Scientific and Clinical Advances Advisory Committee (SCAAC) – Matters arising

Monday 7th October 2024

Date	Action	Responsibility	Due date	Progress to date
03/06/2024	The Executive to add lines on PGT-P testing to the **Embryo testing and treatments for disease** patient-facing webpage, including why PGT-P may be offered, the risks and concerns therein, and explaining that PGT-P is not permitted in the UK.	Molly Davies, Scientific Policy Officer	07/10/2024	The **Embryo testing and treatments for disease webpage** has been updated to inform prospective patients that PGT-P is not permitted in the UK. Lines have been reviewed by Frances Flinter.
03/06/2024	The Executive to review **patient-facing website information** on ICSI to ensure it is clear that there is no evidence-base to support the use of ICSI for non-male factor infertility and it is not endorsed by professional body guidelines.	Molly Davies, Scientific Policy Officer	07/10/2024	The 'Intracytoplasmic sperm injection (ICSI)' treatment **webpage** has been updated to make clear to patients that ICSI for non-male factor infertility is not evidence-based.
03/06/2024	The Executive to commission an expert literature review on evidence of the use of testosterone supplementation as a treatment add-on.	Dina Halai, Head of Policy	03/02/2025	The add-ons will be brought back to the Committee for rating once an expert literature review has taken place.

Mitochondrial Donation

Details about this paper

Area(s) of strategy this paper relates to:	Shaping the future and the best care
Meeting:	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item:	5
Paper number:	HFEA (07/10/2024) 005
Meeting date:	07 October 2024
Author:	Molly Davies, Scientific Policy Officer (HFEA)
Annexes	Annex A - The process referred to in the mitochondrial donation regulation

Output from this paper

For information or recommendation?	For recommendation
Recommendation:	Members are asked to: • Consider the progress of the mitochondrial donation programme as presented by the team at Newcastle Fertility Centre at Life; • Consider the progress of research into mitochondrial donation techniques; • Advise if they are aware of any other recent developments; • Advise on any significant implications for licensing and regulation arising out of the scientific developments; and • Review whether any outputs from the HFEA are required.
Resource implications:	TBC
Implementation date:	TBC
Communication(s):	TBC
Organisational risk:	Low

1. Introduction

1.1. Following an extensive review of the evidence and policy issues by the HFEA, in February 2015 the UK Parliament approved the Human Fertilisation and Embryology (Mitochondrial Donation) Regulations to permit the use of maternal spindle transfer (MST) and pronuclear transfer (PNT), collectively 'mitochondrial donation treatment' or 'mitochondrial replacement therapy', to avoid serious mitochondrial disease. This was the first time such treatments had been legislated for as part of a regulatory regime anywhere in the world. The Regulations, which came into force on 29 October 2015, enable licensed fertility clinics in the UK to apply to the HFEA for a licence to perform mitochondrial donation treatments. However, the Authority agreed that it would only accept applications once an independent panel of experts was satisfied that the techniques were sufficiently safe and efficacious to move from research to clinical treatment.

1.2. In July 2016, the expert panel was reconvened to assess the current state of the research and recommended that, having addressed the earlier recommendations set out in their 2014 report, it was appropriate to offer mitochondrial donation techniques as a risk reduction treatment in carefully selected patients. In December 2016, the Authority made the decision to approve the use of mitochondrial donation in certain, specific cases where PGD is inappropriate or likely to be unsuccessful.

1.3. Before a clinic is allowed to carry out either of these techniques, they need to follow the two-stage licensing process: firstly, to apply to the HFEA for a general licence for MST and/or PNT, and then to seek authorisation to undertake the treatment in the case of a particular patient. All applications for treatment are assessed on an individual basis against the tests set out in the law and only after independent advice from experts.

1.4. Only the Newcastle Fertility Centre at Life has been granted a licence to conduct research and treat patients using mitochondrial donation techniques, being licensed to perform clinical treatment with PNT under a treatment and storage licence, and research into MST under a research licence. As of 22 August 2024, 32 patients have been given approval for mitochondrial donation treatment by the HFEA Statutory Approvals Committee. All applications for mitochondrial donation that have been approved by the HFEA Statutory Approvals Committee (SAC) can be found on this page following the links to 'reports archive' and Licence Committee minutes 'other'. Currently, no clinics in the UK have applied to the HFEA to perform clinical treatments with MST. More information on requirements relating to Mitochondrial donation can be found in the HFEA Code of Practice.

1.5. In March 2022, the Australian Parliament passed a Bill, largely modelled on the UK law, to allow the use of mitochondrial donation to prevent the transmission of severe mitochondrial disease. The Mitochondrial Donation Law Reform (Maeve's Law) Act 2022 took effect on 1 October 2022. The National Health and Medical Research Council of Australia is administering the Mitochondrial Donation Pilot Program through the MitoHOPE Program under the leadership of Monash University. It is anticipated that the clinical trial will start enrolling participants in late-2024.

1.6. Mitochondrial donation has been considered as a high-priority topic by the SCAAC since February 2017 when the Committee agreed to monitor further developments in the scientific and clinic literature relating to mitochondrial donation techniques. Literature on the topic has been considered as part of the horizon scanning prioritisation function of the SCAAC since 2017, with

briefings on the topic being given in the February 2017, February 2018 and February 2021 horizon scanning papers. Representatives from Newcastle Fertility Centre at Life have additionally presented on the progress of their Mitochondrial Donation Programme during the SCAAC's January 2022 and July 2023 meetings. A group of SCAAC members visited Newcastle in December 2023. An update on the visit was given at the February 2024 meeting.

1.7. As previously mentioned, an expert panel was last convened to assess the state of the research in July 2016.This paper presents research in the scientific and clinic literature relating to mitochondrial donation techniques published between 30 August 2016[1] and 30 August 2024 and provides a summary of the findings described in published studies, but not an assessment of study validity.

2. Applications of Maternal Spindle Transfer and Pronuclear Transfer

2.1. Maternal spindle transfers (MST) and pronuclear transfer (PNT) have the potential to avoid transmitting serious mitochondrial disease from mother to child. In its clinical application, MST involves transferring the nuclear DNA from an oocyte with abnormal mitochondria and placing it into an oocyte with healthy mitochondria. PNT involves transferring the pronuclei from an embryo that has abnormal mitochondria and placing them into an embryo that has healthy mitochondria.

2.2. One challenge for MST and PNT techniques is the possibility of introducing mitochondrial DNA heteroplasmy into cells of the resultant child. Normally, mitochondrial DNA within individual cells is homogenous, however, in patients who suffer from mitochondrial disease, a mixture of mutated and non-mutated mitochondrial disease can lead to variable expression of disease, depending on the proportion of mutated mitochondrial DNA in different tissues. Whilst introducing healthy mitochondria from a donated source is a successful treatment for preventing the transmission of the majority of mutated mitochondria, small amounts of residual mitochondria may be transferred. If these residual mitochondria contain defective mutations and subsequently replicate, they could lead to unpredictable effects with the persistence/resurgence of mitochondrial disease in the child. As mitochondrial diseases typically manifest when the proportion of mutated mitochondria reach a certain threshold, ongoing monitoring of outcomes in patients is critical to ensure the safety and long-term efficacy of mitochondrial donation treatments.

2.3. The first account of a live birth in Mexico following MST to overcome the transmission of Leigh syndrome was reported in the press 2016. Publication of a case report in 2017, confirmed the birth of a healthy boy with a neonatal mitochondrial DNA (mtDNA) mutation load of 2.36-9.23% (Zhang *et al.*, 2017a).

2.4. The UK media have since reported on the successful live birth of at least one child born following mitochondrial donation treatment performed at Newcastle Fertility Centre at Life in 2023. In response, the HFEA released a statement indicating that further information on the treatment

[1] Literature published since the Scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception (published November 2016) has been considered. Search terms have been broadened to encompass research looking at alternative methods for performing mitochondrial donation and wider applications of existing therapies.

outcomes will be published by the centre in peer reviewed journal. As of 11 September 2024, no information has been published by the Newcastle Fertility Centre.

2.5. The case study by (Tang *et al.*, 2022a) provides additional support for the application of MST and early PNT to overcome the transmission of homoplastic mitochondrial DNA mutations from mother to child. In the study five metaphase II oocytes were used for maternal spindle transfer and one in vitro matured metaphase I oocyte underwent early PNT. All MST reconstructed oocytes fertilised and cleaved with one progressing to the blastocyst stage, whereas the reconstructed PNT zygote reached the morula stage. Samples were found to show an average carryover rate of 2.9%. Further findings of this study are discussed in section 4 below.

2.6. The use of pronuclear transfer in non-human primates has since been reported in a study by (Li *et al.*, 2024). In this study, researchers generated four healthy cynomolgus monkeys using PNT. All individuals survived over 2 years and were reported to have minimal mitochondrial DNA carryover (3.8% - 6.7%) alongside stable heteroplasmy of the initial proportion of maternal mtDNA during development.

2.7. Longitudinal germline transmission of mitochondrial DNA following maternal spindle transfer in rhesus macaque populations has been investigated by (Ma *et al.*, 2021) who found that growth, general health and fertility of offspring is unremarkable and similar to controls. Most animals stably maintained the initial ratio of parental mitochondrial DNA heteroplasmy and donor mitochondria was transmitted from mothers to offspring; however, in one individual initially negligible maternal mtDNA heteroplasmy increased to <17% in some internal tissues and organs, implying the possibility of mtDNA mutation recurrence.

2.8. To support clinical decision making associated with offering mitochondrial donation treatment vs. PGT-M where there is a medical indication, Pickett et al., 2019 developed an algorithm to predict the proportion of future children with safe heteroplasmy levels < 18% based on a given maternal heteroplasmy.

2.9. Research into mitochondrial donation techniques has been reviewed by (Greenfield *et al.*, 2017), (Craven *et al.*, 2017), (Siristatidis *et al.*, 2022), and (Farnezi *et al.*, 2020).

3. Alternative methods for performing mitochondrial donation

3.1. Alternative methods of performing mitochondrial donation in a clinical setting are currently unlawful in the UK. However, research into alternative methods to perform and refine mitochondrial donation techniques can be performed under a UK embryo research licence.

3.2. The review article by (Craven *et al.*, 2017) summarises other potential techniques to perform mitochondrial replacement, including research into polar body [nuclear] transfer (PBT) and germinal vesicle nuclear transfer (GVT):

Polar body nuclear transfer (PBT)

3.3. As described in the review, polar bodies are byproducts extruded from oocytes and zygotes during the first and second meiotic divisions. The first polar body (PB1) is diploid and extruded from the oocyte on completion of the first meiotic division. The second polar body (PB2) is haploid and is extruded from the zygote following the second meiotic division. Both structures are utilised in techniques of PBT which involves either: (1) removal of PB1 from the MII oocyte followed by

transfer to an enucleated MII oocyte and fertilisation (PB1T), or (2) removal of the PB2 from a fertilised PN stage zygote and transfer to a recipient zygote with a removed pronucleus (PB2T).

3.4. Techniques of PBT were considered by the panel of experts (co-ordinated by the HFEA) as an addendum to the third scientific review of safety and efficacy of methods to avoid mitochondrial disease. The panel recommended that additional studies be undertaken both in the basic research field to improve understanding of the biology of human mitochondria especially during development, and on translational research aimed specifically at providing further safety and efficacy information on PBT. Upon the fourth review in 2016, techniques were noted to show promise as a means to minimise mitochondrial DNA (mtDNA) carryover and avoid mitochondrial disease, but that use was limited by the wording of the regulations (The process referred to in the mitochondrial donation regulation can be found at annex A).

3.5. (Ma *et al.*, 2017) have since described the generation of normal diploid zygotes following the transfer of genomes from PB1 into an enucleated donor MII oocyte (PB1T) and fertilisation of reconstructed oocytes. Whilst PB1T generated zygotes developed to blastocysts less frequently (42%) than controls (75%), genome-wide genetic, epigenetic, and transcriptional analyses of PB1T generated zygotes and control ESCs indicated comparable numbers of structural variations and markedly similar DNA methylation and transcriptome profiles. Authors concluded that rescue of PB1 genetic material via introduction into donor cytoplasm may offer a source of oocytes for infertility treatment or mitochondrial replacement therapy for mtDNA disease. Possible application in patients of advanced age or with decreased ovarian reserve is also highlighted.

3.6. To investigate whether the second polar body could also be used as a nuclear donor, (Wu *et al.*, 2017b) established a protocol for the reconstruction of human oocytes or zygotes using first or second polar bodies as donors. A total of 19 good-quality blastocysts from 75 PB1T embryos were obtained and cryopreserved. Of the 17 thawed blastocyst, 10 were euploid and displayed an average mitochondrial DNA carryover of 0.26%. Using PB2T techniques, researchers generated 14 high quality blastocysts from 51 embryos at a comparable efficiency to PB1T methods. 9 blastocysts were euploid with an average mitochondrial DNA carryover of 0.37%. Mitochondrial DNA carryover was maintained at low levels during long-term *in vitro* proliferation and differentiation of embryonic stem cells, suggesting that PBT can be a promising approach to treat mitochondrial-related diseases.

3.7. Following research by (Li *et al.*, 2023) established a spindle-protrusion-retained second polar body separation technique to allow for earlier second polar body transfer for the avoidance of DNA damage accumulation. The optimised method allowed for further elimination of mitochondrial carryover in reconstructed oocytes through a physically based residue removal method. Researchers obtained a close to normal proportion of normal-karyotype blastocyst in both mice and humans. Mouse embryonic stem cells and live-born pups contained almost undetectable mitochondrial DNA carryover.

3.8. The comparative study by (Tang *et al.*, 2019) investigated the efficiency of four different nuclear transfer techniques to overcome mitochondrial disease in NZB/OlaHsd and B6D2F1 mouse models. In addition to looking at MST and PNT techniques, researchers looked at two novel protocols for optimisation of the second polar body transfer technique using mouse and human oocytes. Comparable blastocyst rates among PB1T, PB2T-b, ST and PNT embryos were

recorded, with lower mitochondrial carryover levels being reported in PB1T and PB2T embryos than those generated from MST and PNT.

3.9. Application of first polar body transfer has since been applied to generate a healthy macaque monkey, highlighting a useful non-human primate model for evaluating safety and efficacy of PBT as a method of mitochondrial replacement. Researchers (Wang *et al.*, 2021), found stable low-level mitochondrial DNA heteroplasmy (<5%) in ear and blood samples of the offspring over a 1.5-year period with no evidence of upward mitochondrial DNA drift.

Pre-pronuclear transfer

3.10. Researchers (Wu *et al.*, 2017a) hypothesised that, as the female pre-pronucleus in humans forms at 3.5-4 hours post fertilisation and will not separate completely from the second polar body within 6 hours when it will thereafter be enclosed by the pronuclear envelope, if removed early the pre-pronucleus could be easily isolated with the extruding second polar body to avoid the use of cytoskeleton disruptors in PNT. Applying this strategy, researchers generated 6 good-quality euploid blastocysts which has an average mitochondrial DNA carryover of 0.36%. By generating embryonic stem cell lines, researchers showed that the ratio of mitochondrial DNA carryover remained low and relatively stable in all tested samples.

Germinal vesicle nuclear transfer

3.11. The method of GVT involves removing the germinal vesicle (the nucleus of an immature oocyte arrested in prophase I of the first meiosis) and transferring it to an enucleated oocyte, followed by in vitro maturation and fertilisation.

3.12. Although GVT has been considered as a technique to prevent transmission of mutant mitochondria, the need to manipulate the oocyte during the early stages of development and progress through in vitro maturation is thought to lead to lower yields of blastocyst when using this technique (Craven *et al.*, 2017; Darbandi *et al.*, 2017). As such, research into GVT in recent years has predominantly focused on investigating mechanisms of oocyte meiosis and maturation, including to increase oocyte competence as discussed below (Tanaka and Watanabe, 2018; Darbandi *et al.*, 2020).

Mitochondrial DNA gene editors

3.13. Research summarised within this subsection refers to mitochondrial gene editing rather than mitochondrial donation and therefore sits more closely with another topic prioritised by the SCAAC on heritable genome editing, but we have included research within this paper for completeness. It is important to note that regulation does not permit editing of the mitochondrial genome in reproduction.

3.14. In 2020, (Mok *et al.*, 2020) described the engineering of a mitochondrial DNA gene editor known as DddA-derived cytosine base editors (DdCBE) which have the ability to catalyse C•G-to-T•A conversions in human mtDNA with high target specificity and product purity. This technique has since been applied in embryo genome editing to prevent transmission of mutant maternal DNA.

3.15. Initial feasibility studies in mice were presented by (Guo *et al.*, 2021a) who selected several sites in mouse mitochondrial DNA as DdCBE targets, representative of G-to-A pathogenic mutations in humans. Microinjection of the mRNAs of DdCBE halves in the mouse zygotes or 2-cell embryo successfully generated edited founder mice with a base conversion rate ranging from 2.48% to

28.51%. These were successfully transmitted to offspring of female founders with differing mutation loads. The DdCBE base editor has further been utilised in zebrafish (Guo *et al.*, 2021b), mice (Lee *et al.*, 2021) and rats (Qi *et al.*, 2021, 2023) for human mitochondrial disease modelling and as a therapeutic approach.

3.16. (Wei *et al.*, 2022b) reported on DdCBE-mediated mitochondrial base editing in human embryos with three pronuclei. After in vitro transcription, authors injected the DdCBE mRNA into clinically discarded human embryos with three pronuclei to check protein expression and cellular localisation. Immunostaining results revealed high expression of the DddA-TALE fusion deaminase pairs and proper co-localization in the blastomere cell of human embryos. Injection of human embryos at the cleavage stage was found to significantly improved base editing efficiency of DdCBE, supporting up to 60% cytosine conversion, compared with the injection at the zygote stage (<10%).

