In vitro derived gametes

Strategic delivery:	☒ Setting standards	☐ Increasing and informing choice	☐ Demonstrating efficiency economy and value
Details:			
Meeting	Scientific and Clinical Advances Advisory Committee		
Agenda item	6		
Paper number	SCAAC(10/16)02		
Meeting date	17 October 2016		
Author	Anna Quinn, Scientific Policy Officer		
Output:			
For information or decision?	For information		
Recommendation	Members are asked to: • consider the progress in research (since February 2011) into in vitro derived gametes; and • advise the executive if they are aware of any other recent developments		
Resource implications	None		
Implementation date	None		
Communication(s)	None		
Organisational risk	☐ Low	☒ Medium	☐ High
Annexes	None		

1. Introduction

1.1. Human eggs and sperm (germ cells) are derived from a type of cell called primordial germ cells. They are produced in the ovaries of a woman or testes of a man by a process called gametogenesis. 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. Eggs and sperm derived from such cells in the laboratory are called in vitro derived gametes.

1.2. The legislation in the UK (the Human Fertilisation and Embryology Act 1990, as amended) prohibits the use of in vitro derived gametes in treatment. Section 1(4) of the Act defines gametes as "*live human eggs, including cells of the female germ line at any stage of maturity*…" or "*live human sperm, including cells of the male germ cell line at any stage of maturity*". The explanatory note to the Act states that the term "*gametes*" in the Act "*has been amended to expressly encompass not only mature eggs and sperm, but also immature gametogenic cells such as primary oocytes and spermatocytes*". Section 3ZA requires that eggs or sperm permitted for treatment are "*produced by or extracted from the ovaries of a woman/testes of a man".*

1.3. Whilst in vitro derived gametes cannot be used in treatment in the UK, they can be used for research purposes, for example, research into germ cell development and cell differentiation. Researchers in the UK need an HFEA research licence if they wish to investigate whether human eggs and sperm derived in vitro could undergo fertilisation and the early stages of embryo development. It is therefore important that the HFEA is aware of the progress of research in this area.

1.4. The committee last reviewed research on in vitro derived gametes in 2009 and later in 2011. The committee thought that one of the main hindrances to in vitro derived gametes was incorrect imprinting. It was suggested that transplanting egg and sperm precursor cells to their normal environment in the ovaries/testes for the later stages of maturation could help to resolve this. However, the transplantation of human gamete precursor cells (derived in vitro) was not at the time a viable, safe approach. Despite progress, no research published at the time convincingly showed that human embryonic stem cells could be differentiated in vitro into mature human eggs or sperm.

1.5. This review highlights developments in animal and human studies with a focus on developments in 2015/2016.

2. Animal studies

2.1. In 2012, Hayashi et al. showed that mouse female embryonic stem (ES) cells and induced pluripotent stem (iPS) cells can develop into primordial germ cell-like cells (PGCLC). When combined with female gonadal somatic cells as reconstituted ovaries, these cells undergo X-reactivation, imprint erasure and cyst formation and exhibit meiotic potential. Upon transplantation under the mouse ovary, the PGCLCs in the reconstituted ovaries mature into germinal vesicle-stage oocytes, which then contribute to fertile offspring after in vitro maturation and fertilisation. This study provides the foundation for further research into the properties of female germ cells and development of germ cells in vitro.

2.2. A review by Kurimoto & Saitou (2015) reflects on progress made using mouse pluripotent stem cells to generate germ cells in vitro. The authors explain how they have succeeded in reconstituting the specification and subsequent development of germ cells in culture in both males and females. ES cells/ iPS cells are induced into cells which resemble the outer layer of the embryo (epiblast-like cells) and then into PGCLCs, which contribute to sperm cell development (spermatogenesis) and egg cell development (oogenesis), and to fertile offspring. The authors note that recent progress has been made in inducing human PGCLCs from ES cells/ iPS cells and this creates an opportunity to develop our understanding of the mechanisms of human germ cell development in vitro.

2.3. Recently, a study by Morohaku et al. (2016) demonstrated the first complete in vitro generation of fertile oocytes from mouse primordial germ cells. The culture system used in this study used an estrogen-receptor antagonist that promotes normal follicle formation and was able to support meiosis, oocyte growth and genomic imprinting. Oocytes generated in this in vitro system were used to create fertile offspring. The in vitro system described by Morohaku et al. allows consistent observation and manipulation throughout the course of oogenesis which in turn will improve our understanding of this process.

