In vitro derived gametes

Strategic delivery:	☒ Safe, ethical, effective treatment	☐ Consistent outcomes and support	☐ Improving standards through intelligence
Details:			
Meeting	Scientific and Clinical Advances Advisory Committee (SCAAC)		
Agenda item	6		
Paper number	SCAAC (08/06/2020) 006		
Meeting date	08 June 2020		
Author	Victoria Askew, Policy Manager		
Output:			
For information or recommendation?	For information		
Recommendation	Members are asked to: • consider the progress in research (since October 2016) into in vitro derived gametes; and • advise the executive if they are aware of any other recent developments; and • review whether any outputs from the HFEA are required addressing the use of in vitro derived gametes.		
Resource implications	N/A		
Implementation date	N/A		
Communication(s)	N/A		
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 (PGCs). 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 PGCs, embryonic stem cells (ESCs) 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 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 in research, 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 October 2016. The committee discussed the need for transplanting in vitro derived gametes back into the ovary or testes in the later stages of maturation to allow imprinting to occur, and that ideally when culturing in vitro derived gametes the supporting somatic cells would also be derived in vitro. The committee also questioned to what extent mouse models are an accurate model for human development.

1.5. An expert external speaker attended the October 2016 SCAAC meeting and informed the committee that progress in research in 2016 had been able to derive gametes without implantation back into ovaries or testes, however stated that these results needed to be reproduced. The speaker explained to the committee that there are differences in regulation of the pluripotent network between mice and humans, as well as differences between germ cell transcriptome and post-implantation development. However, animal models are required in the UK and pigs can be an affordable and accessible model for human development. The speaker acknowledges that a robust methodology for deriving germ cells from somatic cells was yet to be developed and that successful derivation of high-quality cells in humans would still be a number of years away.

1.6. At the February 2020 SCAAC meeting one Committee member commented that the pace of research for in vitro derived gametes is not as fast as in other topics such as genome editing, and milestones are happening slowly, but the committee still need to be aware of developments.

1.7. This review highlights key developments in animal and human studies with a focus on developments since October 2016.

2. Animal studies

2.1. A study conducted by Zhou et al. (2016) reported complete in vitro meiosis from ESC-derived primordial germ cell like cells (PGCLCs). Co-culture of PGCLCs with neonatal testicular somatic cells and sequential exposure to morphogens and sex hormones reproduced key hallmarks of meiosis, including erasure of genetic imprinting, chromosomal synapsis and recombination, and correct nuclear DNA and chromosomal content in the resulting haploid cells. Intracytoplasmic

injection (ICSI) of the resulting spermatid-like cells into oocytes produced viable and fertile offspring, showing that this stepwise approach could functionally recapitulate male gametogenesis in vitro.

2.2. Hikabe et al. (2016) reported the first successful reconstitution in vitro of the entire process of oogenesis from mouse pluripotent stem cells. Fully potent mature oocytes were generated in culture from ESC and induced pluripotent stem cells derived from both embryonic fibroblasts and adult tail tip fibroblasts. Pluripotent stem cell lines were then re-derived from the eggs that were generated in vitro, thereby reconstituting the full female germline cycle in a dish.

2.3. In 2016, Ishikura et al. found that when aggregated with reconstituted testes in vitro, PGCLCs induced from mouse ESCs differentiate into spermatogonia-like cells in vitro and were expandable as cells that resemble germline stem cells (GSCs). The findings showed the GSC-like cells (GSCLCs), but not PGCLCs, colonised adult testes and contribute to spermatogenesis and fertile offspring. Compared to GSCs derived from neonatal testes, GSCLCs exhibited a limited capacity for spermatogenesis. Whole-genome analyses revealed that the GSCLCs exhibited aberrant methylation at vulnerable regulatory elements, including those critical for spermatogenesis, which may restrain their spermatogenic potential.

2.4. Oblette et al. (2017) undertook the first study assessing the differences in nuclear quality between fresh and frozen/thawed (using either controlled slow freezing (CSF) or solid surface vitrification (SSV)) mouse spermatozoa cultured in vitro with in vivo controls. No significant difference was found in rate of aneuploidy, DNA fragmentation or chromatin condensation between either the fresh or frozen/thawed in vitro cultured spermatozoa and in vivo controls. However, the yield of spermatozoa from in vitro culture is lower than that in vivo. The culture conditions will need to be further optimised to allow greater yields for the analysis of epigenetic modifications, embryonic development and spermatozoa at the ultrastructural level by transmission electron microscopy.