3.17. Testing of DdCBE for base editing of mitochondrial DNA in human embryos with three pronuclei was also explored by (Chen *et al.*, 2022). DdCBE pairs targeting pathogenic mutation sites located on human mitochondrial genes were in vitro transcribed to mRNAs by a pre-set promoter and injected into the cytoplasm of human three pronuclear embryos. The results showed that the mutation loads in edited embryos ranged from 1.36% to 58.97% depending upon the site edited and polymerase used for amplification. Authors noted that off-target events could be detected along the mitochondrial genome in cells transfected with DdCBE, suggesting that techniques require further optimisation prior to clinical application. Concerns over off-target effects have also been recorded by (Wei *et al.*, 2022a) and (Lei *et al.*, 2022).

3.18. Precision of these methods has since been further refined by (Lee *et al.*, 2023) who engineered high-fidelity DddA-derived cytosine base editors (HiFi-DdCBEs) with minimal off-target activity by substituting alanine for amino acid residues at the interface between the split $DddA_{tox}$ halves. Following genome sequencing unwanted off-target C-to-T conversions in human mitochondrial DNA were avoided, improving the potential therapeutic application of these techniques. Optimisation of mitochondrial DNA base editing by DdCBEs continues to be explored (Qiu *et al.*, 2024; Wei *et al.*, 2024).

MitoCeption

3.19. MitoCeption is a mitochondria transfer/transplant technique based on the artificial transfer/transplant of mitochondria (AMT/T) to a recipient cell using a thermic shock and centrifugation (Cabrera *et al.*, 2019). To explore whether this technique could be applied for the transfer of exogenous mitochondria to oocytes, (Cabrera *et al.*, 2022) used a xenogeneic study model (human mitochondrial to murine oocytes) to induce the internalisation of exogenous mitochondria in a dose-dependent manner to recipient oocytes in comparison to coincubation. Fluorescence microscopy showed that exogenous and transferred mitochondria by MitoCeption were internalized in oocytes and zygotes and could give rise to healthy pups. This method has been proposed as a potential non-invasive method which could be explored further in the context of assisted reproduction.

4. Wider applications of mitochondrial donation therapies

4.1. Application of mitochondrial replacement techniques have also been proposed as a treatment to improve the quality of oocytes in patients with infertility associated with poor oocyte quality and/or fertilisation failure, as summarised in the review article by (Rodríguez-Varela and Labarta, 2022).

4.2. Using a mouse model, (Yamada and Egli, 2017) demonstrated that nuclear genome transfer (PNT and MST) from a developmentally compromised in vitro aged oocyte to a developmentally competent oocyte was feasible. This transfer could restore developmental competence of oocytes left unfertilised for 20 hours post retrieval, resulting in healthy live births. Increased developmental potential was explained primarily by the correction of abnormal cytokines at anaphase of meiosis and mitosis, a reduction in chromosome segregation errors, and by normalisation of chromosome passenger complex component localisation. Authors concluded that it is therefore possible that mitochondrial replacement therapies may have utility in improving fertility treatment outcomes.

4.3. Such conclusions have since been explored in studies by (Tang *et al.*, 2020) and (Costa-Borges *et al.*, 2020). Using two mouse models, (Tang *et al.*, 2020) demonstrated that PNT and to a lesser extent MST were able to restore embryonic developmental potential in a mouse model of reproductive ageing and embryonic developmental arrest. The proof-of-concept was additionally demonstrated by (Costa-Borges *et al.*, 2020) who showed that in mice replacement of the entire cytoplasm following MST overcame embryo developmental arrest characteristic of non-manipulated oocytes. Resulting mice showed low heteroplasmy levels in multiple organs at adult age, normal histology and fertility. Mice were followed for five generations, revealing that heteroplasmy was reduced in second generation mice and was undetectable in the subsequent generations.

4.4. Four methods of mitochondrial replacement to improve oocyte competence were compared in the case control study by (Darbandi *et al.*, 2020), these included GVT, synchronous ooplasmic transfer and asynchronous ooplasmic transfer using cryopreserved MII oocytes or waste MII oocytes. In the GVT none of the oocytes fused owing to problems in gathering the nuclear structure, however results indicated that other methods of ooplasmic transfer – specifically asynchronous transfer using cryopreserved MII oocytes – may have application in improving oocyte quality.

4.5. Application of PB1T has also been reported for the improvement of poor embryo development resulting from a case of severe embryo fragmentation. Firstly, (Zhang *et al.*, 2017b) generated reconstructed oocytes using fresh and cryopreserved first polar bodies (PB1T) transferred to enucleated metaphase II oocytes, while fresh second polar bodies were removed from fertilised oocytes and used instead of the female pronucleus in donor zygotes. Reconstructed oocytes were then fertilised by ICSI and cultured to the blastocyst stage, producing two fresh and one cryopreserved PBT1 diploid blastocyst (n=6). Cryopreserved PBT1 was subsequently performed for one patient with a history of severe embryo fragmentation. When compared to untreated oocytes, reconstructed oocytes produced three chromosomally normal blastocysts.

4.6. (Zhang *et al.*, 2016) presented a case report of PNT using donor oocytes for a single 30-year-old patient with two previous failed IVF cycles characterised by embryo arrest at the two-cell stage. During the cycle, researchers fertilised eight out of 12 patient oocytes and 12 of 15 donor oocytes;

Patient pronuclei were transferred into an enucleated donor cytoplasm resulting in seven reconstructed zygotes. After the transfer of five reconstructed embryos, a triplet pregnancy was achieved and subsequently reduced to a twin pregnancy. Complications during the pregnancy and premature birth resulted in fetal demise of both twins, however, genetic fingerprinting of the fetal tissue confirmed that the nuclear DNA matched that of the patients and the mtDNA matched that of the oocyte donor. Authors concluded that PNT could aid women experiencing arrested embryo development following IVF.

4.7. Alongside presenting data supporting the use of MST and early PNT techniques to efficiently prevent transmission of homoplastic mtDNA mutations in humans, the case study by (Tang *et al.*, 2022b) presents additional proof-of-concept data supporting the use of MST to overcome fertilisation failure after ICSI due to oocyte quality in humans. Two couples who had previously experienced multiple failed ICSI cycles, characterised by failed fertilisation attributed to a female factor, were enrolled into the study. In this limited population, MST performed prior to ICSI fertilisation resulted in 4/5 and 6/6 fertilized oocytes, providing evidence that MST can overcome failed fertilisation for patients with oocyte-related cause.

4.8. In 2023, (Costa-Borges *et al.*, 2023) undertook a pilot trial to explore the feasibility of using MST as a treatment for infertility in cases of repeat IVF failure. The study focused on women <40 years, with previous IVF attempts characterised by a pattern of low fertilisation rates and/or impaired embryo development. Patients did not have indications of mitochondrial disease. Following twenty-eight MST cycles, which resulted in 19 embryo transfers and 7 clinical pregnancies, 6 children were born. DNA fingerprinting confirmed that the nuclear DNA of MST children was inherited from both parents, without any contribution from the oocyte donor. Paediatric follow-up of the children, performed at intervals from birth to 12-24 months of age, revealed normal development. However, whilst 5 of the children were found to have mtDNA derived almost exclusively from the donor, 1 child showed an increase in the maternal mtDNA haplotype (mitochondrial reversal), accounting for 30% to 60% of the total at birth.

Autologous mitochondria transfer

4.9. To avoid the introduction of a third source of DNA into the oocyte, autologous mitochondrial transfer techniques have been developed. Instead of introducing other cytoplasmic components into the reconstituted oocyte, these techniques typically transfer isolated mitochondria organelles, with the aim of increasing the number of healthy organelles within the oocyte, sometimes referred to as oocyte rejuvenation.

4.10. Autologous mitochondrial transfer differs from that of classic mitochondrial donation therapies to avoid mitochondrial disease as replacement mitochondrial can be sourced from cells of the mother. These techniques are therefore unsuitable for treating maternally inherited mitochondrial diseases. Cell type sources include ovarian stem cells, immature oocytes, granulosa cells and non-ovarian stem cells (Kristensen *et al.*, 2017a; Wolf *et al.*, 2017; Rodríguez-Varela *et al.*, 2021). However, the selection of suitable cells as the source of mitochondria remains controversial (Zhang *et al.*, 2023).

4.11. In 2015, Woods and Tilly developed and commercially launched a variant technique of mitochondrial replacement, termed Autologous Germline Mitochondrial Energy Transfer (AUGUMENT), intended to promote bioenergetic revitalisation of eggs whose capacity for fertilisation and embryogenesis has been compromised by ageing. The technique involved

injecting autologous mitochondria extracted from oocyte precursor cells, which were isolated from an ovarian cortex biopsy, into the patient's own oocytes during the ICSI procedure (Woods and Tilly, 2015).

4.12. Initially clinical reports of AUGUMENT application indicated improvements in outcomes for patients, with one two-centre study reporting a 3- to 6- and 11- to 18-fold increases in clinical pregnancy rates (Fakih, 2015). Encouraging results were also described by (Oktay *et al.*, 2015) Oktay et al. (2015) who reported significantly improved fertilisation rates and embryo grades in a small population of patients (n=10). However, in 2019 a randomized control trial conducted by (Labarta *et al.*, 2019) failed to show improvements in prognosis for patients with previous IVF failure, who then underwent ICSI with autologous mitochondrial transfer when compared to ISCI controls. Despite the fact that no differences were observed in fertilisation rates between the groups, the blastocyst formation rate per zygote was significantly lower in the AUGUMENT group than in the control group and the study was discontinued. Clinical application of AUGUMENT have since been suspended in the USA.

4.13. Techniques of autologous mitochondria transfer have more recently been utilised to transfer mitochondria from non-ovarian stem cells, including adipose-derived stem cells (Yang *et al.*, 2023). Using this technique (Wang *et al.*, 2017) demonstrated that transfer of autologous mitochondria from this source was able to rescue oocyte quality and infertility in aged mice. However, (Sheng *et al.*, 2019) found that transfer of mitochondrial from aged adipose-derived stem cells did not mitigate the poor fertilisation and embryonic development rates of aged oocytes.

4.14. Using naturally aged mice as a model, (Zhang *et al.*, 2021a) demonstrated the feasibility of injecting induced pluripotent stem-cell-derived mitochondria into fertilised oocytes from ageing females. Specifically, this procedure can rescue impaired developmental potential of embryos from ageing females, resulting in an enhanced implantation rate. This however was not seen where mitochondria had been sourced from mouse embryo fibroblast cells, indicating that a synchronous mitochondrial state may be important when supplementing mitochondria in oocytes.

4.15. (Morimoto *et al.*, 2023) applied this technique to human cells, transferring autologous mitochondria extracted from oogonia stem cells to mature oocytes of patients with recurrent failures at the time of ICSI. Good-quality embryo rates, transferable embryo rates and embryo quality score was found to significantly increase after mitochondrial transfer. In the 13 babies born, no abnormalities were reported. Of the tested sample (4 mothers and 5 children) the mitochondrial DNA sequences were identical to the respective maternal sequences at 83 examined sites.

4.16. Transgenerational effects of autologous mitochondrial transfer in mice were investigated by (St. John *et al.*, 2019), whereby researchers assessed litter size, ovarian reserve, weight gain and conducted a histopathological analysis across three generations. Significant increases in litter size and the number of primordial follicles in the ovary matched by changes in global gene expression patterns were reported. Histopathological analysis further revealed that cardiac structure was compromised in first- and second-generation offspring. Conversely, (Kankanam Gamage *et al.*, 2024) found comparable body growth rates across three generations of mice whose oocytes had been supplemented with autologous mitochondria during fertilisation, with no significant histopathological abnormalities detected.

4.17. Downstream effects on the transcriptional profiles of pigs generated through autologous and heterologous mitochondrial DNA transfer were investigated by (Okada *et al.*, 2023). Transcriptome analyses revealed that genes involved in immune response and glyoxylate metabolism were commonly affected in brain, heart and liver tissues by mitochondrial DNA introduction, suggesting that these techniques may influence the expression of genes in adult tissues. DNA methylation and gene expression profiles of blastocysts were also investigated, revealing a significant impact on both gene expression and DNA methylation profiles (Okada *et al.*, 2022).

4.18. (Jiang *et al.*, 2023) recently investigated other potential sources of autologous mitochondria for treating infertility associated with advanced maternal age. Researchers compared autologous adipose, marrow and urine mesenchymal stromal cells as a source of mitochondria. They then highlighted that non-invasively derived urine-derived mesenchymal stem cells act as a useful source for autologous mitochondria when treating infertile females with advanced age or repeated pregnancy failure. Other sources of non-invasive autologous mitochondrial sources include induced pluripotent stem cells (Zhang *et al.*, 2021b) and umbilical cord mesenchymal stem cell induced into granulosa cells, as discussed by (Tang *et al.*, 2022c).

4.19. Autologous mitochondria transfer has also been explored to restore post-fertilisation development which has decreased due to mitochondrial damage following cryopreservation. This research has highlighted additional applications for such techniques (Kankanam Gamage *et al.*, 2022).

4.20. The following review articles further summarise the current progress on autologous mitochondria transfer: (Kristensen *et al.*, 2017b), (Cecchino *et al.*, 2018), (Mobarak *et al.*, 2019), (Cozzolino *et al.*, 2019), (Tilly and Woods, 2020), (Jiang and Shen, 2022), and (Clemente-Suárez *et al.*, 2023).

Mitochondria as diagnostic and therapeutic targets

4.21. Mitochondria have also been investigated as potential diagnostic and therapeutic targets to improve outcomes of assisted reproductive treatment (Podolak *et al.*, 2022). The review article by (Yildirim and Seli, 2024a) summarises research on several pharmacological compounds which have been investigated as having the potential to enhance oocyte quality and pregnancy outcomes by targeting mitochondrial function. These include Coenzyme Q10, Mitoquinone, Resveratrol, Rapamycin and Nicotinamide mononucleotide (Yildirim and Seli, 2024a).

4.22. Mitochondrial copy number and assessment of NADH and flavin adenine dinucleotide content have also been explored as potential predictors of reproductive senescence and embryo competence, as discussed by (Yildirim and Seli, 2024b) in their 2024 review. As summarised, attempts to predict embryo competence are currently limited.

Guidelines

4.23. Mitochondrial replacement therapy to improve the quality of the oocytes in women with difficulties in conceiving is considered a treatment add-on by the European Society of Human and Reproductive Medicine (ESHRE) (Lundin *et al.*, 2023). As there is insufficient evidence of a benefit on pregnancy outcomes or safety, the use of mitochondrial replacement therapy for such purposes is not recommended. As the application of mitochondrial donation therapy for the treatment of infertility is prohibited in the UK, it is not rated by the HFEA as a treatment add-on.

5. Conclusions

5.1. Since 2016, mitochondrial donation has been legally permitted in the UK under the HFE Act to avoid serious mitochondrial disease and regulated by the HFEA. Progress with the mitochondrial donation programme at Newcastle Fertility Centre at Life has resulted in 32 patients being given approval for PNT treatment.

5.2. Research into alternative techniques of mitochondrial donation, such as PBT, are still in experimental stages. However, that research indicates that such techniques may be able to address concerns with mitochondrial heteroplasmy seen across established methods. The HFE Act would need to be updated to allow for clinical application of some of these techniques.

5.3. Novel approaches in heritable genome editing have also been proposed to prevent the transmission of mutant maternal DNA, highlighting a potential future alternative to existing methods. However, as with nuclear and epigenetic genome editing, there are several technical and ethical challenges which will need to be overcome before such techniques may be applied for treatment.

5.4. Mitochondrial donation therapies have additionally begun to be explored more broadly as techniques to improve oocyte quality and rescue developmental competence, specifically in fertility treatment. These techniques have shown some potential to address failures in assisted reproduction treatments associated with failed fertilisation and embryo arrest. Research into autologous mitochondrial replacement techniques offer a personalised approach when utilising therapies for such purposes. Moreover, advancements in mitochondrial replacement therapies have expanded the possibilities for using mitochondria as both diagnostic markers and therapeutic tools in fertility treatment.

6. Recommendations

6.1. Members are asked to:

- Consider the progress of the mitochondrial donation programme as presented by the team at Newcastle Fertility Centre at Life, covering embryological and clinical aspects, and any follow up data on children born using PNT;
- Consider the progress of research into mitochondrial donation techniques, eg PBT techniques, and alternative applications of mitochondrial donation treatment;
- Advise if they are aware of any other recent developments;
- Advise on any significant implications for licensing and regulation arising out of the scientific developments eg should PBT techniques be considered for approval as a technique of MRT in the UK; and
- Review whether any outputs from the HFEA are required e.g. is there a need to add additional information for patients to **HFEA's mitochondrial donation treatment webpage** to highlight that therapies cannot be used for infertility treatment.

7. References

Cabrera F, Castañeda V, Morales E, Velarde F, Ortega M, Leon-Sosa A, Jorgensen C, Caicedo A. Early evidence of the artificial transfer/transplant of mitochondria to oocytes and zygotes by MitoCeption. *Mitochondrion* [Internet] 2022;**65**:102–112. Mitochondrion.

Cabrera F, Ortega M, Velarde F, Parra E, Gallardo S, Barba D, Soto L, Peña G, Pedroza LA, Jorgensen C, *et al.* Primary allogeneic mitochondrial mix (PAMM) transfer/transplant by MitoCeption to address damage in PBMCs caused by ultraviolet radiation. *BMC Biotechnol* [Internet] 2019;**19**:. BMC Biotechnol.

Cecchino GN, Seli E, Alves da Motta EL, García-Velasco JA. The role of mitochondrial activity in female fertility and assisted reproductive technologies: overview and current insights. *Reprod Biomed Online* [Internet] 2018;**36**:686–697. Reprod Biomed Online.

Chen X, Liang D, Guo J, Zhang J, Sun H, Zhang X, Jin J, Dai Y, Bao Q, Qian X, *et al.* DdCBE-mediated mitochondrial base editing in human 3PN embryos. *Cell Discov* [Internet] 2022;**8**:. Cell Discov.

