Committee Paper

Committee:	Scientific and Clinical Advances Advisory Committee
Meeting Date:	8 September 2009
Agenda Item:	4
Paper Number:	SCAAC(09/09)01
Paper Title:	Update on in vitro derived gametes
Author:	Rachel Fowler
For Information or Decision?	Information
Resource Implications:	None
Implementation:	N/A
Communication:	For information only
Organisational Risk:	Low
Recommendation to the Committee:	Members are asked to note this update on research into *In vitro* derivation of gametes and advise the Executive if they are aware of any other advances in research of this technique.
Evaluation:	N/A

1. Lay summary

1.1. Human germ cells (sperm and eggs) are derived from a type of stem cell called primordial germ cells. They are derived via the process of gametogenesis in the testes and ovaries of men and women. Researchers are investigating whether it is possible to carry out gametogenesis in the laboratory using primordial germ cells, embryonic stem cells or other human cells. Sperm and eggs derived from such cells in the laboratory are called in vitro derived gametes.

1.2. In vitro derived gametes can be used for research purposes, eg, research into germ cell development and cell differentiation. In vitro derived gametes could also be used in treatment. For people who are unable to produce their own eggs or sperm, in vitro derived gametes are potentially an opportunity for them to have children that are genetically related to them. It is important to note that the HFE Act 1990 (as amended) prohibits the use of in vitro derived gametes for treatment purposes.

2. Introduction

2.1. In November 2008, SCAAC advised the HFEA that in vitro derived gametes should be a high priority for the Committee and the Authority during 2008/9. The Committee asked to be

periodically updated with relevant research developments and this paper summarises research for the period November 2008 to September 2009.

2.2. During this period research into in vitro derived gametes has focused on techniques using pluripotent stems cells rather than somatic cells. The creation of in vitro derived sperm has been reported and although the degree of similarity to in vivo sperm is not great, this and other research has illustrated significant advances in the technique of deriving gametes in vitro.

3. Research

Pluripotent stem cells

3.1. Researchers have demonstrated in mice that it is possible to differentiate embryonic stem cells (ES cells) into germ cells. Male germ cells have subsequently been shown to differentiate into male gametes and to be capable of producing live male offspring[1,2]. Oocyte maturation remains more problematic although recent research reports the full maturation of ESC-derived oocytes by transplantation into an ovarian niche.[3]

3.2. Research in humans has demonstrated that human ES (hES) cells can differentiate into germ cells. Furthermore, research led by Nayernia at the North-East England Stem Cell Institute in Newcastle, reported earlier in the year the *in vitro* derivation of mature human sperm from such germ cells[4]. The group re-programmed hES cells into germline stem cells and then directed their differentiation into sperm cells. The in vitro derived (IVD) sperm were reported to be haploid and express some normal characteristics of mature human sperm, including tail growth and motility. However, other researchers in the field expressed the view that although the study represented an advance in the technique of *in vitro* derivation of human gametes, the IVD sperm were still a long way from being truly the same as human sperm produced *in vivo*.

3.3. Similar work on deriving human gametes *in vitro* from pluripotent stem cells has been carried out by a group at Cambridge University led by McLaren[5]. In recent work the group reported the generation of induced primordial germ cells (iPG cells) from embryonic germ cells and embryonic stem cells. The gene expression pattern of the resulting iPG cells suggested that they were similar to primordial germ cells created *in vivo* and by co-culture with hamster ovary cells some entered into meiosis.

1.1. Other recent research, carried out by Park et al (2009)[6], showed that culturing hES cells on human fetal gonadal stromal cells significantly improves the efficiency of generating iPG cells from

[1] Nayernia K et al. (2006) In Vitro-Differentiated Embryonic Stem Cells Give Rise to Male Gametes that Can Generate Offspring Mice. *Developmental Cell* 11: 125-132

[2] Kang L et al (2009) iPS Cells Can Support Full-Term Development of Tetraploid Blastocyst-Complemented Embryos. Cell Stem Cell 5(2) 135-138

[3] Nicholas C R et al (2009) Transplantation directs oocyte maturation from embryonic stem cells and provides a therapeutic strategy for female infertility. *Human Molecular Genetics.* Aug 20 2009 [Ahead of print]

[4] Nayernia et al. (2009) In Vitro Derivation of Human Sperm from Embryonic Stem Cells. *Stem Cells Dev*. 7th July 2009 (ahead of print)

[5] Equizabal C et al (2009) Generation of primordial germ cells from pluripotent stem cells. *Differentiation.* 14 Aug 2009 (ahead of print)

[6] Park 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. *Stem Cells* 27(4): 783 -795

them. The group also showed that iPG cells initiate imprint erasure from differentially methylated imprinted regions by day seven of differentiation. However, iPG cells derived from human induced pluripotent stem cells do not initiate imprint erasure as efficiently as those derived from hES cells.