2.5. A study by Dumont et al. in 2017 investigated the effects of cryopreservation and in vitro culture on the expression of proteins involved in apoptotic or autophagic pathways in the pre-pubertal testicular tissue of mice. The impact of cryopreservation procedures was minimal at the end of the culture. However, in vitro culture modified apoptosis- and autophagy-related relative protein levels compared to in vivo controls. The authors argue that a disturbance in the balance between pro- and anti-apoptotic proteins expression during in vitro culture could be one of the reasons for altered apoptosis in less effective (or impaired) spermatogenesis.

2.6. Rondanino et al. (2017) used immature fresh and frozen/thawed testicular tissue of mice to analyse whether an intact blood–testis barrier (BTB), which is essential for the progression of spermatogenesis, could be replicated in in vitro culture. In vitro culture exhibited the correct expression and localisation of major BTB components (CLDN11, CX43 and ZO-1), Sertoli cell maturation, as well as the progression of spermatogenesis at the same pace as in vivo. However, CLDN3 expression was decreased and meiotic and post-meiotic progression was altered in cultured testicular tissues. There was an increased BTB permeability and a decreased expression of the androgen receptor regulated gene Rhox5 at the end of the culture period in comparison with in vivo controls. The completion of in vitro spermatogenesis occurred in seminiferous tubules with an intact BTB, both expressing and lacking CLDN3.

2.7. Investigations by Ohta et al. (2017) showed that the combined application of Forskolin and Rolipram, which stimulate cyclic adenosine monophosphate (cAMP) signalling via different mechanisms, expands PGCLCs up to around 50-fold in culture. The expanded PGCLCs maintain robust capacity

for spermatogenesis. During expansion, PGCLCs comprehensively erased their DNA methylome, including parental imprints, in a manner that precisely recapitulated genome-wide DNA demethylation in gonadal germ cells, while essentially maintaining their identity as sexually uncommitted PGCs, apparently through appropriate histone modifications. By establishing a paradigm for PGCLC expansion, the system reconstituted the epigenetic "blank slate" of the germ line, an immediate precursory state for sexually dimorphic differentiation.

2.8. A recent review by Maker et al. (2019) considered publications investigating the mechanisms leading to germ cell development in mammals, particularly in mice and non-human primates, as well as the applicability of these animal models to human germ cell development. Mouse models have provided essential mechanistic insight into the process of germ cell lineage development. However, there are several structural differences between mice and humans during early embryogenesis that hinder the extrapolation of findings made in murine models to what may occur in humans. Recent studies using human or non-human primate embryos and human-induced pluripotent stem cell (hiPSC)-derived germ cells shed light on key cellular and genetic mechanisms governing germ cell development in humans. Utilising the knowledge obtained from studying germ cell development in different animal models, induction methods established by various laboratories now permit partial reconstitution of human gametogenesis in vitro.

3. Human studies

3.1. A study conducted by Jia et al. (2017) investigated whether human umbilical cord stem cells and their condition media (HUMSC-CM) could improve the development of frozen-thawed in-vitro cultured ovarian tissues compared with a serum-free culture medium (SF-CM). The results indicated that the HUMSC-CM group provided a better protecting effect on the in vitro culture of the cryopreserved ovarian tissue compared to the SF-CM group. There was a significantly increased rate of morphological normal primordial follicles in HUMSC-CM on day 2 to day 4 of culture. Significant increases were also seen in micro vessel density and significant decreases in percentage of apoptotic follicles on day 1, 5 and 7.

3.2. In 2017, de Michele et al. cultured frozen/thawed samples of immature testicular tissue (ITT) from three pre-pubertal boys to determine whether organotypic culture systems were able to provide the appropriate testicular microenvironment for in-vitro maturation. The study found that organotypic culture systems provided a microenvironment capable of preserving seminiferous tubule (ST) integrity and Leydig cell (LC) functionality and inducing Sertoli cell (SC) maturation. However, samples were small with ITT from only three prepubertal boys cultured in the long term organotypic system.

3.3. McLaughlin et al. (2018) demonstrated for the first time that development of human oocytes from primordial/unilaminar stages to resumption of meiosis (Metaphase II) and emission of a polar body could be achieved within a serum free multi-step culture system. This study only included 10 samples; however, it provides proof of concept that complete development of human oocytes could occur in vitro.

3.4. Sun et al. demonstrated for the first time in 2018 the successful and efficient generation of functional human haploid spermatids from human spermatogonial stem cells (SSC) by three dimensional-induced system in vitro. The study revealed that human SSCs entered meiosis in vitro, underwent several key processes of the in vivo meiosis including chromosomal synapsis and recombination,

and DSB repair, and finally differentiated into haploid spermatids with normal chromosome numbers. Significantly, haploid spermatids generated from human SSCs via three dimensional induced system were capable of fertilizing mouse oocytes, which subsequently enabled the development of hybrid embryos.