Clemente-Suárez VJ, Martín-Rodríguez A, Yáñez-Sepúlveda R, Tornero-Aguilera JF. Mitochondrial Transfer as a Novel Therapeutic Approach in Disease Diagnosis and Treatment. *Int J Mol Sci* [Internet] 2023;**24**:. Int J Mol Sci.

Costa-Borges N, Nikitos E, Späth K, Miguel-Escalada I, Ma H, Rink K, Coudereau C, Darby H, Koski A, Dyken C Van, *et al.* First pilot study of maternal spindle transfer for the treatment of repeated in vitro fertilization failures in couples with idiopathic infertility. *Fertil Steril* [Internet] 2023;**119**:964–973. Fertil Steril.

Costa-Borges N, Spath K, Miguel-Escalada I, Mestres E, Balmaseda R, Serafín A, Garcia-Jimenéz M, Vanrell I, González J, Rink K, *et al.* Maternal spindle transfer overcomes embryo developmental arrest caused by ooplasmic defects in mice. *Elife* [Internet] 2020;**9**:. Elife.

Cozzolino M, Marin D, Sisti G. New Frontiers in IVF: mtDNA and autologous germline mitochondrial energy transfer. *Reprod Biol Endocrinol* [Internet] 2019;**17**:. Reprod Biol Endocrinol.

Craven L, Tang MX, Gorman GS, Sutter P De, Heindryckx B. Novel reproductive technologies to prevent mitochondrial disease. *Hum Reprod Update* [Internet] 2017;**23**:501–519. Hum Reprod Update.

Darbandi S, Darbandi M, Agarwal A, Khorshid HRK, Sadeghi MR, Esteves SC, Sengupta P, Dutta S, Fathi Z, Zeraati H, *et al.* Comparing four laboratory three-parent techniques to construct human aged non-surrounded nucleolus germinal vesicle oocytes: A case-control study. *Int J Reprod Biomed* [Internet] 2020;**18**:425–438. Int J Reprod Biomed.

Darbandi S, Darbandi M, Khorshid HRK, Shirazi A, Sadeghi MR, Agarwal A, Al-Hasani S, Naderi MM, Ayaz A, Akhondi MM. Reconstruction of mammalian oocytes by germinal vesicle transfer: A systematic review. *Int J Reprod Biomed* [Internet] 2017;**15**:601. Shahid Sadoughi University of Medical Sciences and Health Services.

Fakih MH. The AUGMENTSM Treatment: Physician Reported Outcomes of the Initial Global Patient Experience. 2015;

Farnezi HCM, Goulart ACX, Santos A Dos, Ramos MG, Penna MLF. Three-parent babies: Mitochondrial replacement therapies. *JBRA Assist Reprod* [Internet] 2020;**24**:189–196. JBRA Assist Reprod.

Greenfield A, Braude P, Flinter F, Lovell-Badge R, Ogilvie C, Perry ACF. Assisted reproductive technologies to prevent human mitochondrial disease transmission. *Nature Biotechnology 2017 35:11* [Internet] 2017;**35**:1059–1068. Nature Publishing Group.

Guo J, Chen X, Liu Z, Sun H, Zhou Y, Dai Y, Ma Y, He L, Qian X, Wang J, *et al.* DdCBE mediates efficient and inheritable modifications in mouse mitochondrial genome. *Mol Ther Nucleic Acids* [Internet] 2021a;**27**:73–80. Mol Ther Nucleic Acids.

Guo J, Zhang X, Chen X, Sun H, Dai Y, Wang J, Qian X, Tan L, Lou X, Shen B. Precision modeling of mitochondrial diseases in zebrafish via DdCBE-mediated mtDNA base editing. *Cell Discov* [Internet] 2021b;**7**:. Cell Discov.

Jiang Z, Shen H. Mitochondria: emerging therapeutic strategies for oocyte rescue. *Reprod Sci* [Internet] 2022;**29**:711–722. Reprod Sci.

Jiang Z, Shi C, Han H, Fu M, Zhu H, Han T, Fei J, Huang Y, Jin Z, He J, *et al.* Autologous non-invasively derived stem cells mitochondria transfer shows therapeutic advantages in human embryo quality rescue. *Biol Res* [Internet] 2023;**56**:. Biol Res.

John JC St., Makanji Y, Johnson JL, Tsai TS, Lagondar S, Rodda F, Sun X, Pangestu M, Chen P, Temple-Smith P. The transgenerational effects of oocyte mitochondrial supplementation. *Sci Rep* [Internet] 2019;**9**:. Sci Rep.

Kankanam Gamage SU, Hashimoto S, Miyamoto Y, Nakano T, Yamanaka M, Kitaji H, Takada Y, Matsumoto H, Koike A, Satoh M, *et al.* Supplementation with autologous adipose stem cell-derived mitochondria can be a safe and promising strategy for improving oocyte quality. *J Assist Reprod Genet* [Internet] 2024;**41**:. J Assist Reprod Genet.

Kankanam Gamage US, Hashimoto S, Miyamoto Y, Nakano T, Yamanaka M, Koike A, Satoh M, Morimoto Y. Mitochondria Transfer from Adipose Stem Cells Improves the Developmental Potential of Cryopreserved Oocytes. *Biomolecules* [Internet] 2022;**12**:. Biomolecules.

Kristensen SG, Pors SE, Andersen CY. Improving oocyte quality by transfer of autologous mitochondria from fully grown oocytes. *Hum Reprod* [Internet] 2017a;**32**:725–732. Hum Reprod.

Kristensen SG, Pors SE, Andersen CY. Diving into the oocyte pool. *Curr Opin Obstet Gynecol* [Internet] 2017b;**29**:112–118. Curr Opin Obstet Gynecol.

Labarta E, los Santos MJ de, Herraiz S, Escribá MJ, Marzal A, Buigues A, Pellicer A. Autologous mitochondrial transfer as a complementary technique to intracytoplasmic sperm injection to improve embryo quality in patients undergoing in vitro fertilization-a randomized pilot study. *Fertil Steril* [Internet] 2019;**111**:86–96. Fertil Steril.

Lee H, Lee S, Baek G, Kim A, Kang BC, Seo H, Kim JS. Mitochondrial DNA editing in mice with DddA-TALE fusion deaminases. *Nature Communications 2021 12:1* [Internet] 2021;**12**:1–6. Nature Publishing Group.

Lee S, Lee H, Baek G, Kim JS. Precision mitochondrial DNA editing with high-fidelity DddA-derived base editors. *Nat Biotechnol* [Internet] 2023;**41**:378–386. Nat Biotechnol.

Lei Z, Meng H, Liu L, Zhao H, Rao X, Yan Y, Wu H, Liu M, He A, Yi C. Mitochondrial base editor induces substantial nuclear off-target mutations. *Nature* [Internet] 2022;**606**:804–811. Nature.

Li CY, Liu XC, Li YZ, Wang Y, Nie YH, Xu YT, Zhang XT, Lu Y, Sun Q. Generation of mitochondrial replacement monkeys by female pronucleus transfer. *Zool Res* [Internet] 2024;**45**:292–298. Zool Res.

Li W, Liao X, Lin K, Cai R, Guo H, Ma M, Wang Y, Xie Y, Zhang S, Yan Z, *et al.* Earlier second polar body transfer and further mitochondrial carryover removal for potential mitochondrial replacement therapy. *MedComm (Beijing)* [Internet] 2023;**4**:. MedComm (2020).

Lundin K, Bentzen JG, Bozdag G, Ebner T, Harper J, Clef N Le, Moffett A, Norcross S, Polyzos NP, Rautakallio-Hokkanen S, *et al.* Good practice recommendations on add-ons in reproductive medicine. *Human Reproduction* [Internet] 2023;**38**:2062–2104. Oxford Academic.

Ma H, Dyken C Van, Darby H, Mikhalchenko A, Marti-Gutierrez N, Koski A, Liang D, Li Y, Tippner-Hedges R, Kang E, *et al.* Germline transmission of donor, maternal and paternal mtDNA in primates. *Hum Reprod* [Internet] 2021;**36**:493–505. Hum Reprod.

Ma H, O'Neil RC, Marti Gutierrez N, Hariharan M, Zhang ZZ, He Y, Cinnioglu C, Kayali R, Kang E, Lee Y, *et al.* Functional Human Oocytes Generated by Transfer of Polar Body Genomes. *Cell Stem Cell* [Internet] 2017;**20**:112–119. Cell Stem Cell.

Mobarak H, Heidarpour M, Tsai PSJ, Rezabakhsh A, Rahbarghazi R, Nouri M, Mahdipour M. Autologous mitochondrial microinjection; a strategy to improve the oocyte quality and subsequent reproductive outcome during aging. *Cell Biosci* [Internet] 2019;**9**:. Cell Biosci.

Mok BY, Moraes MH de, Zeng J, Bosch DE, Kotrys A V., Raguram A, Hsu FS, Radey MC, Peterson SB, Mootha VK, *et al.* A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. *Nature* [Internet] 2020;**583**:631–637. Nature.

Morimoto Y, Gamage USK, Yamochi T, Saeki N, Morimoto N, Yamanaka M, Koike A, Miyamoto Y, Tanaka K, Fukuda A, *et al.* Mitochondrial Transfer into Human Oocytes Improved Embryo Quality and Clinical Outcomes in Recurrent Pregnancy Failure Cases. *Int J Mol Sci* [Internet] 2023;**24**:. Int J Mol Sci.

Okada T, McIlfatrick S, Hin N, Aryamanesh N, Breen J, John JC St. Mitochondrial supplementation of Sus scrofa metaphase II oocytes alters DNA methylation and gene expression profiles of blastocysts. *Epigenetics Chromatin* [Internet] 2022;**15**:. Epigenetics Chromatin.

Okada T, Penn A, John JC St. Mitochondrial DNA Supplementation of Oocytes Has Downstream Effects on the Transcriptional Profiles of Sus scrofa Adult Tissues with High mtDNA Copy Number. *Int J Mol Sci* [Internet] 2023;**24**:. Int J Mol Sci.

Oktay K, Baltaci V, Sonmezer M, Turan V, Unsal E, Baltaci A, Aktuna S, Moy F. Oogonial Precursor Cell-Derived Autologous Mitochondria Injection to Improve Outcomes in Women With Multiple IVF Failures Due to Low Oocyte Quality: A Clinical Translation. *Reprod Sci* [Internet] 2015;**22**:1612–1617. Reprod Sci.

Pickett SJ, Blain A, Ng YS, Wilson IJ, Taylor RW, McFarland R, Turnbull DM, Gorman GS. Mitochondrial Donation - Which Women Could Benefit? *N Engl J Med* [Internet] 2019;**380**:1971–1972. N Engl J Med.

Podolak A, Woclawek-Potocka I, Lukaszuk K. The Role of Mitochondria in Human Fertility and Early Embryo Development: What Can We Learn for Clinical Application of Assessing and Improving Mitochondrial DNA? *Cells* [Internet] 2022;**11**:. Cells.

Qi X, Chen X, Guo J, Zhang X, Sun H, Wang J, Qian X, Li B, Tan L, Yu L, *et al.* Precision modeling of mitochondrial disease in rats via DdCBE-mediated mtDNA editing. *Cell Discov* [Internet] 2021;**7**:. Cell Discov.

Qi X, Tan L, Zhang X, Jin J, Kong W, Chen W, Wang J, Dong W, Gao L, Luo L, *et al.* Expanding DdCBE-mediated targeting scope to aC motif preference in rat. *Mol Ther Nucleic Acids* [Internet] 2023;**32**:1–12. Mol Ther Nucleic Acids.

Qiu J, Wu H, Xie Q, Zhou Y, Gao Y, Liu J, Jiang X, Suo L, Kuang Y. Harnessing accurate mitochondrial DNA base editing mediated by DdCBEs in a predictable manner. *Front Bioeng Biotechnol* [Internet] 2024;**12**:. Front Bioeng Biotechnol.

Rodríguez-Varela C, Herraiz S, Labarta E. Mitochondrial enrichment in infertile patients: a review of different mitochondrial replacement therapies. *Ther Adv Reprod Health* [Internet] 2021;**15**:263349412110235. Ther Adv Reprod Health.

Rodríguez-Varela C, Labarta E. Role of Mitochondria Transfer in Infertility: A Commentary. *Cells* [Internet] 2022;**11**:1867. Multidisciplinary Digital Publishing Institute (MDPI).

Sheng X, Yang Y, Zhou J, Yan G, Liu M, Xu L, Li Z, Jiang R, Diao Z, Zhen X, *et al.* Mitochondrial transfer from aged adipose-derived stem cells does not improve the quality of aged oocytes in C57BL/6 mice. *Mol Reprod Dev* [Internet] 2019;**86**:516–529. Mol Reprod Dev.

Siristatidis C, Mantzavinos T, Vlahos N. Maternal spindle transfer for mitochondrial disease: lessons to be learnt before extending the method to other conditions? *Hum Fertil* [Internet] 2022;**25**:838–847. Taylor & Francis.

Tanaka A, Watanabe S. Can cytoplasmic donation rescue aged oocytes? *Reprod Med Biol* [Internet] 2018;**18**:128–139. Reprod Med Biol.

Tang M, Boel A, Castelluccio N, Cardona Barberán A, Christodoulaki A, Bekaert B, Popovic M, Meerschaut F Vanden, Sutter P De, Menten B, *et al.* Human germline nuclear transfer to overcome mitochondrial disease and failed fertilization after ICSI. *J Assist Reprod Genet* [Internet] 2022a;**39**:609–618. J Assist Reprod Genet.

Tang M, Boel A, Castelluccio N, Cardona Barberán A, Christodoulaki A, Bekaert B, Popovic M, Meerschaut F Vanden, Sutter P De, Menten B, *et al.* Human germline nuclear transfer to overcome mitochondrial disease and failed fertilization after ICSI. *J Assist Reprod Genet* [Internet] 2022b;**39**:609–618. J Assist Reprod Genet.

Tang M, Guggilla RR, Gansemans Y, Jeught M Van Der, Boel A, Popovic M, Stamatiadis P, Ferrer-Buitrago M, Thys V, Coster R Van, *et al.* Comparative analysis of different nuclear transfer techniques to prevent the transmission of mitochondrial DNA variants. *Mol Hum Reprod* [Internet] 2019;**25**:797–810. Mol Hum Reprod.

Tang M, Popovic M, Stamatiadis P, Jeught M van der, Coster R van, Deforce D, Sutter P de, Coucke P, Menten B, Stoop D, *et al.* Germline nuclear transfer in mice may rescue poor embryo development associated with advanced maternal age and early embryo arrest. *Hum Reprod* [Internet] 2020;**35**:1562–1577. Hum Reprod.

Tang S, Yang N, Yu M, Wang S, Hu X, Ni H, Cai W. Noninvasive autologous mitochondria transport improves the quality and developmental potential of oocytes from aged mice. *F S Sci* [Internet] 2022c;**3**:310–321. F S Sci.

Tilly JL, Woods DC. The obligate need for accuracy in reporting preclinical studies relevant to clinical trials: autologous germline mitochondrial supplementation for assisted human reproduction as a case study. *Ther Adv Reprod Health* [Internet] 2020;**14**:263349412091735. Ther Adv Reprod Health.

Wang Z, Li Y, Yang X, Wang Y, Nie Y, Xu Y, Zhang X, Lu Y, Zhang T, Liu Q, *et al.* Mitochondrial replacement in macaque monkey offspring by first polar body transfer. *Cell Res* [Internet] 2021;**31**:233–236. Cell Res.

Wang ZB, Hao JX, Meng TG, Guo L, Dong MZ, Fan LH, Ouyang YC, Wang G, Sun QY, Ou XH, *et al.* Transfer of autologous mitochondria from adipose tissue-derived stem cells rescues oocyte quality and infertility in aged mice. *Aging* [Internet] 2017;**9**:2480–2488. Aging (Albany NY).

Wei Y, Jin M, Huang S, Yao F, Ren N, Xu K, Li S, Gao P, Zhou Y, Chen Y, *et al.* Enhanced C-To-T and A-To-G Base Editing in Mitochondrial DNA with Engineered DdCBE and TALED. *Adv Sci (Weinh)* [Internet] 2024;**11**:. Adv Sci (Weinh).

Wei Y, Li Z, Xu K, Feng H, Xie L, Li D, Zuo Z, Zhang M, Xu C, Yang H, *et al.* Mitochondrial base editor DdCBE causes substantial DNA off-target editing in nuclear genome of embryos. *Cell Discov* [Internet] 2022a;**8**:. Cell Discov.

Wei Y, Xu C, Feng H, Xu K, Li Z, Hu J, Zhou L, Wei Y, Zuo Z, Zuo E, *et al.* Human cleaving embryos enable efficient mitochondrial base-editing with DdCBE. *Cell Discov* [Internet] 2022b;**8**:. Cell Discov.

Wolf DP, Morey R, Kang E, Ma H, Hayama T, Laurent LC, Mitalipov S. Concise Review: Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer: A Horse in the Race? *Stem Cells* [Internet] 2017;**35**:26–34. Stem Cells.

Woods DC, Tilly JL. Autologous Germline Mitochondrial Energy Transfer (AUGMENT) in Human Assisted Reproduction. *Semin Reprod Med* [Internet] 2015;**33**:410–421. Semin Reprod Med.

Wu K, Chen T, Huang S, Zhong C, Yan J, Zhang X, Li J, Gao Y, Zhao H, Chen ZJ. Mitochondrial replacement by pre-pronuclear transfer in human embryos. *Cell Research 2017 27:6* [Internet] 2017a;**27**:834–837. Nature Publishing Group.

Wu K, Zhong C, Chen T, Zhang X, Tao W, Zhang J, Li H, Zhao H, Li J, Chen ZJ. Polar bodies are efficient donors for reconstruction of human embryos for potential mitochondrial replacement therapy. *Cell Research 2017 27:8* [Internet] 2017b;**27**:1069–1072. Nature Publishing Group.

Yamada M, Egli D. Genome Transfer Prevents Fragmentation and Restores Developmental Potential of Developmentally Compromised Postovulatory Aged Mouse Oocytes. *Stem Cell Reports* [Internet] 2017;**8**:576–588. Stem Cell Reports.