2.4. Afsartala et al. (2016) isolated mouse amniotic mesenchymal stem cells (AM-MSCs) from mouse embryonic membrane. These cells were then induced for differentiation into male germ cells using a two stage method. The results indicated that a number of the AM-MSCs successfully differentiated into germ cells. Based on these results, the authors suggest that AM-MSCs could be a potential source of adult pluripotent stem cells for in vitro generation of germ cells and cell-based therapies for treatment of infertility.

2.5. Murakami et al. (2016) investigated the role of NANOG (a pluripotency factor found in the inner cell mass of blastocysts) in unipotent primordial germ cells in mice. Naïve pluripotent ES cells first developed epiblast-like cells which then differentiate to germ cell-like cells. The authors reported the surprising result that

Nanog alone induced PGCLCs in epiblast-like cells, independently of BMP4. They go on to propose that the epigenome is reset during establishment of epiblast-like cells, noting genome-wide changes in NANOG binding pattern between ES cells and epiblast-like cells.

3. Human studies

3.1. A recent review by Surani (2015) highlights that research has unpicked the mechanism of human primordial germ cell specification and ability to reset the epigenome for totipotency. The article discusses that regulators of human primordial germ cell specification also initiates resetting of the epigenome, resulting in a comprehensive erasure of DNA methylation, erasure of imprints and X reactivation in early human primordial germ cells in vivo. The review states that within the extreme hypomethylated environment of the early human germline are sections of DNA (loci) that are resistant to DNA methylation, with subsequent predominant expression in neutral cells. The article concludes that these loci provide a model for studies on the mechanism of epigenetic inheritance, and their response to environmental factors. The article highlights that these studies reveal differences with the mouse model, which are probably due to differences in the regulation of human pluripotency, and in post implantation development at gastrulation, thus emphasising the need to produce studies in the human model.

3.2. Tang et al. demonstrated in 2015 that early programming for human primordial germ cells is distinct from that in mice, with co-expression of somatic specifiers and naïve pluripotency genes. This unique gene regulatory network, drives comprehensive germline DNA methylation by repressing DNA methylation pathways and activating TET-mediated hydroxymethylation. This study provides insights on early human germline transcriptional network and epigenetic reprogramming that subsequently impacts human development and disease.

3.3. In 2015, Irie et al. showed the specification of human PGCLCs from germline competent pluripotent stem cells. It was found that SOX17 was the key regulatory gene for development towards a human primordial germ cell-like fate, whilst BLIMP1 repressed endodermal and other somatic genes during specification of these cells. The authors noted this was an unexpected result as *SOX17* has no detectable role in the specification of mouse primordial germ cells. This work acts as a foundation for further studies on resetting the epigenome in human PGCLCs and human primordial germ cells for totipotency and the transmission of genetic and epigenetic information.

3.4. Ge et al. (2015) demonstrated that human germ cell-like cells could be successfully derived in vitro by differentiation of human fetus skin-derived stem cells from fetus skin tissue. The authors showed that the in vitro derived germ cell-like cells expressed the same biomarkers as the equivalent cells in vivo, the

human fetal skin-derived stem cells were also shown to have the potential to differentiate into male or female germ cell-like cells under appropriate conditions.

3.5. In their 2016 study, Lai et al. used endometrial mesenchymal stem cells (stem cells which can develop into skeletal and connective tissue) derived from menstrual blood to develop germ cells in vitro. The endometrial mesenchymal stem cells were induced to differentiate into germ cells in a differentiation medium supplemented with 20% human follicular fluid. The induced cell aggregates contained not only oocyte-like structures but also cells expressing follicle stimulating hormone receptor and luteotropic hormone receptor, and produced estrogen and progesterone regulated by gonodatropin, suggesting that granulosa-like and theca-like cells were also induced. The authors suggest that endometrial mesenchymal stem cells may act as an in vitro system for investigating maturation of ovarian follicles in humans.