3.5. A review by Rombaut et al. published in 2018 summarised the current evidence base for known regulators of the development, survival, proliferation and differentiation of male gametes. This includes factors of the TGFβ superfamily, metabolites of vitamin A, growth hormones, sex steroids and, most importantly, somatic cell support. The authors suggest that by designing a stepwise differentiation protocol, including all proven contributing regulators, and therefore mimicking more closely human in vivo spermatogenesis and its temporo-spatial organisation a higher differentiation efficiency for in vitro spermatogenesis could be obtained.

3.6. Recently, Yamashiro et al. (2020) published a protocol to differentiate hiPSCs into oogonia in vitro. hiPSCs were induced into incipient mesoderm-like cells (iMeLCs) using activin A and a WNT pathway agonist. iMeLCs, or, alternatively, human pluripotent stem cells (hPSCs) cultured with divergent signalling inhibitors, were induced into hPGC-like cells (hPGCLCs) in floating aggregates by cytokines including bone morphogenic protein 4 (BMP4). hPGCLCs were aggregated with mouse embryonic ovarian somatic cells to form xenogeneic reconstituted ovaries, which were cultured under an air–liquid interface condition for around four months for hPGCLCs to differentiate into oogonia and immediate precursory states for oocytes. To date, this is the only approach that generates oogonia from hPGCLCs.

3.7. Kurek et al. conducted a review in 2020 of the literature regarding in vitro derivation of human male germ cells from hPSCs. The authors concluded that so far, robust and efficient differentiation approaches are limited, especially when aiming for more advanced stages of male germ-cell differentiation. A comparison of protocols between studies is difficult because of the variety of culture approaches and because of the variety of cell lines used for differentiation, all possessing a specific profile from the start. Although expression of mature male-specific markers as well as the generation of haploid cells has been shown, morphological similarities to cells at more advanced stages of spermatogenesis, and functionality, are still missing so far. Future in vitro differentiation protocols will most likely have to include multi-step approaches simulating crucial phases of germ-cell development. Current protocols do not allow sufficient differentiation of fully matured spermatids, and even if possible, testing the ultimate functionality by fertilising oocytes and producing offspring will present us with many ethical conflicts, which need to be addressed very carefully.

4. Conclusions

4.1. SCAAC last considered in vitro derived gametes in October 2016. The committee discussed the need for transplanting in vitro derived gametes back into the ovary or testes in the later stages of maturation and questioned to what extent mouse models are an accurate model for human development. An external expert speaker presented at the meeting concluded that a robust methodology for deriving germ cells from somatic cells was yet to be developed and that successful derivation of high-quality cells in humans would still be a number of years away.

4.2. Since the committee last discussed in vitro derived gametes, research in this area has made some promising progress. However, there are still many fundamental technical challenges that need to be overcome before in vitro gametogenesis could be considered for used in clinical practice. Currently,

in vitro gametogenesis is inefficient when compared to in vivo controls. It is also evident that in vitro derived gametes contain epigenetic abnormalities and, germ cell development has currently only been possible in humans up to the early stages, with meiosis technically difficult to achieve. In mice, the generation of fertile offspring from in vitro gametogenesis had been achieved, however, it is important to consider the applicability of these methodologies to humans. Despite this, there are serious ethical and safety concerns that make research in humans challenging and necessitates the use of animal models.

5. Recommendations

5.1. Members are asked to:

- consider the progress in research since into in vitro derived gametes; and
- advise the Executive if they are aware of any other recent developments; and
- review whether any outputs from the HFEA are required addressing the use of in vitro derived gametes.

6. References

Dumont, L., Chalmel, F., Oblette, A., Berby, B., Rives, A., Duchesne, V., Rondanino, C., & Rives, N. (2017). Evaluation of apoptotic- and autophagic-related protein expressions before and after IVM of fresh, slow-frozen and vitrified pre-pubertal mouse testicular tissue. Molecular human reproduction, 23(11), 738–754. https://doi.org/10.1093/molehr/gax054

Hikabe, O., Hamazaki, N., Nagamatsu, G., Obata, Y., Hirao, Y., Hamada, N., Shimamoto, S., Imamura, T., Nakashima, K., Saitou, M., & Hayashi, K. (2016). Reconstitution in vitro of the entire cycle of the mouse female germ line. *Nature*, *539*(7628), 299–303. https://doi.org/10.1038/nature20104