Yang Y, Zhang C, Sheng X. Mitochondrial Transfer from Mouse Adipose-Derived Mesenchymal Stem Cells into Aged Mouse Oocytes. *J Vis Exp* [Internet] 2023;**2023**:. J Vis Exp.

Yildirim RM, Seli E. Mitochondria as therapeutic targets in assisted reproduction. *Human Reproduction* [Internet] 2024a;Available from: https://dx.doi.org/10.1093/humrep/deae170.

Yildirim RM, Seli E. Mitochondria as determinants of reproductive senescence and competence: implications for diagnosis of embryo competence in assisted reproduction. *Human Reproduction* [Internet] 2024b;Available from: https://dx.doi.org/10.1093/humrep/deae171.

Zhang C, Tao L, Yue Y, Ren L, Zhang Z, Wang X, Tian J, An L. Mitochondrial transfer from induced pluripotent stem cells rescues developmental potential of in vitro fertilized embryos from aging females†. *Biol Reprod* [Internet] 2021a;**104**:1114–1125. Biol Reprod.

Zhang C, Tao L, Yue Y, Ren L, Zhang Z, Wang X, Tian J, An L. Mitochondrial transfer from induced pluripotent stem cells rescues developmental potential of in vitro fertilized embryos from aging females†. *Biol Reprod* [Internet] 2021b;**104**:1114–1125. Biol Reprod.

Zhang J, Liu H, Luo S, Lu Z, Chávez-Badiola A, Liu Z, Yang M, Merhi Z, Silber SJ, Munné S, *et al.* Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. *Reprod Biomed Online* [Internet] 2017a;**34**:361–368. Reprod Biomed Online.

Zhang J, Zhuang G, Zeng Y, Grifo J, Acosta C, Shu Y, Liu H. Pregnancy derived from human zygote pronuclear transfer in a patient who had arrested embryos after IVF. *Reprod Biomed Online* [Internet] 2016;**33**:529–533. Reprod Biomed Online.

Zhang Q, Hao JX, Liu BW, Ouyang YC, Guo JN, Dong MZ, Wang ZB, Gao F, Yao YQ. Supplementation of mitochondria from endometrial mesenchymal stem cells improves oocyte quality in aged mice. *Cell Prolif* [Internet] 2023;**56**:. Cell Prolif.

Zhang SP, Lu CF, Gong F, Xie PY, Hu L, Zhang SJ, Lu GX, Lin G. Polar body transfer restores the developmental potential of oocytes to blastocyst stage in a case of repeated embryo fragmentation. *J Assist Reprod Genet* [Internet] 2017b;**34**:563–571. J Assist Reprod Genet.

8.

Permitted egg: process

***4.**—(1) The process referred to in regulation 3(a) consists of the following two steps.*

(2) In step 1—

(a)either—

(i)all the nuclear DNA of an egg ("egg A") is removed, or

(ii)all the nuclear DNA of egg A other than polar body nuclear DNA is removed; and

(b)either—

(i)all the nuclear DNA of another egg ("egg B") is removed, or

(ii)all the nuclear DNA of egg B other than polar body nuclear DNA is removed.

(3) In step 2 all the nuclear DNA of egg B which is not polar body nuclear DNA is inserted into egg A.

Human stem cell-based embryo models (SCBEMs)

Details about this paper

Area(s) of strategy this paper relates to:	Shaping the future
Meeting	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item	6
Paper number	HFEA (07/10/2024) 006
Meeting date	7 October 2024
Author	Dina Halai, Head of Regulatory Policy, Scientific Molly Davies, Scientific Policy Officer
Annexes	Annex A: Scientific literature review Annex B: Definition of embryos, eggs and sperm in the HFE Act Annex C: Differences between SCBEMs and human embryos as outlined in the G-SCBEM Code of Practice

Output from this paper

For information or recommendation?	For recommendation
Recommendation	Members are asked to advise on: • Applications of SCBEMs in research; • Limits on SCBEM research; and • How should the Act be updated to better define and differentiate between SCBEMs and "live human embryos".
Resource implications	TBC
Implementation date	TBC
Communication(s)	TBC
Organisational risk	☐ Low ☒ Medium ☐ High

1. Introduction

1.1. Stem cell-based embryo models (SCBEMs) are not explicitly regulated by the Human Fertilisation and Embryology Act 1990 (the HFE Act). This paper will outline the scientific considerations for bringing human SCBEMs within the HFE Act to 'future proof' it, so that it is better able to accommodate future scientific developments and new technologies within this field, and provide a summary of recent research developments in this area.

1.2. The SCAAC will be asked to give their advice and/or make recommendations concerning human SCBEMs, focusing on the scientific and technical aspects including potential benefits and drawbacks.

1.3. Any advice or recommendations received from SCAAC will feed into the development of an Authority paper for the January 2025 Authority meeting. At that meeting the Authority will consider both the scientific/technical case as well as the wider ethical arguments to come to a consensus on proposals.

1.4. Any change in the Act is for Government and Parliament. However, it continues to be important for the HFEA to lead the discussions and understand the scientific, legal and ethical implications concerning SCBEMs.

2. Background

2.1. The HFEA published proposals for modernising the HFE Act in November 2023. One of the four areas where recommendations were made was in future scientific developments and innovation. The recommendations made:

- The Act should explicitly give the HFEA greater discretion to support innovation in treatment and research.
- The Act should be amended to 'future proof' it, so that it is better able to accommodate future scientific developments and new technologies.

2.2. One of the areas identified under future scientific developments was human stem-cell based embryo models (SCBEMs) which are outside the regulatory categories of the HFE Act. Definition of embryos, eggs and sperm in the HFE Act can be found in annex B. The HFEA are responsible for the regulation of human embryo research; the HFE Act's 2008 amendments do not explicitly ban research on human SCBEMs, but the application of embryo models in fertility treatment is not allowed. Furthermore, the Human Reproductive Cloning Act 2001 (legislation.gov.uk) '*prohibits the placing in a woman of a human embryo which has been created otherwise than by fertilisation'*. There is currently no legal prohibition preventing transfer of a human SCBEMs into the uterus of a non-human animal.

2.3. Given the potential of human SCBEMs to be used in human research and the benefits they might offer in improving our understanding of early development of embryos, the SCAAC continue to monitor follow-up studies through its horizon scanning processes.

2.4. Currently, research using human embryos requires a research licence from the HFEA. The Authority considers applications for research licences and may grant a licence if the research can be considered necessary and desirable to achieve at least one of the following principal purposes as defined in the HFE Act (2008):

- (a) increasing knowledge about serious disease or other serious medical conditions,
- (b) developing treatments for serious disease or other serious medical conditions,
- (c) increasing knowledge about the causes of any congenital disease or congenital medical condition that does not fall within paragraph (a),
- (d) promoting advances in the treatment of infertility,
- (e) increasing knowledge about the causes of miscarriage,
- (f) developing more effective techniques of contraception,
- (g) developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation, or
- (h) increasing knowledge about the development of embryos.

2.5. The topic of SCBEMs (originally known as synthetic human entities with embryo-like features) was first introduced to the SCAAC as a high-priority topic in February 2018. The SCAAC last considered research in this area as part of its horizon scanning process in February 2024 and concluded that, although in humans it is impossible to know what the developmental potential is, it is essential for the committee to understand these models' capabilities to ensure any new regulations can appropriately capture the evolution of these models. The SCAAC also agreed that it is important to reassure society that there are specific scientific goals for doing work on embryo models.

2.6. Progress of research into human embryonic and embryonic-like stem cells was also considered by the SCAAC in June 2024 where the Committee noted that new methods to derive stem cell lineages are improving in consistency, however, concerns with epigenetic errors and the introduction of mutations persist. If such errors prevail in initial hESC-like cell lineages, development of subsequent SCBEM or organoids may not be truly representative of human development. A barrier for research into SCBEM and newly derived cell lineages remains a lack of high-quality research using human embryos, specifically with regards to the second week of development and beyond.

3. What are SCBEMs?

3.1. SCBEMs is an umbrella term for a variety of structures with different features and uses in research. Some terms or categories refer to the stage that is being simulated (e.g. blastoid and gastruloid), others relate to specific embryonic parts or tissues (such as neuruoloids). Stem cell-based embryo models can therefore be generated with varying degrees of completeness and reflecting different stages of development.

3.2. SCBEMs are mainly derived from human pluripotent stem cells, and are organised structures which closely mimic key developmental processes of early-stage human embryos, including cellular heterogeneity and patterning. In replicating the early developmental stages, SCBEMs open avenues for research which are otherwise limited by technical and ethical limitations when using human embryos. Unlike embryos, SCBEM can be manipulated and studied under controlled conditions, bringing with them the potential for novel insights into embryonic development, the process of implantation and development of disease. Another advantage of SCBEMs is that they have the potential to be scalable, which opens opportunities such as toxicology testing.

3.3. Efforts have also been made to organise SCBEMs into categories based on ethically significant characteristics, however there is still debate that some models cannot straightforwardly be classified as one or the other. The International Society for Stem Cell Research (ISSCR) guidance 2021 relies on a distinction between integrated and non-integrated models:

- "Non-integrated stem cell-based embryo models: These stem cell-based embryo models will experimentally recapitulate some, but not all aspects of the peri-implantation embryo, for example differentiation of the embryonic sac or embryonic disc in the absence of extraembryonic cells. These stem cell-based embryo models do not have any reasonable expectations of specifying additional cell types that would result in formation of an integrated embryo model. Gastruloids are an example of a non-integrated stem cell-based embryo model.
- Integrated stem cell-based embryo models: These stem cell-based embryo models contain the relevant embryonic and extra-embryonic structures and could potentially achieve the complexity where they might realistically manifest the ability to undergo further integrated development if cultured for additional time in vitro. A guiding principle of review should be that the integrated stem cell- based embryo models should be used to address a scientific question deemed highly meritorious by a rigorous review process. Blastoids are an example of an integrated stem cell model."

3.4. Though other methods may be used in future, human SCBEMs are currently developed from either human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), or from cells that are in the process of reprogramming but that have not yet reached full iPSC identity. In the UK, hESCs can be obtained from donated embryos, or from the UK Stem Cell Bank (the sole public repository of all UK-derived human embryonic stem cell lines). iPSCs can be obtained from donated tissue and resource banks such as the Human Induced Pluripotent Stem Cells Initiative.

3.5. While there is currently no evidence of successful models of the zygote or early cleavage stage embryo, a range of models have been produced which mimic early embryonic development to varying degrees. These can be broadly broken down into models of blastoids, gastruloids, and axioloids.

3.6. Blastoids refer to stem cell-based embryo models that morphologically resemble the pre-implantation embryo. This is akin to embryonic day 5 – 8 of human blastocyst development, containing cell types of the trophectoderm, hypoblast and epiblast. These models have allowed for improved understanding of cell fate determination, implantation mechanisms and early embryo-uterine interactions.

3.7. Peri-gastruloids, gastruloids and structured stem cell-based embryo models (SEM, such as bilaminoids, heC-embryoids and E-assembloids), recapitulate more advanced developmental stages which mimic the process of early gastrulation; where the embryo undergoes germ layer formation (ectoderm, mesoderm, endoderm) and body axis specification. In human embryos this process occurs between approximately day 10 and 14 post fertilisation.

3.8. Axioloids and somitoids represent a more specialised model that focuses on replicating the body axis formation of the embryo. These models simulate the early steps of creating the anterior-posterior and dorsal-ventral axes, which are crucial for defining the overall body plan of the developing embryo. In studying axioloids, researchers can gain insights into spinal cord development, nervous system organization and the mechanisms that underlie body symmetry and segmentation.

4. Summary of research developments

4.1. Annex A provides a review of the literature on SCBEM published between 1st January 2023 and 31st August 2024. Updates in organoid research relevant to the remit of the HFEA will be considered separately in February 2025 as part of the regular prioritisation of horizon scanning topics.

4.2. Despite their potential, embryo models currently depend on the self-organisation of pluripotent stem cells which are limited by their uncontrolled self-organisation and low reproducibility resulting in insufficient complexity and fidelity. As a result, they appear to have limited potential to develop on to the implantation and post-implantation stages. To improve the utility of SCBEMs, researchers are currently attempting to optimise protocols to guide organisation of stem cells into structures resembling more complete models that can be accurately reproduced at scale. This requires a detailed understanding of developmental pathways, cellular plasticity, and lineage specification which, as research into SCBEMs expands, are becoming increasingly understood.

4.3. Limitations have been noted, particularly with respect to reproducibility, efficiency and the extent to which the correct tissues (including appropriate proportions of cell types) were present, but these reports have raised expectations that more complete models of post-implantation embryos may be achievable in the near future.

4.4. Such models will still require assessment and validation against human embryos to ensure they are a scientifically valid replacement (Peng et al, 2022), and there is no current evidence to suggest that SCBEMs can be equivalent or have the potential to replace the use of human embryos in research. In addition, it is likely that human embryos will always be needed as a research tool, even if SCBEMs and other new cells may reduce their use.

5. Wider challenges for SCBEMs

5.1. Wider challenges for SCBEMs relate to validation and benchmarking, including:

5.1.1. While much has been learned from studies in animal models, there are significant differences between species in embryonic development.

5.1.2. There is much we do not know, particularly about post-implantation stage embryonic development. Many human embryos fail to develop and it is not clear or predictable why or when this will happen.

5.1.3. Stem cell line culturing methods at present allow accumulation of genetic and epigenetic changes.

5.1.4. Access to human embryos is limited in numbers and barriers arise as a result of the current system for donation and distribution of embryos in the UK, which requires donation to a specific research project. One of other proposals for modernising the HFE Act covers the recommendation to amend the HFE Act in relation to embryo donation for research purposes. The changes being considered would allow patients donating embryos to consent to research embryo banking rather than a specific project – we will not visit this proposal on research consent as part of this paper.

5.1.5. The quality of embryos available are affected by the way they are obtained (eg what is left after higher quality embryos have been used in IVF treatment); and the effect of storage and processing.

5.1.6. The prohibition on embryo culture beyond 14 days, which limits validation of embryo models designed to go beyond 14 days. This limits the ability to undertake research on post 14-day developments using embryo models.

5.1.7. Embryos donated for research after miscarriages and pregnancy terminations are available from about day 28. However, the condition of this tissue is variable, they are extremely scarce between post-conception weeks 4 and 8, and unlikely to be equivalent to a healthy live embryo in utero.

5.2. For research using SCBEMs to progress, it will be important to validate models against embryos of equivalent stages to ensure that the model is equivalent to its in vivo counterpart, or at least to define which aspects can be recapitulated and which aspects cannot. At this time, there is no current evidence which suggests that SCBEM are equivalent to, or have the potential to replace, the use of human embryos in research. Therefore, although SCBEM have been argued to offer an *alternative* to human embryos for use in research, many researchers would argue that models should be seen as *complementary* to human embryos.

6. Regulatory and governance landscapes

6.1. SCBEMs are relatively new and, as yet, do not neatly fit existing regulatory structures in the UK or in other jurisdictions. Regulation has responded incrementally to the developing science but, given their novelty, SCBEMs are not explicitly referred to in legislation. This section outlines the relevant national and international regulatory and governance landscapes that apply to SCBEMs.

6.2. The different regulatory regimes in the UK governing pluripotent stem cells (hESCs and hiPSCs) from which SCBEMs can be generated are:

6.2.1. The HFE Act governs research on embryos, but governance ceases when the hESCs (human embryonic stem cells) are made into stem cell lines. HFEA licences for embryo research involving the derivation of hESCs require licencees to deposit a sample of each cell line generated in the UK Stem Cell Bank (UKSCB). Licencees are not permitted by the HFEA to carry out secondary research projects on ES cells or to transfer ES cell lines to third parties without the approval of the UKSCB Steering Committee. The UKSCB oversees research, including research generating SCBEMs from hESCs. A Code of Practice for the use of Human Stem Cell Lines, published in 2010, provides guidance on best practice for those working with stem cell lines and specifies oversight mechanisms for research involving hESCs. The UKSCB, set up in 2003 and funded by the National Institute for Health Research (NIHR), is the sole public repository for UK hESC lines. It undertakes research to improve the standardisation, quality and safety of human pluripotent stem cell (hPSC)-based products and oversees the use and sharing of quality controlled stem cell lines.

6.2.2. The Human Tissue Act 2004 in England and Wales and Northern Ireland and the Human Tissue (Scotland) Act 2006 govern the use and storage of tissue and cells that come from the human body. This is relevant to hiPSCs (human induced pluripotent stem cells) which are skin, blood or other body cells reprogrammed into an embryonic-like pluripotent state. The Human Tissue Authority (HTA) issues licenses under the Human Tissue Act 2004 in England, Wales and Northern Ireland and performs certain tasks on behalf of the Scottish government. The donation

of tissue for the production of hiPSCs must be in accordance with the requirements for tissue use for research. The consent provided by the donor would need to be based on appropriate information about the intention of the research. Once the stem cell line is established, the Acts and licensing regimes do not apply. hiPSC lines can be deposited at the UKSCB but are not subject to the same level of oversight by the UKSCB Steering Committee, and all research involving human tissue should have Research Ethics Committee (REC) approval, but this is not a legal requirement.

6.2.3. The Medicines and Healthcare products Regulatory Agency (MHRA) regulates research outputs from stem cell research for medicinal use. The Gene Therapy Advisory Committee (GTAC) is the UK national Research Ethics Committee (REC) for research involving gene therapy or stem cell therapy. Any researchers wishing to undertake gene therapy or stem cell therapy clinical trials must apply to GTAC for ethical approval.