1.2. Research into in vitro derived gametes using pluripotent stem cells has investigated not just the use of ES cells as described above but also other stem cells, eg, mesenchymal stem cells. Mesenchymal stem cells have been re-programmed and differentiated into sperm and oocyte germ cells in both the mouse[7, 8] and the human[9]. A recent report illustrates their potential in humans to differentiate into sperm-like cells[10].

Somatic cells

1.3. Research into in vitro derived gametes has also looked at using somatic cells, although there is less research in this area compared to the use of pluripotent stem cells. Fusion of an embryonic stem cell with a somatic cell has been shown to reprogram the hybrid cell genome into one that allows differentiation into primordial germ cells *in vitro*, Lavagnolli *et al* (2008)[11]. These cells can then undergo further differentiation into germ like cells and could provide personalised stem cells applicable to regenerative medicine and assisted reproductive technologies.

1.4. In reviewing the technique of somatic cell haploidisation, Nagy et al (2008)[12] concluded that it was impeded by difficulties in ensuring accurate chromosome segregation and prevention of epigenetic defects in imprinted genes of the somatic cell nucleus. These need to be overcome before the technique provides a valid method for deriving gametes *in vitro*.

4. Timescale for introduction

4.1. Members of SCAAC and the HFEA Horizon Scanning Panel have previously estimated a timescale for deriving gametes *in vitro* of between five and ten years. In May 2008 some SCAAC members expressed the view that the timescale for deriving sperm would be shorter than for oocytes.

4.2. This year the Hinxton Group published a review of research into in vitro derived gametes from pluripotent stem cells[13] and 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. They also suggested that creation of

---

[7] Nayernia (2006) Derivation of male germ cells from bone marrow stem cells. *Lab Invest* 86(7):654-63

[8] Bukovsky (2007) Bone marrow derived cells and alternative pathways of oogenesis in adult rodents. *Cell cycle* 6(18):2306-9

[9] Drusenheimer (2007) Putative human male germ cells from bone marrow stem cells. *Soc Reprod Fertil Suppl* 63:69-76

[10] Hua J et al (2009) Characterization of mesenchymal stem cells (MSCs) from human fetal lung: Potential differentiation of germ cells. Tissue Cell. Aug 1 2009 (ahead of print)

[11] Lavagnolli et al. (2008) Nuclear reprogramming of somatic cells by embryonic stem fusion yields germ cell in vitro. *ESHRE* session 59

[12] Nagy Z et al. (2008) Symposium: Genetic and epigenetic aspects of assisted reproduction. Development of artificial gametes. *Reproductive BioMedicine Online* 16(4): 539-544

[13] Mathews et al (2009) Pluripotent stem cell-derived gametes: truth and (potential) consequences. *Cell stem cell* 5(1): 11-14

oocytes from cells carrying the X and Y chromosomes and sperm from cells carrying two copies of the X chromosome would be very difficult.

5. Regulation

5.1. Under the HFE Act 1990 (as amended) in vitro derived gametes cannot be used for treatment purposes. Under the Act only permitted gametes can be used in treatment. These are defined as eggs or sperm which have been produced by or extracted from the ovaries of a woman or testes of a man, and whose nuclear or mitochondrial DNA has not been altered. In vitro derived gametes are not permitted gametes because they have not been produced by or extracted from the ovaries of a woman or testes of a man.

5.2. In vitro derived gametes can however be used for research purposes and the derivation of in vitro derived gametes for research does not require a licence from the HFEA. A licence would only be required if researchers wished to use in vitro derived gametes to create an embryo to test whether they were capable of fertilisation.

6. Risks

6.1. In May 2008, members of SCAAC expressed the opinion that the process of deriving gametes *in vitro* was very complex and the safety issues were largely unknown. Further knowledge was thought to be required around gametogenesis and in particular epigenetics.

6.2. A similar view was given by the HFEA Horizon Scanning Panel in June 2009 and the Hinxton Group in May 2009. It was the Panel's view that animal models, investigation of the meiotic process and long term studies of offspring resulting from in vitro derived gametes were required before the technique could be considered successful or potentially used in treatment. It was similarly the view of the Hinxton Group that it would be critical to assess the quality of in vitro derived gametes.

7. Conclusion

7.1. Research has continued to progress into the derivation of gametes *in vitro* and appears to be most advanced and focused on the method of deriving gametes from pluripotent cells, in particular sperm from ES cells.

7.2. SCAAC members are asked to note this update on research into in vitro derived gametes and advise the Executive if they are aware of any other advances in research of this technique.