Ishikura, Y., Yabuta, Y., Ohta, H., Hayashi, K., Nakamura, T., Okamoto, I., Yamamoto, T., Kurimoto, K., Shirane, K., Sasaki, H., & Saitou, M. (2016). In Vitro Derivation and Propagation of Spermatogonial Stem Cell Activity from Mouse Pluripotent Stem Cells. *Cell reports*, *17*(10), 2789–2804. https://doi.org/10.1016/j.celrep.2016.11.026

Jia, Y., Shi, X., Xie, Y., Xie, X., Wang, Y., & Li, S. (2017). Human umbilical cord stem cell conditioned medium versus serum-free culture medium in the treatment of cryopreserved human ovarian tissues in in-vitro culture: a randomized controlled trial. Stem cell research & therapy, 8(1), 152. https://doi.org/10.1186/s13287-017-0604-4

Kurek, M., Albalushi, H., Hovatta, O., & Stukenborg, J. B. (2020). Human Pluripotent Stem Cells in Reproductive Science-a Comparison of Protocols Used to Generate and Define Male Germ Cells from Pluripotent Stem Cells. *International journal of molecular sciences*, *21*(3), 1028. https://doi.org/10.3390/ijms21031028

McLaughlin, M., Albertini, D. F., Wallace, W., Anderson, R. A., & Telfer, E. E. (2018). Metaphase II oocytes from human unilaminar follicles grown in a multi-step culture system. Molecular human reproduction, 24(3), 135–142. https://doi.org/10.1093/molehr/gay002

Makar, K., & Sasaki, K. (2019). Roadmap of germline development and in vitro gametogenesis from pluripotent stem cells. *Andrology*, 10.1111/andr.12726. Advance online publication. https://doi.org/10.1111/andr.12726

de Michele, F., Poels, J., Weerens, L., Petit, C., Evrard, Z., Ambroise, J., Gruson, D., & Wyns, C. (2017). Preserved seminiferous tubule integrity with spermatogonial survival and induction of

Sertoli and Leydig cell maturation after long-term organotypic culture of prepubertal human testicular tissue. Human reproduction (Oxford, England), 32(1), 32–45. https://doi.org/10.1093/humrep/dew300

Oblette, A., Rives, N., Dumont, L., Rives, A., Verhaeghe, F., Jumeau, F., & Rondanino, C. (2017). Assessment of sperm nuclear quality after in vitro maturation of fresh or frozen/thawed mouse pre-pubertal testes. Molecular human reproduction, 23(10), 674–684. https://doi.org/10.1093/molehr/gax048

Ohta, H., Kurimoto, K., Okamoto, I., Nakamura, T., Yabuta, Y., Miyauchi, H., Yamamoto, T., Okuno, Y., Hagiwara, M., Shirane, K., Sasaki, H., & Saitou, M. (2017). In vitro expansion of mouse primordial germ cell-like cells recapitulates an epigenetic blank slate. The EMBO journal, 36(13), 1888–1907. https://doi.org/10.15252/embj.201695862

Rombaut, C., Mertes, H., Heindryckx, B., & Goossens, E. (2018). Human in vitro spermatogenesis from pluripotent stem cells: in need of a stepwise differentiation protocol?. Molecular human reproduction, 24(2), 47–54. https://doi.org/10.1093/molehr/gax065

Rondanino, C., Maouche, A., Dumont, L., Oblette, A., & Rives, N. (2017). Establishment, maintenance and functional integrity of the blood-testis barrier in organotypic cultures of fresh and frozen/thawed prepubertal mouse testes. Molecular human reproduction, 23(5), 304–320. https://doi.org/10.1093/molehr/gax017

Sun, M., Yuan, Q., Niu, M., Wang, H., Wen, L., Yao, C., Hou, J., Chen, Z., Fu, H., Zhou, F., Li, C., Gao, S., Gao, W. Q., Li, Z., & He, Z. (2018). Efficient generation of functional haploid spermatids from human germline stem cells by three-dimensional-induced system. Cell death and differentiation, 25(4), 749–766. https://doi.org/10.1038/s41418-017-0015-1

Yamashiro, C., Sasaki, K., Yokobayashi, S., Kojima, Y., & Saitou, M. (2020). Generation of human oogonia from induced pluripotent stem cells in culture. Nature protocols, 15(4), 1560–1583. https://doi.org/10.1038/s41596-020-0297-5

Zhou, Q., Wang, M., Yuan, Y., Wang, X., Fu, R., Wan, H., Xie, M., Liu, M., Guo, X., Zheng, Y., Feng, G., Shi, Q., Zhao, X. Y., Sha, J., & Zhou, Q. (2016). Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells In Vitro. Cell stem cell, 18(3), 330–340. https://doi.org/10.1016/j.stem.2016.01.017