6.3. In 2021, the International Society for Stem Cell Research, a global non-profit independent organisation, issued guidelines supportive of SCBEM research and distinguishing current SCBEMs from embryos. It recommended that SCBEM research is subject to review, approval and monitoring through a specialised oversight process to assess the scientific rationale and merit of the research and its ethical permissibility. The ISSCR in 2021 distinguished between integrated and non-integrated SCBEMs, as set out above. The ISSCR Guidelines state that, based on the current state of science, models should not be considered embryos from either a biological or legal perspective. The ISSCR distinguishes three categories of research: those that do not raise ethical issues, those that require vigilance, and those that should be prohibited. In the latest edition of the ISSCR ethical guidelines, both types of embryo models (integrated and non-integrated) are considered to suggest a gradation of ethical concerns. According to the ISSCR, research using integrated models must be approved by ethics committees, while research using non-integrated models need only be notified to the same committees. The ISSCR guideline is currently under review.

6.4. In 2024, a voluntary Governance of Stem Cell-Based Embryo Models Code of Practice for the UK ('the G-SCBEM Code') was produced by Cambridge Reproduction working in partnership with the Progress Educational Trust. This code builds on the ISSCR guidance but does not adopt the distinction between integrated and non-integrated models and considered findings from a public dialogue on the governance of research involving SCBEMs. It sets out fundamental research principles including the requirement that SCBEMs are only as 'complex' or 'integrated' as needed to achieve the research objectives, and that they are researched for the minimum time necessary. The Code sets out that transferring SCBEMs into the uterus of a living person or other animal is not permitted. It does not, however, set an upper limit for how long SCBEMs can be kept in culture. The stated reason is that this avoids "oversimplified limits" and the impracticability of applying "unified limits to all types of embryo model." Instead, limits should be set on all research projects on a case-by-case basis.

6.5. In compliance with ISSCR guidance, the G-SCBEM Code recommends that a review committee is established, and a register maintained. This register would record studies and make basic details available to the public, subject to oversight committee concerns about confidentiality and intellectual property disclosure. The oversight committee, when it is formed, is intended to review applications, assess compliance with the Code, and confirm culture time limits for each individual research project being reviewed.

6.6. The G-SCBEM code has been widely welcomed and may be a step to fill the regulatory gap. It outlines an approach that could increase confidence in research whilst retaining the flexibility needed to respond to developments. However, some warn of limited legitimacy given the extensive discretion given to the oversight committee. In addition, it is argued that a fixed upper limit on embryo model culture time is needed to strengthen the regulatory response.

6.7. At the European level, few countries intend to adopt specific legislation on embryo models, with the exception of the Netherlands. Most countries refer to the ethical guidelines issued by the ISSCR, which are a reference for researchers around the world, especially in countries where research on embryos and hESC is not regulated by law.

6.8. The Health Council in the Netherlands is, as far as we are aware, the only European country with proposals to revise legislation (the Dutch Embryo Act 2002) to define limits of culture time and define both embryos and the SCBEM. Following a request from the Ministry of Health and Welfare, the Health Council of the Netherlands established a temporary committee to look at embryo research. This committee considered the use of "non-conventional embryos", which were referred to as "embryo like structures" (ELS). The Committee was of the opinion that integrated ELS (designed to represent whole embryos) qualified for protection under the Dutch Embryo Act as their potential to become a person could not be ruled out. In the Netherlands, under the Dutch Embryo Act, a Central Oversight Committee reviews, then approves or rejects research projects using human embryos. One of the criteria considered is whether the scientific insights could be obtained without using human embryos. This opens up the possibility of rejecting human embryo use in favour of SCBEMs in the future once science has sufficiently advanced. It will therefore be necessary to establish how "sufficiently advanced to replace human embryo use in research" can be defined.

6.9. Proposals recommend a 28- day culture limit for both embryo research and integrated SCBEMs that might one day have potential to develop into a human being. These models would be designated "non-conventional embryos". It is the Health Council's contention that the knowledge gap between 14-28 days makes SCBEMs a particularly relevant tool, but that thereafter the gains do not outweigh the risks. As for the appropriate limit, the proposed Dutch model has suggested that 28 days would balance utility in research and the point of limb bud development when some sections of society would see the SCBEM as becoming more human. It aligns with their support for extension of the 14-day rule for embryos to 28 days. As such, it would have benefits of consistency and clarity.

6.10. There has been a change of government in the Netherlands since the report was published and the new government has not given any indication that it wishes to consider or act on the report's recommendations.

6.11. In 2023, the Conseil d'Orientation in France set out an opinion on the regulation of SCBEMs. It differentiated between three ways of viewing the status of SCBEMs (or 'embryoids' as the Counseil translation prefers) and mapped this onto different appropriate regulatory responses:

- "1. restrictive position: embryoids are not embryos, but techniques will improve and the goal is to achieve equivalence. Consequently, research on embryoids should already be regulated in the same way as research on embryos.
- 2. permissive position: embryoids are not embryos, they are cultured cells. No special framework should be provided, but the same rules should apply as for all research on cell lines.

- 3. intermediate position: embryoids are not embryos, but they model early embryonic development and enable scientific and medical advances. Therefore, they deserve a specific framework that should be more flexible than that for embryo research, but more stringent than that for research on traditional cell lines."

In common with the ISSCR, the Conseil d'Orientation in France supports the intermediate position. The Conseil has the view that even if non-human animal models acquire properties that make them impossible to distinguish from naturally conceived embryos, the human SCBEM can be distinguished from the human embryo. This is because the SCBEM originates from stem cells rather than fertilisation, and SCBEMs are at no point intended to serve the goal of procreation. The French Conseil d'Orientation report also states that while integrated embryo models cannot currently acquire the ability to become a foetus, they may do so in future. The Conseil retains the view that embryoids should not be considered embryos, unlike the Dutch Health Council.

6.12. In Australia, some SCBEMs (see for more information: Determining whether an embryo model is regulated by the ERLC | NHMRC) are encompassed in their statutory definition of 'embryo' and as a result are subject to the same regulatory framework. Elsewhere, however, there is broad consensus that SCBEMs are not currently embryos, and as such a bespoke regulatory response is needed that balances a precautionary approach to risk and the public benefits that might flow from it.

6.13. The Nuffield Council on Bioethics are currently conducting a rapid review project to assess and advise on the ethical and regulatory issues raised by research using human stem cell-based embryo models with the intention to provide robust, actionable recommendations for decision makers working in this area by the end of 2024. The questions they are exploring include:

- Do these models raise specific ethical considerations and could they merit the requirement for special protections?
- Are current UK governance mechanisms suitable and sufficient?
- How might these governance mechanisms need to evolve as science advances and embryo models become more sophisticated?

7. Conclusions

7.1. SCBEMs do not replicate complete embryos, but might at some point in the future develop morally relevant features and could potentially be developed into more complex models.

7.2. It is also the case that some forms of SCBEMs will gain scientific value if they can mimic aspects of early embryonic development in as complete a manner as possible. SCBEMs might be considered to have greater value as a research tool with the potential to increase knowledge about early human development, than as an avenue for research which may result in findings or applications that could further human health and wellbeing. There may also be potential to replace, reduce reliance on, or increase efficiency of human embryo or non-human animal research insofar as their use is considered more ethically problematic than the use of SCBEMs. Research involving such stem cell-based embryos will still need to use bone fide human embryos as reference models.

7.3. The 14- day rule, which applies clearly to the human embryo created by fertilisation is ill-suited to an entity that has no 'day zero' due to its stem cell-based origins and develops in a non-linear

fashion. A culture might start at the equivalent of day 21 or 28, for example, or contain elements equivalent to day 7 and elements equivalent to day 14.

7.4. Most of the current research on SCBEMs is early research – that is, it is at the stage of generating knowledge and understanding of the underlying mechanisms of early human development. The pace of development has accelerated in recent years, and it is anticipated that this will continue as more studies become available to build on.

7.5. The potential of stem cell research was unforeseen when the HFE Act was debated in 1990 and 2008. At present the HFE Act governs human embryos and gametes. Despite their biological similarity to in vivo-derived embryos, SCBEMs are not currently explicitly regulated by the HFE Act. Recent developments in stem-cell based embryo models in particular have attracted considerable media coverage. As noted above, the interest in SCBEM research has also led to the development of a voluntary framework to govern the creation and use of such models in the UK (the G-SCBEM Code), which has been broadly welcomed as practical first step and may be sufficient. However, given the lack of sanctions associated in any voluntary model and questions about the legitimacy of oversight, it may not assuage concerns about inappropriate research or use of human SCBEMs. Such concerns may mean that it might be appropriate to amend the HFE Act to permit some form of statutory regulation of human SCBEMs in the future. Any future regulation would need to be proportionate to the issues at stake, rather than replicating the regulatory scheme that exists for human embryos.

7.6. Two public dialogues have been conducted in the UK:

7.6.1. One on research involving early human embryos published in October 2023 led by The Human Developmental Biology Initiative in order to better understand society's current hopes and concerns for embryo research to ensure that research continues to be carried out responsibly, transparently and with societal consent.

7.6.2. And another on the governance of research involving SCBEMs published in April 2024 led by Cambridge Reproduction (an interdisciplinary initiative that brings together researchers across Cambridge) to inform development of the G-SCBEM Code.

7.7. The foundation of regulatory flexibility and high levels of public trust in the scientific development of emerging technologies form a strong backdrop to the development of a suitable regulatory response to SCBEMs. Governance should aim to promote scientifically robust and ethical research whilst reassuring the public that harms will be mitigated.

8. Questions for the committee

SCAAC members are asked to consider the following scientific questions related to SCBEMs:

8.1. Do you know about any other research or recent developments in the field of SCBEMs that our literature review has not covered?

Applications of SCBEMs in research

8.2. What are or can be the most useful applications of SCBEMs in research?

- Can SCBEMs replace the use of human embryos in research?
- Are there scientific reasons why it might be valuable to create a SCBEM that goes on to develop capacities to feel pain/awareness?

- One day it may be possible to develop an integrated embryo model that could (scientifically) be implanted in-utero. What would we consider to be "prohibited research"? eg transfer of a SCBEM in to a human or other non-human animal, full ectogenesis etc.

Limits on SCBEM research

8.3. What, if any, limits should be placed on SCBEM research that can be easy to determine?

- Do the committee agree that given how SCBEMs are created/develop to date, it could be considered unreasonable for the 14-day rule to apply to SCBEMs?
- Should an upper limit be applied for all SCBEM research that refers to a development point/specific feature instead? What could this be? eg development of a heartbeat or the central nervous system, or sensing pain, or before the embryo becomes a foetus (this occurs at day 56 – 60)
- In addition, should a case-by-case limit be determined based on the minimum time needed to achieve the valuable scientific objective proposed?
- Is there, or could there be, a category of models requiring additional regulatory oversight than other tissues?

SCBEMs vs live human embryos

8.4. If some SCBEMs become indistinguishable from live human embryos, those SCBEMs could potentially be brought within the embryo licensing regime. However, doing so would impose the strict limitations on research for those SCBEMs as are currently in place for human embryo research eg the 14-day rule.

- Could SCBEMs in the future be indistinguishable from a live human embryo – and what would a good model of the embryo look like?
- If SCBEMs are brought within the current embryo licensing scheme, how will this impact the research?

8.5. What are the scientific characteristics or developments that distinguish SCBEMs from human embryos and explain why different ethical approaches are used? We know that the most unviable/necrotic embryo with a negligible/zero chance of establishing a pregnancy is still afforded the same status in law.

8.6. How should the HFE Act be updated to better define and differentiate between SCBEMs and “live human embryos”? Current definitions in the HFE Act are outlined in annex B. The focus in the Act is on “live” and “human” embryos, terms which are to be given a “purposive” rather than literal meaning.

- How can the current definition of the live human embryo be amended so that it is distinct from SCBEMs?
- How can SCBEMs be defined in the HFE Act to incorporate all types of human SCBEMs?
  - The G-SCBEM Code of Practice outlines the differences between SCBEMs and human embryos (see annex C). Might the SCAAC want to align with them or offer a different description/definition?
  - Should intention and origin form part of the definition?

8.7. If SCBEMs are not currently viewed as ‘human embryos’, then it could be debated that the HFE Act does not prevent the transfer of SCBEMs to a human. Should the HFE Act be amended to explicitly ban the transfer of SCBEMs to humans? The SCAAC may want to consider the need for clarity and public reassurance.

9. Annex A: Scientific literature review

9.1. This annex provides a review of the literature on SCBEM published between 1st January 2023 and 31st August 2024, building upon the seminal work from the Weizmann Institute of Science and the University of Cambridge (2022) in creating self-organising structures from stem cells (Oldak et al., 2023; Weatherbee et al., 2023).

9.2. SCBEM are primarily derived from pluripotent stem cells such as human embryonic stem cells or induced pluripotent stem cell cultures. However, deriving accurate SCBEMs from pluripotent stem cells involves overcoming challenges related to replicating extraembryonic tissues, such as the placenta, which totipotent cells in the embryo would naturally produce. As methods to create and maintain human embryonic stem cells are considered a separate topic by the SCAAC, papers are not detailed here. A summary of relevant development is noted below:

9.2.1. 'Alternative methods to derive embryonic and embryonic like stem cells' was last discussed in June 2024 in which developments in deriving new populations of expanded and extended potential stem cells (EPSC), extraembryonic cell lineages and eight-cell like cells were discussed.

9.2.2. Whilst pluripotent stem cells can be reprogrammed to exhibit properties similar to human embryonic stem cells and recapitulate some features of early embryonic development in vitro, stem cells are not yet able to replace embryonic stem cells in their entirety. Ongoing challenges with epigenetic memory, which can bias differentiation potential, and genetic instability during prolonged culture, impact the reliability and reproducibility of SCBEM production. These factors, alongside difficulties in mimicking the precise cell signalling environments and ensuring the formation of functionally organised tissues, continue to limit the accuracy of SCBEMs in recapitulating true embryonic development.

9.3. Updates in organoid research relevant to the remit of the HFEA will be considered separately in February 2025 as part of the prioritisation of horizon scanning topics; however, this paper refers to the use of endometrial organoids which have been applied to model the process of implantation using SCBEM.

Morula-like cells

9.4. The morula describes the cluster of 16-32 totipotent cells of the late cleavage-stage embryo which typically forms around days 3-4 post fertilisation after multiple divisions of the fertilised egg.

9.5. The recent development of eight-cell like cells (8CLC) (Yu et al., 2022; Taubenschmid-Stowers et al., 2022; Mazid et al., 2022; Moya-Jódar et al., 2023) and expanded and extended potential stem cells (EPSC) (Gao et al., 2019), alongside stem cell lines reprogrammed to naivety, mimic some aspects of blastomeres during the early-cleavage stage. However, complete morula-like models of embryonic development would mimic the complex cellular arrangements and transitions characteristic of this clustered structure.

9.6. Recent work by (Fernandez-Rial & Fidalgo, 2024) demonstrate that using strategic induction of signal transducer and activator of transcription 3 (STAT3) phosphorylation, coupled with carefully defined culture conditions, mouse pluripotent cells could be reprogrammed into a transient morula-like cell state. The resulting morula-like cells closely mirror their in vivo counterparts, exhibiting not only molecular resemblance but also the ability to differentiate into both embryonic and extraembryonic lineages.

9.7. In vitro generation of mouse transient morula-like cells molecularly distinct from that of embryonic stem cells was also reported by (Li et al., 2023a). Morula-like cells were shown to be able to generate blastoids and morula-like cell-derived embryoids contain cell types found in natural embryos at early gastrulation. Morula-like cells introduced into morulae were found to segregate into epiblast, primitive endoderm, and trophectoderm, fates in blastocyst chimeras and have a molecular signature indistinguishable from that of host embryo cells.

9.8. The protocol for generating mouse morula-like cells resembling 8- 16-cell stage embryo cells is described by (Li et al., 2024), who lays out the steps for induction (via increasing Stat3 activation) and the isolation of morula-like cells. Authors then go onto detail procedures for segregating cells into blastocyst cell fates to create embryo-like structures.

Blastoids

9.9. Typically, current stem-cell models bypass the organisational step characteristic of the morula, self-organising directly into a blastocyst-like structure containing components resembling the inner cell mass, an outer cell layer resembling the trophoblast, and a fluid-filled cavity.

9.10. In optimising protocols for the generation of blastoids, researchers are attempting to improve the efficiency, reproducibility and fidelity of blastoid generation, aiming to create models that more closely mimic human and non-human blastocysts. Refining these models will in turn improve their ability to recapitulate early embryonic processes such as implantation and early placental development, while addressing current limitations in cellular organisation and developmental potential. Across the past 20 months, several methodologies have been proposed to generate human and non-human blastoids with varying efficiencies.

9.11. (Yu et al., 2023) described an optimized protocol for the efficient generation of large quantities of high-fidelity human blastoids from naïve pluripotent stem cells. Through proteomics analysis, researchers identified phosphosite-specific signatures potentially involved in the derivation and/or maintenance of signalling states in the human blastoid. In addition, endometrial stomal effects were found to promote the survival, proliferation, and syncytialization of trophoblast cells during co-culture with blastoids and blastocysts. Through single-cell RNA sequencing a population of cells resembling early migratory trophoblast were identified.

9.12. (Karvas et al., 2023) report on the culture of human blastoids on thick three-dimensional extracellular matrices, which replicate the hallmarks of early post-implantation development. This includes: epiblast lumenogenesis, rapid expansion and diversification of trophoblast lineages, and robust invasion of extravillous trophoblast cells by day 14. Extended culture resulted in localised activation of the primitive streak marker TBXT and the emergence of embryonic germ layers by day 21. Modulation of WNT signally was found to alter the balance between epiblast and trophoblast fates in the post-implantation blastoids.

9.13. (Heidari Khoei et al., 2023) describe a protocol to efficiently achieve the morphology of the blastocyst and form linages according to the pace and sequence of blastocyst development in human blastoid models. Resultant blastoids are transcriptionally reflective of the pre-implantation blastocyst. As described below, authors go onto replicate the process of implantation using an organoid of the endometrium.

9.14. (Tu et al., 2023) present an alternative strategy to generate human blastoids from heterogeneous intermediates resembling natural blastocysts in morphological architecture, composition of cell lineages, transcriptome and lineage differentiation potential. When cultured in an in vitro 3D

culture system, blastoids reflect features of human peri-implantation and pregastrulation development.

