

**Human Fertilisation and Embryology Authority
The Scientific and Clinical Advances Group**

Committee:	Scientific and Clinical Advances Group
Meeting Date:	14 th June 2007
Agenda Item:	7
Paper Number:	SCAG(06/07)03
Paper Title:	<i>In vitro</i> derived gametes
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For Information or Decision?	Decision
Resource Implications:	Accounted for in the business plan (horizon scanning)
Recommendation to the Committee:	<p>Members are asked to:</p> <ul style="list-style-type: none"> • Consider the developments outlined regarding the production of gametes <i>in vitro</i> from embryonic stem cells and somatic cell haploidization • Consider whether they wish to review their view on these techniques and the likelihood of these gametes being safe to use

1. Introduction

1.1 The potential use of *in vitro* derived gametes was identified through the horizon scanning process as a technique that may have an impact on assisted reproduction in the near future. It was discussed at the September 2005 and September 2006 SCAG meetings.

1.2 The two main techniques for producing gametes *in vitro* are the development of gametes from embryonic stem cells and somatic cell haploidization. Germinal vesicle transfer has also been considered by SCAG. The HFEA Horizon Scanning Panel, at their 2005 meeting, agreed that embryonic stem cells would be the most likely source of *in vitro* derived gametes.

1.3 Members at the September 2006 SCAG meeting thought that the development of gametes from embryonic stem cells was not yet at a stage for therapeutic use but that the HFEA would be likely to receive a research application for the production of male gametes within a year. The development of eggs from stem cells was thought to be further off than sperm, but SCAG should reconsider the issue again in a year. SCAG discussed whether an individual could have a child using just their genetic material but noted that it was not possible for male germ cells to be produced from female

cells because the presence of two X chromosomes is incompatible for spermatogenesis.

1.4 Members of the Ethics & Law Committee considered the issues surrounding *in vitro* derived gametes in January 2007. The Committee identified the key issues to be investigated further as the scientific unknowns, consent issues and definitions of parenthood. Members agreed that creating embryos from *in vitro* derived gametes would require a licence from the HFEA.

1.5 The aim of this paper is for SCAG to consider the new developments regarding the production of gametes, *in vitro*, from embryonic stem cells and somatic cell haploidization. Members are asked to consider whether they wish to review the view the Group came to in September 2006. If the Group's view on these techniques and the likelihood of these gametes being safe to use varies greatly from the previous conclusion then this will be communicated to the Ethics and Law Committee. SCAG's views will be incorporated into the second annual horizon scanning report which will be written at the end of the year.

2. Developing gametes from embryonic stem cells

2.1 Embryonic stem cells appear to be able to differentiate into germ cells of various stages spontaneously and quickly. This is probably due to either the inherent nature of embryonic stem cells or the microenvironment of the culture conditions. Exactly how such gamete-like cells are generated during stem cell culture remains unclear.

2.2 Studies on mice have derived primordial germ cells (PGCs) *in vitro* from embryonic stem cells (ESCs) that may form oocyte-like cells and develop into blastocyst-like structures (Hubner *et al* 2003, Lacham-Kaplan *et al* 2006, Payer *et al* 2006), or mature into spermatids that can support embryo formation when injected into oocytes (Toyooka *et al* 2003, Geijsen *et al* 2004, Kehler *et al* 2005). Nayernia *et al* (2006a) produced viable transgenic offspring from ESC-derived male gametes. 12 live mice were born but these exhibited abnormalities and died prematurely. The study did not establish whether offspring carried a full haploid contribution from ESC-derived sperm.

2.3 Investigations with human ESCs are more preliminary. When SCAG considered this issue previously, limited work had been carried out on human ESCs. Spontaneous or induced differentiation in culture of human ESCs was shown to produce PGCs (Clark *et al* 2004, Aflatoonian & Moore 2005). An ESHRE abstract by Aflatoonian *et al* (2005) indicated that human ESCs could differentiate into PGCs, and occasionally early spermatid cells (but not oocytes), under appropriate culture conditions. This work has not yet been published.

2.4 Studies since then have reviewed and investigated more effective ways of differentiating PGCs from human ESCs (Clark & Reijo Pera 2006, Aflatoonian & Moore 2006). Lyle Armstrong, at the University of Newcastle, is currently working on differentiating human ESC into primordial oocytes, but is not ready to publish anything yet. His group can isolate PGCs from ESCs and are developing techniques to encourage them to progress towards oogenesis. Karim Nayernia, also at the University of Newcastle, is interested in deriving sperm from human ESCs. Mature or functional gametes have not yet been derived from human ESCs.