3.6. Investigations by Medrano et al. (2016) showed that human somatic cells (male foreskin fibroblasts and mesenchymal cells) could be converted directly to a meiotic germ cell-like phenotype by inducing them with a combination of selected key germ cell developmental factors. In this study ectopic expression of the germ line-related genes PRDM1, PRDM14, LIN28A, DAZL, VASA and SYCP3 induced direct conversion of somatic cells into a germ cell-like phenotype in vitro. Approximately 1% of these converted cells were able to complete meiosis, demonstrated by their haploid status and the expression of several post-meiotic markers.

3.7. A review by Mouka et al. (2016) reflects on advances in deriving germ cells in vitro from pluripotent stem cells. The review highlights that fertile mouse eggs and sperm have been derived in vitro using mouse ES cells or mouse iPS cells. However, it was noted that these studies used an in vivo induction system that is difficult to replicate in humans. The authors conclude that further research is needed to develop culture systems that replicate the gonadal niche environment in humans, which in turn requires studies to investigate how developing germ cells interact with their microenvironment. Progress in this field is evolving rapidly and in vitro derived gametes remain a potential solution for those people who are unable to produce their own mature germ cells.

4. Conclusions

4.1. SCAAC last considered in vitro derived gametes in February 2011 and the committee concluded that there was no published literature available at that time which convincingly showed human embryonic stem cells could be differentiated into mature eggs or sperm.

4.2. Since the committee last discussed in vitro derived gametes, research in this area has progressed significantly and has been recognised in the media on a number of occasions. Human germ cell-like cells have now been derived from a

number of different stem cell sources including iPS cells, ES cells and mesenchymal stem cells. Mechanisms have been proposed to explain how epigenetic reprogramming occurs when cells are induced to a germ cell-like cell fate. Progress has also been made in deriving not only sperm cells but also egg cells in vitro; in mice these in vitro derived oocytes have been used to generate fertile offspring.

5. Recommendations

5.1. Members are asked to:

- consider the progress in research (since February 2011) into in vitro derived gametes; and
- advise the Executive if they are aware of any other recent developments.

6. References

Afsartala, Z. et al., 2016. Amniotic membrane mesenchymal stem cells can differentiate into germ cells in vitro. *In vitro cellular & developmental biology. Animal*.

Cutas, D. & Smajdor, A., 2016. "I am Your Mother and Your Father!" In Vitro Derived Gametes and the Ethics of Solo Reproduction. *Health care analysis : HCA : journal of health philosophy and policy*.

Ge, W. et al., 2015. Differentiation of early germ cells from human skin-derived stem cells without exogenous gene integration. *Scientific reports*, 5, p.13822.

Hayashi, K. et al., 2012. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. *Science (New York, N.Y.)*, 338(6109), pp.971–5.

Irie, N. et al., 2015. SOX17 is a critical specifier of human primordial germ cell fate. *Cell*, 160(1-2), pp.253–268.

Kurimoto, K. & Saitou, M., 2015. Mechanism and Reconstitution In Vitro of Germ Cell Development in Mammals. *Cold Spring Harbor symposia on quantitative biology*, 80, pp.147–54.

Lai, D. et al., 2016. Differentiation of human menstrual blood-derived endometrial mesenchymal stem cells into oocyte-like cells. *Acta biochimica et biophysica Sinica*, p.gmw090–..

Medrano, J. V et al., 2016. Human somatic cells subjected to genetic induction with six germ line-related factors display meiotic germ cell-like features. *Scientific reports*, 6(October 2015), p.24956..

Morohaku, K. et al., 2016. Complete in vitro generation of fertile oocytes from mouse primordial germ cells. *PNAS*, 113(32), pp.9021–9026.

Mouka, A. et al., 2016. In Vitro Gamete Differentiation from Pluripotent Stem Cells as a Promising Therapy for Infertility. *Stem cells and development*, 25(7), pp.509–21.

Murakami, K. et al., 2016. NANOG alone induces germ cells in primed epiblast in vitro by activation of enhancers. *Nature*, 529(7586), pp.403–407.

Surani, M.A., 2015. Human Germline: A New Research Frontier. *Stem cell reports*, 4(6), pp.955–60.

Tang, W.W.C. et al., 2015. A unique gene regulatory network resets the human germline epigenome for development. *Cell*, 161(6), pp.1453–1467.