9.15. (Wei et al., 2024) describe a detailed step-by-step protocol for robust and high-efficiency generation of human blastoids from naïve pluripotent stem cells.

9.16. (Luo & Yu, 2024) developed a three-dimensional, two-step induction system for generating human blastoids using human expanded potential stem (EPS) cells which recapitulate the key developmental processes and cell lineages of human blastocysts. Extended in vitro culture of blastoids can result in post-implantation embryonic structures.

9.17. (Tan, Liu & Polo, 2024) describe a step-by-step protocol for the generation of induced human blastoids (iBlastoids) via somatic reprogramming of human dermal fibroblast cells and the subsequent generation of iBlastoids using reprogramming intermediates. Several characterisation and functions assays that can be used are discussed.

9.18. (Pennarossa et al., 2024) describe an approach to combine miR-200-mediated reprogramming and mechanical stimuli to create three-dimensional spherical aggregates that are phenotypically similar to those of natural embryos. By exploiting the miR-200 family property in dermal fibroblasts, a high plasticity state was induced. Cells were either driven towards a trophectoderm lineage using an ad hoc induction protocol or inner cell mas-like spheroids through encapsulation into a polytetrafluoroethylene micro bioreactor, before being co-cultured and transferred to wells to form blastoids.

9.19. To elucidate the underlying mechanisms by which human naïve pluripotent stem cells generate blastoids, (Guo et al., 2024) studied self-renewing human naïve pluripotent stem cells in three-dimensional suspension culture. The spontaneous formation of blastoids which mimic the structure, size and transcriptome characteristics of early stage blastoids capable of progressing to post-implantation stages, was found to be conferred by the glycogen synthase kinase-3 signalling inhibitor IM-12 present in the self-renewing medium. Upregulation of oxidative phosphorylation-associated genes by IM-12 underly the capacity of pluripotent stem cells to spontaneously generate blastoids. E5 embryo like intermediates co-express SOX2/OCT4 and GATA6 and by day 3 specify trophoblast date which coincides with cavity and blastoid formation, suggesting that 3D culture triggered dedifferentiation of pluripotent stem cells into early embryo-like intermediates competent to segregate to blastocyst fates.

9.20. The use of other totipotent-like stem cells was investigated by (Zhang et al., 2023) using a mouse model. Researchers demonstrated that spliceosome repression-induced totipotent blastomere-like cells form blastocyst-like structures within ~80% of microwells. Additionally, they generated blastoids initiating from a single cell. Such blastoids were found to express specific markers of constituent cell lineages of a blastocyst and resemble blastocyst in cell-lineage allocation. Despite being composed of fewer primitive endoderm-like cells, blastoids shared a similar profile to natural embryos. Blastocysts were demonstrated to develop beyond the implantation stage in vitro and induce decidualisation in vivo.

9.21. The role of senescence on four-factor induced reprogramming was investigated by (Grigorash et al., 2023) who used genetic and chemical approaches to manipulate senescent cells. Results indicate that removal of $p16^{High}$ cells result in four factor induced reprogramming of somatic cells to totipotent-like stem cells which express markers of both pluripotency and the two-cell embryonic state and readily formed implantation-competent blastoids. Senescence-dependent

regulation of nicotinamide N-methyltransferase was identified as a key mechanism controlling the S-adenosyl-L-methionine levels during four factor induced reprogramming required for expression of the two-cell genes and acquisition of an extraembryonic potential.

9.22. (Luo et al., 2024) also explored the impact of senescence on implantation ability. Using a novel three-dimensional system with totipotent blastomere-like cells authors constructed a totipotent blastomere-like blastoids and senescence-related embryo models derived from oxidative stress-induced totipotent blastomere-like cells. Blastoids were found to exhibit characteristic blastocyst morphology, cell lineages, and a higher consistency in developmental rate, demonstrating the ability to develop post-implantation structures in vitro and successfully implant into mouse uteri. However, senescence impaired the implantation potential of the blastoids, mimicking characteristics of impaired implantation ability associated with advanced maternal age. Analysis of differentially expressed genes in lineage-like cells of the blastoids and deciduae identified potential targets for addressing implantation potential.

9.23. (Luijkx et al., 2024) developed a blastoid model to efficiently induce monochrionic twins, termed 'twin blastoids', containing a cystic GATA3+ trophectoderm-like epithelium encasing two distinct inner cell masses. Morphological and morphokinetic analyses revealed that twinning occurs during the cavitation phase via splitting of the OCT4+ pluripotent core. Each inner cell mas contained its own NR2F2+ polar trophectoderm-like region, ready for implantation.

9.24. (De Santis et al., 2024) show that upon in vitro attachment, human blastoids self-organise a BRA^{+} population and undergo gastrulation. Single-cell RNA sequencing of models replicates the transcriptomic signature of the human gastrula and analysis of developmental timing reveals that in both blastoid models and natural human embryos the onset of gastrulation can be traced to timescales equivalent to twelve days post fertilisation.

9.25. (Chen et al., 2023) report on the presence of RNA-binding protein Musashi-1 (MSI1) short C-terminal proteins in early mouse embryos and mouse embryonic stem cells, but not in human embryonic stem cells, under conventional culture conditions. MSI1-C is induced upon naive induction and facilitates human embryonic stem cells naive pluripotency acquisition, elevating the pluripotency of primed human embryonic stem cells toward a formative-like state. This study identifies MSI1-C as an essential regulator in embryonic stem cells pluripotency states and early embryonic development.

9.26. (Huang et al., 2024) demonstrate that treating mouse embryonic stem cells with sodium butyrate increases the population of two cell-like cells and enables direct reprogramming of embryonic stem cells into trophoblast stem cells without transition through a two cell like state. Mechanistically sodium butyrate inhibits histone deacetylase activities in the LSd1-HDAC1/2 corepressor complex, with increased acetylation in regulatory regions of both two-cell and trophoblast stem cell specific genes though to promote their expression. NaB-treated cells acquire the capacity to generate blastoid-like structures that can develop beyond the implantation stage in vitro and form deciduae in vivo.

9.27. (Wang et al., 2023) applied a computational framework named TimeTalk generated from mouse scRNA-seq datasets to decipher early embryo development-related ligand-receptor pairs in blastocyst and blastoids. In doing so researchers found that blastoid models share the core communication pathways with the epiblast and primitive endoderm lineages in the blastocysts.

9.28. (Linneberg-Agerholm et al., 2024) identified an in vitro population similar to the extra-embryonic primitive endoderm in vivo that exhibits the embryonic and extra-embryonic potency and can form blastoids. Commitment in the primitive endoderm is suppressed by JAK/STAT signalling, collaborating with OCT4 and the sustained expression of a subset of pluripotency-related transcription factors that safeguard an enhancer landscape permissive for multi-lineage differentiation.

9.29. (Liu et al., 2023) used single-cell transcriptomic analysis to reveal that trophectoderm-like structures of extended pluripotent stem cell derived blastocysts were primarily composed of primitive endoderm-related cells instead of trophectoderm related cells. Inhibition of primitive endoderm-related cell differentiation through inhibition of MEK signalling or knockout of Gata6 in extended pluripotent stem cells markedly suppressed blastoid formation. The study demonstrated that blastocyst like structures reconstituted by combining extended pluripotent stem cell derived bilineage embryo like structures with tetraploid embryos or trophectoderm cells could implant normally and develop into live fetuses.

9.30. Generation of blastoids in other animal models has also been achieved, including those of the bovine, pig and cynomolgus monkey models (Pinzón-Arteaga et al., 2023; Xiang et al., 2024).

9.31. The generation of non-human blastoids was described by (Li et al., 2023b). In generating cynomolgus monkey blastoids, researchers described the development to the embryonic disc, with structures of the yolk sac, chorionic cavity, amnionic cavity, primitive streak and connecting stalk through prolonged in vitro culture; thus, revealing the capacity of blastoids to undergo gastrulation in vitro. Transference of non-human blastoids to cynomolgus monkey surrogates additional demonstrated the ability of blastoids to achieve pregnancies, as indicated by progesterone levels and early gestational sacs. This work has been subsequently reviewed by (Kwon, 2024) and (Anwised et al., 2023).

9.32. Research generating and studying blastoids and embryoids has been reviewed by the following articles: (Oura, Hamilton & Wu, 2023; Liu & Polo, 2024; Ávila-González et al., 2023; Cockerell et al., 2023; Kim, Kim & Shin, 2023; Zhou et al., 2023; David et al., 2023; Qin et al., 2023; Saadeldin et al., 2024; Zhang, Reis & Simunovic, 2023; Biondic et al., 2023). A comment on the current status of research is given by (Moinard, Pasque & David, 2023).

Embroids and Gastruloids

9.33. Limited research generated from search.

9.34. (Hislop et al., 2024) present a genetically inducible stem cell-derived embryoid model of early post-implantation human embryogenesis that captures the reciprocal co-development of embryonic tissue and the extra-embryonic endoderm and mesoderm niche with early haematopoiesis. The extra-embryonic layer in these embryoids lacks trophoblast and shows advanced multilineage yolk sac tissue-like morphogenesis that harbours a process similar to distinct waves of haematopoiesis, including the emergence of erythroid-, megakaryocyte-, myeloid- and lymphoid-like cells.

Axioloid

9.35. Limited research generated from search.

Assembloids

9.36. When combined with other organoid models, such as blastoids, or additional cell or tissue types, such as embryos or immune cells, organoid systems can be used to model cell interactions or molecular signalling pathways. This includes those involved in embryo implantation, endometrial growth, differentiation and disease. A combination of organoid models or organoid-cell culture is sometimes referred to as an 'assembloid'.

9.37. Following the formation and characterisation of human blastoids, (Heidari Khoei et al., 2023) additionally replicated the process of implantation into a hormonally stimulated two-dimensional endometrial organoid, culturing blastoids up to 6 days (the time equivalent of day 13). Although the implantation assay allowed for modelling of the initial step of attachment and repulsion, the invasive processes and interactions with underlying uterine cells were not seen due to lacking other uterine cell types.

9.38. The methods paper by (Rawlings et al., 2024) details the establishment of a three-dimensional endometrial assembloid, comprising a gland-like epithelial organoid in a stromal matrix. Believed to replicate endometrial tissue structure more faithfully than mono-layer co-cultures, endometrial assembloids can be applied to study human embryo-endometrial interactions to enhance understanding of implantation.

9.39. To model the embryo-endometrial interface (Shibata et al., 2024) first developed hormone-responsive endometrial organoids composed of an exposed apical epithelium surface, dense stromal cells and a self-formed endothelial network, termed apical-out (AO-EMO), which emulate the in vitro architecture of endometrial tissue. When cocultured with human blastoids, the three-dimensional assembloid system was able to recapitulate implantation stages spanning apposition, adhesion and invasion.

9.40. The review article by (Cai, Li & Li, 2023) summarises the current applications of endometrial organoid systems for modelling implantation. Incorporating additional components such as stromal cells, immune cells and blood vessels with current endometrial organoids will pave the way to a more complete *in vitro* model of the endometrium. Such a model can in turn be utilised to elucide the molecular mechanisms underlying implantation.

10. Annex B: Definition of embryos, eggs and sperm in the HFE Act

(1) In this Act (except in section 4A or in the term "human admixed embryo")—
*(a)embryo means a **live human embryo** and does not include a human admixed embryo (as defined by section 4A(6)), and*
(b) references to an embryo include an egg that is in the process of fertilisation or is undergoing any other process capable of resulting in an embryo.

(2) This Act, so far as it governs bringing about the creation of an embryo, applies only to bringing about the creation of an embryo outside the human body; and in this Act—
*(a) references to embryos the creation of which was brought about in vitro (in their application to those where fertilisation or any other process by which an embryo is created is complete) are to those where fertilisation **or any other process by which the embryo was created began outside the human body whether or not it was completed there**, and*

(4) In this Act (except in section 4A)—
*(a) references to eggs are to **live human eggs, including cells of the female germ line at any stage of maturity**, but (except in subsection (1)(b)) not including eggs that are in the process of fertilisation or are undergoing any other process capable of resulting in an embryo,*
*(b) references to sperm are **to live human sperm, including cells of the male germ line at any stage of maturity**, and*
(c) references to gametes are to be read accordingly.

A. Definition of "Permitted" Gametes/Embryos (s3ZA)

(2) A permitted egg is one—
*(a) which has been **produced by or extracted from the ovaries** of a woman, and*
(b) whose nuclear or mitochondrial DNA has not been altered.

(3) Permitted sperm are sperm—
*(a) which have been **produced by or extracted from the testes** of a man, and*
(b) whose nuclear or mitochondrial DNA has not been altered.

(4) An embryo is a permitted embryo if—
*(a) it has been **created by the fertilisation of a permitted egg by permitted sperm**,*
(b) no nuclear or mitochondrial DNA of any cell of the embryo has been altered, and
(c) no cell has been added to it other than by division of the embryo's own cells.

11. Annex C: Differences between SCBEMs and human embryos as outlined in the G-SCBEM Code of Practice

*"**2.2 SCBEMs are currently considered to be biologically distinct from human embryos***

SCBEMs differ from human embryos in several ways, most evidently:

• Current SCBEMs are not the products of a process of direct bi-parental fertilisation involving eggs and sperm.

• At present, SCBEMs are relatively basic and do not recreate the full complexity of embryo development. There are differences in rate and order of development as well as differences in molecular processes.

• It is not known to what extent SCBEMs have the potential to undergo normal embryological development.

• Few SCBEMs are generated with the aim of modelling a complete human embryo.

Furthermore, differences from human embryos in cellular composition, in processes that shape the formation of the embryo, and in gene activity and epigenetic features associated with development have all been reported in SCBEMs.

Current SCBEMs do not proceed through developmental stages equivalent to those observed in the first five days of development in embryos that have arisen from fertilisation. Rather, SCBEMs are engineered to initiate development at specific timepoints/stages of development after these earliest events.

The degree to which SCBEMs align with conventional developmental timings is often not clear-cut. It is neither practical nor accurate to apply conventional developmental timings, such as 'days post-fertilisation', to SCBEMs. For all of these reasons, SCBEMs are currently considered to be biologically distinct from human embryos."

12. References

Amadei, G., Handford, C.E., Qiu, C., De Jonghe, J., Greenfeld, H., Tran, M., Martin, B.K., Chen, D.Y., Aguilera-Castrejon, A., Hanna, J.H., Elowitz, M.B., Hollfelder, F., Shendure, J., Glover, D.M. & Zernicka-Goetz, M. (2022) Embryo model completes gastrulation to neurulation and organogenesis. *Nature*. 610 (7930), 143–153. doi:10.1038/S41586-022-05246-3.

Anwised, P., Moorawong, R., Samruan, W., Somredngan, S., Srisutush, J., Laowtammathron, C., Aksoy, I., Parnpai, R. & Savatier, P. (2023) An expedition in the jungle of pluripotent stem cells of non-human primates. *Stem cell reports*. 18 (11), 2016–2037. doi:10.1016/J.STEMCR.2023.09.013.

Ávila-González, D., Gidi-Grenat, M.Á., García-López, G., Martínez-Juárez, A., Molina-Hernández, A., Portillo, W., Díaz-Martínez, N.E. & Díaz, N.F. (2023) Pluripotent Stem Cells as a Model for Human Embryogenesis. *Cells*. 12 (8). doi:10.3390/CELLS12081192.

Biondic, S., Canizo, J., Vandal, K., Zhao, C. & Petropoulos, S. (2023) Cross-species comparison of mouse and human preimplantation development with an emphasis on lineage specification. *Reproduction (Cambridge, England)*. 165 (4), R103–R116. doi:10.1530/REP-22-0144.

Cai, Y., Li, N. & Li, H. (2023) Combining Endometrial Assembloids and Blastoids to Delineate the Molecular Roadmap of Implantation. *Stem cell reviews and reports*. 19 (5), 1268–1282. doi:10.1007/S12015-023-10527-Z.

Chen, Y., Chen, Y., Li, Q., Liu, H., Han, J., Zhang, H., Cheng, L. & Lin, G. (2023) Short C-terminal Musashi-1 proteins regulate pluripotency states in embryonic stem cells. *Cell reports*. 42 (10). doi:10.1016/J.CELREP.2023.113308.

Cockerell, A., Wright, L., Dattani, A., Guo, G., Smith, A., Tsaneva-Atanasova, K. & Richards, D.M. (2023) Biophysical models of early mammalian embryogenesis. *Stem cell reports*. 18 (1), 26–46. doi:10.1016/J.STEMCR.2022.11.021.

David, L., Bruneau, A., Fréour, T., Rivron, N. & Van de Velde, H. (2023) An update on human pre- and peri-implantation development: a blueprint for blastoids. *Current opinion in genetics & development*. 83. doi:10.1016/J.GDE.2023.102125.

Fernandez-Rial, C. & Fidalgo, M. (2024) Induction of Transient Morula-Like Cells in Mice Through STAT3 Activation. *Cellular reprogramming*. 26 (1), 8–9. doi:10.1089/CELL.2023.0116.

Gao, X., Nowak-Imialek, M., Chen, X., Chen, D., Herrmann, D., et al. (2019) Establishment of porcine and human expanded potential stem cells. *Nature Cell Biology 2019 21:6*. 21 (6), 687–699. doi:10.1038/s41556-019-0333-2.

Grigorash, B.B., van Essen, D., Liang, G., Grosse, L., Emelyanov, A., Kang, Z., Korablev, A., Kanzler, B., Molina, C., Lopez, E., Demidov, O.N., Garrido, C., Liu, F., Saccani, S. & Bulavin, D. V. (2023) p16High senescence restricts cellular plasticity during somatic cell reprogramming. *Nature cell biology*. 25 (9), 1265–1278. doi:10.1038/S41556-023-01214-9.