2.5 Customized gametes could theoretically be generated using somatic cell nuclear transfer technology (Master, 2006). A nucleus from a patient's skin cell could be transferred into an enucleated oocyte and induced to begin embryo development by parthenogenesis. ESCs could then be derived from the embryo and differentiated *in vitro* into gametes.

2.6 The process is theoretically possible because human ESCs have been cloned from individuals and there is some evidence that human ESCs can differentiate into PGCs. However the process has not been demonstrated yet and would be labour intensive and inefficient.

3. Somatic cell nuclear transfer (SCNT) and haploidization

3.1 Constructing artificial gametes may be possible by transferring somatic cells into enucleated oocytes and inducing chromosomal halving of their nuclei, known as 'haploidization'. Correct chromosomal segregation is crucial in artificial haploidization. Initial successes have been observed but significant alterations at spindle construction and chromosomal segregation were also described.

3.2 Takeuchi *et al* (2005) injected somatic cell nuclei into enucleated human MII oocytes and induced haploidization by either activation or sperm injection. About 40% of the reconstituted oocytes formed two 'putative haploid' pronuclei but no polar body was extruded. Therefore, with the injection of a spermatozoon, there were three distinct pronuclei. Removal of one of the somatic cell-derived 'haploid' nuclei restored biparental diploidy. Most of the oocytes did not develop fully but some underwent early preimplantation development. Blastomeres isolated from cleaving embryos showed a chaotic distribution of chromosomes.

3.3 Both mature (metaphase II) oocytes and germinal vesicle stage oocytes appear to support haploidization-like reduction of somatic cell nuclei. MII oocytes require a spermatozoon or other stimulus for haploidization to occur and obtaining a normal haploid complement of chromosomes may prove difficult. Germinal vesicle stage oocytes need to undergo *in vitro* maturation, known to be a limiting factor for their further embryonic development and implantation.

3.4 There have not been any further developments reported since SCAG considered this in 2006.

4. Developing gametes from adult stem cells

4.1 There is some evidence that germ cells can be derived (or transdifferentiated) from adult stem cells residing outside of the gonad: from bone marrow (Nayernia *et al* 2006b), peripheral blood cells (Johnson *et al* 2004, 2005) and pig foetal skin cells (Dyce *et al* 2006).

4.2 This could indicate that PGC and germ cell formation *in vitro* is more plastic than previously believed. However detection of germ cell markers may be due to aberrant expression or detection in culture, and not due to true germ cell phenotype.

5. Safety of *in vitro* derived gametes

5.1 It is difficult to determine whether PGC and germ cell development *in vitro* follow similar programmes to those occurring *in vivo*. ESC-derived gametes display similar biological properties to gametes derived from germ cell precursors but the functionality of ESC-derived gametes remains to be established.

5.2 There is major uncertainty about the genetic/epigenetic processing of germ cells *in vitro*. The effect of artificial gamete production on imprinting is still unknown.

6. Timescale

6.1 There is strong evidence that primordial germ cells can be derived readily from mouse ESCs *in vitro* and there is some evidence that similar processes will occur with human ESCs. However evidence is lacking of meiosis and terminal gametogenesis. Gametes are occasionally generated under appropriate culture conditions but this is not robust and their complete developmental competence is uncertain.

6.2 The experiments of Nayernia *et al* (2006a) need to be replicated to try and achieve functional gametes and live offspring.

6.3 Mature gametes have not yet been isolated from human ESCs.

7. Regulatory framework

7.1 There are no prohibitions in the 1990 HFE Act that would prevent the use of *in vitro* derived gametes in treatment. If sperm and/or eggs were derived *in vitro* a licence would be needed to store them. If they were used fresh,

centres would need to be licensed to do so under the EU Tissue and Cells Directive.

7.2 As the techniques of somatic cell haploidization and germinal vesicle transfer are applied to the egg, rather than the embryo, it does not constitute a breach of the Act. However, a licence would be required to create embryos *in vitro* from these gametes.

7.3 The draft Human Tissues and Embryos Bill contains a regulation making power to give Parliament flexibility to introduce the use of *in vitro* derived gametes in the future if so desired (Part 2, section 1(6)).

8. Conclusion

8. Members are asked to:

- Consider the developments outlined regarding production of gametes, *in vitro*, from embryonic stem cells and somatic cell haploidization
- Consider whether they wish to review their view on these techniques and the likelihood of these gametes being safe to use

9. References

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