types of reproductive immunology treatments.</p> <p>British Fertility Society review of evidence for use of medical adjuncts in IVF.</p> <p>A study of the effects of Natural Killer cell level (or the chemicals they release) in the peripheral blood and the uterus and establishment of pregnancy.</p>	<p>Faridi R et al. (2009) Influence of activating and inhibitory killer immunoglobulin-like receptors on predisposition to recurrent miscarriages. <i>Hum Rep</i> 24(7):1758-1764.</p> <p>Guerin L et al. (2009) Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? <i>Hum Rep Update</i> 15(5):517-535.</p> <p>King K et al. (2010) Detailed analysis of peripheral blood natural killer (NK) cells in women with recurrent miscarriage. <i>Hum Rep</i> 25(1):52-58.</p> <p>Nardo L et al. (2009) Medical adjuncts in IVF: evidence for clinical practice. <i>Human Fertility</i> 12(1):1-13.</p> <p>Quenby S et al. (2009) Uterine natural killer cells and angiogenesis in recurrent reproductive failure. <i>Hum Rep</i> 24(1):45-54.</p> <p>Scarpellini F and Sbracia M (2009) Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: a randomised controlled trial. <i>Hum Rep</i> 24(11):2703-2708.</p> <p>Wang W et al. (2009) Improvement of fertility with adoptive CD25+ natural killer cell transfer in subfertile non-obese diabetic mice. <i>RBMO</i> 18(1):95-103.</p> <p>Zammiti et al. (2009) Tumour necrosis factor and lymphotoxin haplotypes in idiopathic recurrent pregnancy loss. <i>Fertility and Sterility</i> 91(5):1903-1908.</p>

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- <sup>2</sup> Kallen B et al. (2005) *In vitro* fertilisation (IVF) in Sweden: risk for congenital malformations after different IVF methods. *Birth Defects Res A Clin Mol Teratol* 73:162-169
- <sup>3</sup> Olsen C K et al. (2005) *In vitro* fertilisation is associated with an increase in major birth defects. *Fertility and Sterility* 84:1308-1315
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