Guo, M., Wu, J., Chen, C., Wang, X., Gong, A., Guan, W., Karvas, R.M., Wang, K., Min, M., Wang, Y., Theunissen, T.W., Gao, S. & Silva, J.C.R. (2024) Self-renewing human naïve pluripotent stem cells dedifferentiate in 3D culture and form blastoids spontaneously. *Nature communications*. 15 (1). doi:10.1038/S41467-024-44969-X.

Heidari Khoei, H., Javali, A., Kagawa, H., Sommer, T.M., Sestini, G., David, L., Slovakova, J., Novatchkova, M., Scholte op Reimer, Y. & Rivron, N. (2023) Generating human blastoids modeling blastocyst-stage embryos and implantation. *Nature protocols*. 18 (5), 1584–1620. doi:10.1038/S41596-023-00802-1.

Hislop, J., Song, Q., Keshavarz F, K., Alavi, A., Schoenberger, R., LeGraw, R., Velazquez, J.J., Mokhtari, T., Taheri, M.N., Rytel, M., Chuva de Sousa Lopes, S.M., Watkins, S., Stolz, D., Kiani, S., Sozen, B., Bar-Joseph, Z. & Ebrahimkhani, M.R. (2024) Modelling post-implantation human development to yolk sac blood emergence. *Nature*. 626 (7998), 367–376. doi:10.1038/S41586-023-06914-8.

Huang, B., Peng, X., Zhai, X., Hu, J., Chen, J., Yang, S., Huang, Q., Deng, E., Li, H., Barakat, T.S., Chen, J., Pei, D., Fan, X., Chambers, I. & Zhang, M. (2024) Inhibition of HDAC activity directly reprograms murine embryonic stem cells to trophoblast stem cells. *Developmental cell*. 59 (16). doi:10.1016/J.DEVCEL.2024.05.009.

Karvas, R.M., Zemke, J.E., Ali, S.S., Upton, E., Sane, E., Fischer, L.A., Dong, C., Park, K. mi, Wang, F., Park, K., Hao, S., Chew, B., Meyer, B., Zhou, C., Dietmann, S. & Theunissen, T.W. (2023) 3D-cultured blastoids model human embryogenesis from pre-implantation to early gastrulation stages. *Cell stem cell*. 30 (9), 1148-1165.e7. doi:10.1016/J.STEM.2023.08.005.

Kim, Y., Kim, I. & Shin, K. (2023) A new era of stem cell and developmental biology: from blastoids to synthetic embryos and beyond. *Experimental & molecular medicine*. 55 (10), 2127–2137. doi:10.1038/S12276-023-01097-8.

Kwon, T. (2024) Role and ethics of cynomolgus monkey (Macaca fascicularis) blastoids in primate developmental biology research. *Journal of medical primatology*. 53 (2). doi:10.1111/JMP.12693.

Li, H., Chang, L., Huang, J. & Silva, J.C.R. (2024) Protocol for generating mouse morula-like cells resembling 8- to 16-cell stage embryo cells. *STAR protocols*. 5 (2). doi:10.1016/J.XPRO.2024.102934.

Li, H., Chang, L., Wu, J., Huang, J., Guan, W., Bates, L.E., Stuart, H.T., Guo, M., Zhang, P., Huang, B., Chen, C., Zhang, M., Chen, J., Min, M., Wu, G., Hutchins, A.P. & Silva, J.C.R. (2023a) In vitro generation of mouse morula-like cells. *Developmental cell*. 58 (22), 2510-2527.e7. doi:10.1016/J.DEVCEL.2023.09.013.

Li, J., Zhu, Q., Cao, J., Liu, Y., Lu, Y., et al. (2023b) Cynomolgus monkey embryo model captures gastrulation and early pregnancy. *Cell stem cell*. 30 (4), 362-377.e7. doi:10.1016/J.STEM.2023.03.009.

Linneberg-Agerholm, M., Sell, A.C., Redó-Riveiro, A., Perera, M., Proks, M., Knudsen, T.E., Barral, A., Manzanares, M. & Brickman, J.M. (2024) The primitive endoderm supports lineage plasticity to enable regulative development. *Cell*. 187 (15), 4010-4029.e16. doi:10.1016/J.CELL.2024.05.051.

Liu, K., Xu, X., Bai, D., Li, Y., Zhang, Y., et al. (2023) Bilineage embryo-like structure from EPS cells can produce live mice with tetraploid trophectoderm. *Protein & cell*. 14 (4), 262–278. doi:10.1093/PROCEL/PWAC029.

Liu, X. & Polo, J.M. (2024) Human blastoid as an in vitro model of human blastocysts. *Current opinion in genetics & development*. 84. doi:10.1016/J.GDE.2023.102135.

Luijkx, D.G., Ak, A., Guo, G., van Blitterswijk, C.A., Giselbrecht, S. & Vrij, E.J. (2024) Monochorionic Twinning in Bioengineered Human Embryo Models. *Advanced materials (Deerfield Beach, Fla.)*. 36 (25). doi:10.1002/ADMA.202313306.

Luo, Y., An, C., Zhong, K., Zhou, P., Li, D., Liu, H., Guo, Q., Wei, W., Pan, H., Min, Z., Li, R., Yu, Y. & Fan, Y. (2024) Exploring the impacts of senescence on implantation and early embryonic development using totipotent cell-derived blastoids. *Journal of advanced research*. doi:10.1016/J.JARE.2024.02.011.

Luo, Y.X. & Yu, Y. (2024) Protocol for the Generation of Human EPS-Blastoids Using a Three-Dimensional Two-Step Induction System. *Methods in molecular biology (Clifton, N.J.)*. 2767, 27–41. doi:10.1007/7651_2022_471.

Mazid, M.A., Ward, C., Luo, Z., Liu, C., Li, Y., et al. (2022) Rolling back human pluripotent stem cells to an eight-cell embryo-like stage. *Nature*. 605 (7909), 315–324. doi:10.1038/S41586-022-04625-0.

Moinard, E., Pasque, V. & David, L. (2023) Having a blast(oid): Modeling human embryo peri-implantation development with blastoids. *Cell stem cell*. 30 (9), 1125–1126. doi:10.1016/J.STEM.2023.08.007.

Moya-Jódar, M., Ullate-Agote, A., Barlabé, P., Rodríguez-Madoz, J.R., Abizanda, G., Barreda, C., Carvajal-Vergara, X., Vilas-Zornoza, A., Romero, J.P., Garate, L., Agirre, X., Coppiello, G., Prósper, F. & Aranguren, X.L. (2023) Revealing cell populations catching the early stages of human embryo development in naive pluripotent stem cell cultures. *Stem cell reports*. 18 (1), 64–80. doi:10.1016/J.STEMCR.2022.11.015.

Oldak, B., Wildschutz, E., Bondarenko, V., Comar, M.Y., Zhao, C., et al. (2023) Complete human day 14 post-implantation embryo models from naive ES cells. *Nature*. 622 (7983), 562–573. doi:10.1038/S41586-023-06604-5.

Oura, S., Hamilton, J.N. & Wu, J. (2023) Recent advances in stem cell-based blastocyst models. *Current opinion in genetics & development*. 81. doi:10.1016/J.GDE.2023.102088.

Pennarossa, G., Arcuri, S., Gandolfi, F. & Brevini, T.A.L. (2024) Generation of Artificial Blastoids Combining miR-200-Mediated Reprogramming and Mechanical Cues. *Cells*. 13 (7). doi:10.3390/CELLS13070628.

Pinzón-Arteaga, C.A., Wang, Y., Wei, Y., Ribeiro Orsi, A.E., Li, L., Scatolin, G., Liu, L., Sakurai, M., Ye, J., Hao Ming, Yu, L., Li, B., Jiang, Z. & Wu, J. (2023) Bovine blastocyst-like structures derived from stem cell cultures. *Cell stem cell*. 30 (5), 611-616.e7. doi:10.1016/J.STEM.2023.04.003.

Qin, J., Li, W., Lv, B., Xue, Z. & Xue, J. (2023) Early differentiation and gene expression characteristics of trophoblast lineages†. *Biology of reproduction*. 108 (5), 709–719. doi:10.1093/BIOLRE/IOAD027.

Rawlings, T.M., Tryfonos, M., Makwana, K., Taylor, D.M., Brosens, J.J. & Lucas, E.S. (2024) Endometrial Assembloids to Model Human Embryo Implantation In Vitro. *Methods in molecular biology (Clifton, N.J.)*. 2767, 63–74. doi:10.1007/7651_2023_495.

Saadeldin, I.M., Ehab, S., Noreldin, A.E., Swelum, A.A.A., Bang, S., Kim, H., Yoon, K.Y., Lee, S. & Cho, J. (2024) Current strategies using 3D organoids to establish in vitro maternal-embryonic interaction. *Journal of veterinary science*. 25 (3). doi:10.4142/JVS.24004.

De Santis, R., Rice, E., Croft, G., Yang, M., Rosado-Olivieri, E.A. & Brivanlou, A.H. (2024) The emergence of human gastrulation upon in vitro attachment. *Stem cell reports*. 19 (1), 41–53. doi:10.1016/J.STEMCR.2023.11.005.

Shibata, S., Endo, S., Nagai, L.A.E., Kobayashi, E.H., Oike, A., Kobayashi, N., Kitamura, A., Hori, T., Nashimoto, Y., Nakato, R., Hamada, H., Kaji, H., Kikutake, C., Suyama, M., Saito, M., Yaegashi, N., Okae, H. & Arima, T. (2024) Modeling embryo-endometrial interface recapitulating human embryo implantation. *Science advances*. 10 (8). doi:10.1126/SCIADV.ADI4819.

Tan, J.P., Liu, X. & Polo, J.M. (2024) Reprogramming fibroblast into human iBlastoids. *Nature protocols*. 19 (8). doi:10.1038/S41596-024-00984-2.

Taubenschmid-Stowers, J., Rostovskaya, M., Santos, F., Ljung, S., Argelaguet, R., Krueger, F., Nichols, J. & Reik, W. (2022) 8C-like cells capture the human zygotic genome activation program in vitro. *Cell stem cell*. 29 (3), 449-459.e6. doi:10.1016/J.STEM.2022.01.014.

Tu, Z., Bi, Y., Zhu, X., Liu, W., Hu, J., Wu, L., Mao, T., Zhou, J., Wang, H., Wang, H., Gao, S. & Wang, Y. (2023) Modeling human pregastrulation development by 3D culture of blastoids generated from primed-to-naïve transitioning intermediates. *Protein & cell*. 14 (5), 337–349. doi:10.1093/PROCEL/PWAC041.

Wang, L., Zheng, Y., Sun, Y., Mao, S., Li, H., Bo, X., Li, C. & Chen, H. (2023) TimeTalk uses single-cell RNA-seq datasets to decipher cell-cell communication during early embryo development. *Communications biology*. 6 (1). doi:10.1038/S42003-023-05283-2.

Wei, Y., Liu, K., Pinzon-Arteaga, C.A., Logsdon, D., Yu, L., Yuan, Y. & Wu, J. (2024) Generation of Human Blastoids from Naive Pluripotent Stem Cells. *Methods in molecular biology (Clifton, N.J.)*. 2767, 1–18. doi:10.1007/7651_2023_485.

Weatherbee, BAT., Gantner, CW., Iwamoto-Stohl, LK., Daza, RM., Hamazaki, N., Shendure, J., Zernicka-Goetz, M. (2023) Pluripotent stem cell-derived model of the post-implantation human embryo. Nature. 2023. doi: 10.1038/s41586-023-06524-4.

Xiang, J., Wang, H., Shi, B., Li, J., Liu, D., Wang, K., Wang, Z., Min, Q., Zhao, C. & Pei, D. (2024) Pig blastocyst-like structure models from embryonic stem cells. *Cell discovery*. 10 (1). doi:10.1038/S41421-024-00693-W.

Yu, L., Logsdon, D., Pinzon-Arteaga, C.A., Duan, J., Ezashi, T., et al. (2023) Large-scale production of human blastoids amenable to modeling blastocyst development and maternal-fetal cross talk. *Cell stem cell*. 30 (9), 1246-1261.e9. doi:10.1016/J.STEM.2023.08.002.

Yu, X., Liang, S., Chen, M., Yu, H., Li, R., Qu, Y., Kong, X., Guo, R., Zheng, R., Izsvák, Z., Sun, C., Yang, M. & Wang, J. (2022) Recapitulating early human development with 8C-like cells. *Cell reports*. 39 (12). doi:10.1016/J.CELREP.2022.110994.

Zhang, M., Reis, A.H. & Simunovic, M. (2023) Human embryoids: A new strategy of recreating the first steps of embryonic development in vitro. *Seminars in cell & developmental biology*. 141, 14–22. doi:10.1016/J.SEMCDB.2022.07.003.

Zhang, P., Zhai, X., Huang, B., Sun, S., Wang, W.J. & Zhang, M. (2023) Highly efficient generation of blastocyst-like structures from spliceosomes-repressed mouse totipotent blastomere-like cells. *Science China. Life sciences*. 66 (3), 423–435. doi:10.1007/S11427-022-2209-3.

Zhou, J., Hu, J., Wang, Y. & Gao, S. (2023) Induction and application of human naive pluripotency. *Cell reports*. 42 (4). doi:10.1016/J.CELREP.2023.112379.

In vitro derived gametes (IVGs)

Details about this paper

Area(s) of strategy this paper relates to:	Shaping the future
Meeting:	SCAAC
Agenda item:	7
Paper number:	HFEA (07/10/2024) 007
Meeting date:	7 October 2024
Author:	Rebecca Taylor, Scientific Policy Manager Mina Mincheva, Policy Manager
Annexes	Annex A: Scientific literature review Annex B: Definition of gametes and regulation of gamete use in the HFE Act

Output from this paper

For information or recommendation?	For recommendation
Recommendation:	Members are asked to advise on: • Current and future use of in-vitro gametes (IVGs) in research and fertility treatment • Scientific considerations to address in relation to reforming the HFE Act to regulate the use of in-vitro gametes in fertility research and treatment
Resource implications:	TBC
Implementation date:	TBC
Communication(s):	TBC

Organisational risk	☐ Low	☒ Medium	☐ High

1. Introduction

1.1. In vitro derived gametes (IVGs) are not explicitly regulated by the Human Fertilisation and Embryology Act 1990 (the HFE Act), however whether they fall under the Act is a question of interpretation of the term gamete is defined (see Annex B). There may therefore be a need to amend the HFE Act to improve clarity. The progress on IVGs raises the question as to whether changes are needed in relation to regulating the research use of IVGs in a safe and ethical manner. This paper will outline the scientific considerations for clarifying the regulation of IVGs within the HFE Act to 'future proof' it, so that it is better able to accommodate future scientific developments and new technologies within this field and provide a summary of recent research developments in this area.

1.2. The SCAAC will be asked to give their advice and/or make recommendations concerning IVGs, focusing on the scientific and technical aspects including potential benefits and drawbacks.

1.3. Any advice or recommendations received from SCAAC will feed into the development of an Authority paper for the January 2025 Authority meeting. At that meeting the Authority will consider both the scientific/technical case as well as the wider ethical arguments to come to a consensus on proposals.

1.4. Any change in the Act is for Government and Parliament; however, it continues to be important for the HFEA to lead the discussions and understand the scientific, legal and ethical implications concerning IVGs.

2. Background

2.1. The HFEA published proposals for modernising the HFE Act in November 2023. One of the four areas for reform identified was in respect of future scientific developments and innovation. The recommendations made were:

- The Act should explicitly give the HFEA greater discretion to support innovation in treatment and research.
- The Act should be amended to 'future proof' it, so that it is better able to accommodate future scientific developments and new technologies.

2.2. One of the areas identified under future scientific developments was in vitro gametes (IVGs), which are not explicitly regulated under the HFE Act.

2.3. IVGs are gametes (eggs or sperm) created in a laboratory (in vitro) by reprogramming other cells, such as embryonic stem cells or skin cells, to become functional egg and sperm cells. This process is known as in vitro gametogenesis (IVG). Approaches to generating gametes in vitro can be broadly divided into two categories depending on the initial source of cells:

- Different two- or three-dimensional cultures can be used to generate mature functional haploid gametes from immature germ cells at various stages of gametogenesis retrieved from the gonads.
- Techniques for the generation of functional haploid gametes from diploid cells, such as embryonic stem cells (ESC) or induced pluripotent stem cells (iPSCs) reprogrammed from somatic cells (e.g. skin cells). These techniques depend on the induction of meiosis in vitro. Another approach in this category is a modified conventional method of somatic cell nuclear

transfer (SCNT), which employs direct reprogramming of diploid somatic cells into haploid gametes by inducing premature cell division (Mikhalchenko et al., 2024). This research technique has been used for generating oocytes. Stem cell-based embryo models may also be an alternative source for primordial germ cell-like cells (PGLC).

2.4. The HFE Act prohibits the use of IVGs 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". The definition of gametes and regulation of gamete use in the HFE Act can be found in annex B. Provisions in the amended (2008) HFE Act permit the Secretary of State to introduce regulations that would allow for the definition of embryo, eggs, sperm and gametes be amended to include things that would not otherwise fall within the definition.

2.5. Human IVGs (unfertilised and not generated into an embryo) fall within ISSCR category 1b research (research permissible without review, but must be reported to designated body for monitoring) as described by the ISSCR **Guidelines for Stem Cell Research and Clinical Translation** (2021).

2.6. The use of immature sperm (i.e. spermatids) is prohibited in clinical practice under the **Authorised processes list** that describes the processes clinics can use to carry out the licensable activities set out in the HFE Act.

2.7. There is currently no agreement among scientists in the field as to the likely timeframe for creating viable human IVGs: some believe it may be possible in the next 2-3 years, while others believe it will take more than a decade. Currently several groups have generated human primordial germ cell-like cells that can develop to the oogonial stage, but none have managed to go further along the process of oogenesis (Frost & Gilchrist, 2024). Researchers are now trying to generate human oocytes from stem cells (Frost & Gilchrist, 2024).

2.8. In addition, considerable progress will be required in relation to the tests needed to characterise and validate such IVGs, many of which have yet to be developed.

2.9. Developing the techniques required for safe and effective IVGs suitable for human reproduction is likely to be "extraordinarily difficult" (Frost & Gilchrist, 2024). To date, reproductive in vitro gametogenesis (IVG) has been achieved only in mice, but not yet in non-human primates. Of the eight steps required to use IVG in reproduction, follicle in vitro development (IVD) is likely to be the most difficult and has thus far failed to lead to the development of competent oocytes starting from ovarian follicles in non-rodent models (Frost & Gilchrist, 2024).

2.10. There is no consensus as to whether the IVG reproduction proof of concept in mice has applicability to non-human primates or humans (National Academies of Sciences, 2023). If it were to be achieved in non-human primates, there would probably need to be follow-up studies of any offspring conceived for several years before any attempt was made to undertake human reproduction using IVG (National Academies of Sciences, 2023). This means that the use of IVGs in human reproduction i.e. as part of fertility treatment, is currently thought to be a long way off, even with the most optimistic estimates of scientific progress.

2.11. The use of IVGs in research is, however, likely to be technically feasible earlier than use in fertility treatment. IVGs could be used in research to further develop understanding of developmental biology for studying the causes of some forms of infertility. and to aid drug development.

2.12. IVGs have long been a topic of interest for the SCAAC. Whilst IVGs 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. The committee last reviewed research in October 2023 and considered ongoing challenges related to human IVG, including gamete developmental competence, efficiency of generating IVGs, safety concerns over correct genetic and epigenetic make-up, culture media, transcription factors and supporting cells that require a thorough understanding of testicular and ovarian biology, and consequently the health implications for resultant offspring. The committee also considered the topic at the most recent horizon scanning prioritisation exercise, which took place in February 2024: the SCAAC identified IVG as a high priority area. This topic was also discussed at the Horizon Scanning meeting at the European Society of Human Reproduction and Embryology 2023 conference. Attendees noted the increased treatment options IVGs may eventually create and the current legal restrictions in the UK and USA.[1]

3. Summary of research developments

3.1. Annex A provides a review of the literature on IVG published between 1st January 2023 and 31st August 2024.

3.2. There have been increasing research efforts to use pluripotent stem cells to generate germ cell precursors, primordial germ cells (PGCs) in animal models, coupled with optimising culture conditions to induce their differentiation and maturation towards the male and female germ line.

3.3. The functionality of IVG (fertilisation of IVG with an in vivo gamete) is yet to be tested and validated.

3.4. Key considerations to advance the field of IVG are gaining a better understanding of ovarian/oocyte and testicular biology, improving the efficiency of iPSC and ESC derivation, developing pluripotent stem cell-based sources for ovarian and testicular somatic cells that support germ cell development, and optimising culture conditions, with a particular focus on three-dimensional cultures.

3.5. It is important to acknowledge that in order to realise the reproductive potential of primary follicles generated from PGCLCs through in vitro oogenesis, the technology will likely need to be coupled with other reproductive biotechnologies, such as follicle in vitro development (IVD), in vitro maturation (IVM) and bi-phasic IVM.

4. Potential benefits and drawbacks of IVG

4.1. The potential benefits of using IVGs in research could include:

- Expanding understanding of developmental biology.
- Increasing the availability of oocytes from stem cells without the need for egg donors, thus enabling greater early-stage embryo research.

[1] The HFEA Chief Executive was a speaker at the **IVG Ethics and Policy Symposium,** which took place in Leiden, The Netherlands on 3-4 June 2024. The Symposium attracted speakers and participants from across Europe and beyond including researchers, clinicians, regulators and commercial organisations.

- Increasing the availability of embryos for use in research (this requires IVGs that can successfully undergo fertilisation to create embryos).
- Gametes derived from stem cells lines would be genetically isogenic (many gametes with the same genotype), which can aid research studies by minimising potentially confounding genetic differences.
- Identifying causes of infertility and congenital conditions arising during gamete development, fertilisation or early embryo development. For example, this could enable researchers to create gametes from iPSCs from individuals with infertility and study them.
- Using eggs and sperm derived by IVG to test the repro-toxicity of drugs on fertility and embryonic development.

4.2. The potential drawbacks of using IVGs in research could include:

- Insufficient reproducibility; in vitro created eggs and sperm do not develop in exactly the same way as human derived gametes and therefore cannot entirely replace them in research; research results cannot be considered reliable.

4.3. The potential benefits to using IVG in fertility treatment could include:

- Enabling people to have a genetically related child when they would not otherwise be able to do so, for example:
    - Same sex couples could have a child genetically related to both parents[2]; proof of concept has already been established in mice (Murakami et al., 2023).
    - Heterosexual couples could overcome infertility of one partner to have a genetically related child.
- Eliminating the need for egg or sperm donors, which would remove or reduce the costs and other challenges associated with donation..
- Obviating the need for patients to undergo invasive and costly egg retrieval as part of IVF, which could reduce some of the risks involved in treatment, as well as making fertility treatment more affordable and accessible
- For people with a risk of passing on serious genetic diseases, the greater number of embryos produced using IVG could facilitate pre-implantation genetic testing to select embryos for transfer. (For many couples undergoing PGT, the small number of embryos available for testing, together with the significant chance that none will be genetically suitable for transfer, are limiting factors). Advances in genome editing may, in the future, enable the generation of disease-free gametes.
- Reducing age barriers to having children.
- Removing the need for people with serious health conditions to stop taking medication while trying to conceive/undergoing IVF.

4.4. The potential drawbacks to using IVG in fertility treatment could include:

- The potential for IVG to introduce germline genetic and epigenetic modifications that could be transmitted across generations.

[2] SCAAC discussions on IVG in October 2023 highlighted that whilst it may in future be possible to generate XX cells from XY induced pluripotent stem cells (iPSCs), the generation of XY cells from XX iPSCs is highly unlikely. This may limit the possibility of a genetically related child for a female same sex couple using IVG.

- The greater number of gametes and embryos being produced through IVG leading to expanded pre-implantation genetic testing (PGT). This could arise through patients wishing to further refine embryo selection without concerns about selection resulting in too few embryos to transfer. In jurisdictions where PGT-P targeting polygenic diseases is permitted[3] or PGT-M (for monogenic conditions) is less strictly regulated than the UK, patients may seek to screen IVG derived embryos for many different conditions with a possible disability bias and/or tipping over into enhancement/selection of desirable traits (eugenics).
- Ethical concerns about the destruction of large numbers of IVG derived embryos through expanded PGT.
- Logistical challenges for clinics due to the need to store much higher numbers of IVG eggs and sperm and IVG derived embryos.
- The reduction in age barriers for parenthood creating new challenges such as higher risk pregnancies in older mothers, and children born to parents more likely to die or become infirm during their childhood.
- An increase in the demand for gestational surrogates.

5. Questions for the committee

The SCAAC are asked to consider the following scientific questions related to in-vitro gametes.

5.1. Do you know about any other research or recent developments in the field of IVGs that our literature review has not covered?

5.2. In relation to possible changes to the HFE Act to better accommodate IVGs:

- Should the definition of gametes be updated to differentiate between IVGs and “live human eggs” and “live human sperm”? Is it important to develop definitions that differentiate between different types of IVGs?
- Are there any scientific reasons to regulate IVGs derived from embryonic stem cells (ESC) and those derived from somatic cells re-programmed into induced pluripotent stem cells (iPSCs) differently?

5.3. In relation to the 14-day time limit on human embryo research in the HFE Act:

- Does the 14-day rule impinge on the development of methods to characterise and/or validate IVGs?
- Could the 14-day rule limit the ability of scientists to use IVG for research purposes including research aimed at using IVG to create embryos?

5.4. Should benchmarks for the characterisation and/or validation of the functional quality of IVGs be different for research use and clinical use?

5.5. What is the likelihood of DNA mutations/epigenetic problems in IVGs making them unsuitable/unsafe for future clinical use?

5.6. Do you think it may be necessary to undertake clinical trials of IVGs for clinical use to ascertain safety and efficacy?

[3] PGT-P is not permitted in the UK.

6. Next steps

6.1. As noted above, we are in the process of developing an Authority paper outlining proposals to revise the HFE Act including to better accommodate future scientific developments. There are different ways to approach this. Research on embryos created using IVGs requires an embryo research licence from the HFEA, however this work could involve a recommendation that the use of IVGs themselves for research or reproduction should be explicitly brought under the remit of the HFE Act. Given the differing pace of scientific progress, it may be appropriate to revise the HFE Act in a way that allows for subsequent secondary legislation through regulations to govern the use of IVGs.

6.2. The proposals may need to further clarify how IVGs are defined under the HFE Act. IVGs are not explicitly regulated by the HFE Act, but whether they fall under the Act is ultimately a question of interpretation of the term gamete as defined in the Act (see Annex B).

6.3. It will also be necessary to consider what provisions may be needed to regulate the research use of IVGs in a safe and ethical manner. Such research is likely to include:

- further investigating developmental biology
- testing the repro-toxicity of drugs
- genetic screening and editing of IVGs
- pre-clinical studies to test the functionality of IVGs

7. Annex A: Scientific literature review

7.1. This annex provides a review of the literature on IVG published between 1st January 2023 and 31st August 2024.

7.2. Primordial germ cells (PGC) are the common precursor for both spermatozoa and oocytes and migrate to the developing primitive gonad during embryonic development, giving rise to the germline. After being specified in the early post-implantation embryo, between approximately embryonic weeks 2 and 3, human PGCs (hPGC) start migration from the extra-embryonic yolk sac wall into the developing genital ridges. During migration through the hindgut and dorsal mesentery, occurring between gestational weeks 4 and 6, hPGCs start a heterogeneous maturation process comprising global DNA demethylation and chromatin reorganisation events. After gonadal colonisation and sex determination, hPGCs continue maturating into pro-spermatogonia or oogonia in the developing testes or ovaries, respectively.

7.3. Researchers are investigating whether it is possible to carry out gametogenesis in the laboratory using different cell sources which can be divided in two broad categories: germline stem cells (the committed germ cell lineage) and pluripotent stem cells – such as embryonic stem cells (ESC) or somatic cells reprogrammed to induced pluripotent stem cells (iPSC) – which are induced to generate primordial germ cell-like cells (PGCLCs).

7.4. Some scientific advances have been made to test the functionality of IVG gametes derived from different pluripotent stem cell sources in mice and primates that employs fertilisation of an in vitro derived gamete with in vivo derived gamete (see literature review below and IVG paper from **SCAAC October 2023**).

7.5. In literature, in vitro gametogenesis is an umbrella term largely referring to using different pluripotent stem cells as a starting material. However, in addition immature germ cells from different stages of the gametogenesis cycle are also used in different cultures settings to attempt to generate mature functional haploid gametes. Therefore, oocytes and spermatozoa derived in the laboratory from immature germ cells or stem cell sources can be referred to as in vitro derived gametes. Another potential source for in vitro derived gamete precursors is stem cell-based embryo models.

7.6. PGCLCs have now been generated for different species (porcine, equine, northern white rhino, marmoset, cynomolgus monkey, rhesus macaque). Additionally, other work has shown that human pluripotent stem cells can generate human PGCLCs.

7.7. Regarding in vitro oogenesis using human gametes, oocytes from small antral follicles are the earliest stage of follicles used as a starting material for Assisted Reproduction Technology (ART) procedures in clinical settings. Current efforts of researchers and biotech companies are centred around generating human oocytes from stem cells to overcome the shortage of high-quality oocytes as a result of infertility or increasing age. The furthest developmental stage researchers have been able to achieve starting from pluripotent stem cells is oogonia, and this is through a coculture system with mouse foetal granulosa cells (review in (Frost & Gilchrist, 2024).

In vitro maturation (IVM) of immature oocytes

7.8. A particular method for supporting maturation of immature oocytes in vitro, IVM, has gained traction in recent years. IVM is a blanket term applied to obtain mature oocytes from immature cumulus–oocyte complexes (COCs) retrieved from antral follicles. Different methods refer to IVM involving some form of ovarian stimulation or gonadotropins and an in vitro maturation step. The

most referred to IVM methods are standard (or clinical) IVM and bi-phasic IVM (Gilchrist et al., 2024).

7.9. Standard/clinical IVM involves maturation in vitro of immature germinal vesicle (GV)-stage intact COCs in one step to MII, from unstimulated or FSH-primed patients (Gilchrist et al., 2024). Bi-phasic IVM entails maturation in vitro of immature GV-stage intact COCs in two steps, from unstimulated or FSH-primed patients. Intact COCs are GV-arrested in step one (called pre-IVM) and matured in step two. In the pre-IVM phase, intact COCs are deliberately arrested at the GV-stage, the purpose of which allow for cytoplasmic maturation of the oocyte (Gilchrist et al., 2024).

7.10. Indications for standard/clinical IVM are patients with polycystic ovarian syndrome (PCOS), cancer patients for fertility preservation before chemotherapy and women who do not respond to gonadotropin stimulation (Gilchrist et al., 2024; Lundin et al., 2023). According to the ESHRE good practice recommendations on add-ons in reproductive medicine (Lundin et al., 2023) the use of standard IVM is not considered an add-on. Any other indication, such as women with regular cycles and normal antral follicle count (AFC), the recommendations consider standard IVM to be an add-on. The current version of bi-phasic IVM, using a c-type natriuretic peptide (CNP)-mediated pre-IVM phase (called CAPA-IVM) has been introduced in human studies in 2020 and is still at experimental level (Gilchrist et al., 2024).

7.11. To date, biphasic IVM has been reported in just three types of patients, including women diagnosed with PCOS, women with a high AFC, and women with gynaecological malignancies (Gilchrist et al., 2024). Though safer (no risk of ovarian hyperstimulation syndrome, OHSS) results from standard IVM are inferior to traditional IVF(Vuong et al., 2020). Standard IVM is routinely used in Belgium at **Brussels IVF fertility** centre for patients with PCOS and some cancer patients. IVM is also listed as an authorised process on the **Authorised processes list** under licensed activity 'Processing gametes'. Bi-phasic IVM is likely to become particularly important in future ART scenarios, such as in vitro follicle development (IVD) and IVG as it has the potential to endow developmental competence in vitro on oocytes that are otherwise developmentally incompetent.

7.12. The current summary of scientific developments provides covers publications on in vitro derived gametes published between 1st September 2023 and 31st August 2024. The review does not include literature search for IVM which presented as a briefing. The Executive notes that the following summary of scientific evidence is not an assessment of studies' validity.

Reviews on scientific advances in culture methods for achieving IVG

7.13. A review by (Romualdez-Tan, 2023) overview developments in IVG using iPSCs. First, the paper presents key concepts from groundwork studies on IVG and briefly discussing techniques for the derivation of iPSCs. The paper discusses current and future clinical implications, focusing on recent advances in IVG research using iPSCs in various stages of gametogenesis as well using stem cell-based embryo models, SCBEM (gastruloids and blastoids) as alternative source for IVG.

7.14. An opinion article by (Frost & Gilchrist, 2024) reviews seminal scientific advances and culture methods in murine in vitro oogenesis using different starting cell source. The authors further discuss the current culture techniques applicable to human IVG and the technological hurdles in translating the successful mouse IVG techniques to human reproductive medicine. These hurdles include understanding ovarian biology, efficiency of iPSC culture, formation and activation of

primordial follicles in vitro, using oocytes from pre- and periantral follicles, and overcoming challenges related to particular in vitro culture stages (e.g. follicle in vitro development, IVD). The authors argue that overcoming the IVD hurdle will require addressing three elements: (i) discovery-based research on early folliculogenesis in large mammals including primates; (ii) substantial investment in resources and effort by multiple sectors including increased access to nonhuman primate and human ovarian tissue for research; and (iii) a marked increase in expenditure in this space.

7.15. A review by (Mizuta & Saitou, 2023) discuss recent advances in the understanding of the mechanisms of foetal oocyte development in mammals, as well as in in vitro oogenesis. The authors discuss that achievement of a complete and in vitro oogenesis system in primates would require two critical steps — 1) efficient generation of an abundant number of oogonia and 2) derivation of ovarian somatic cells from ESCs/iPSCs.

7.16. A review by (Gizer et al., 2024) summarises advancements in in vitro spermatogenesis using iPSCs, highlighting the limitations of current protocols, such as low differentiation efficiency and insufficient functional germ cell yield. The authors introduce a novel iPSC-based approach including cellular, chemical, and physical factors that provide the complex arrangement of testicular seminiferous tubules embedded within a vascularised stroma. The authors argue that bioengineered organoids supported by smart bio-printed tubules and microfluidic organ-on-a-chip systems would offer efficient, precise, personalised platforms for autologous pluripotent stem cell sources to undergo the spermatogenetic cycle, presenting a promising tool for infertile male patients with complete testicular aplasia.

7.17. A study by (Damyanova et al., 2024) review progress in the development and application of spermatogonial stem cell (SSC) technologies for clinical application focusing on cryopreservation, in vitro expansion and differentiation, spermatogonial transplantation, and testis tissue grafting.

7.18. Three other reviews by (Bashiri et al., 2023; Fath-Bayati et al., 2023; Salem et al., 2023) overview the progress made in in vitro spermatogenesis using different approaches such as testis tissue or cell suspension culture and three-dimensional methods (eg scaffolds, hydrogels, organoids and organotypic culture of testicular fragments) and clinical implications of those approaches for male infertility treatment and fertility preservation. Additionally, a review by (Jokar et al., 2023) reviews advances in the application of tissue engineering approaches to cell-tissue culture on synthetic or natural scaffolds supplemented with particular focus on growth factors to promote in vitro spermatogenesis.

7.19. A scoping review by (Kwaspen et al., 2024) examines the transcriptional profiles of human testicular cells across different developmental stages to enhance understanding of testis development and optimise in vitro maturation for fertility restoration, particularly in cases of nonobstructive azoospermia and prepubertal boys. By analysing research related to native and in vitro cultured human testicular cells and single-cell RNA sequencing (scRNA-seq), the review identifies critical changes in gene expression and signalling pathways, such as the AKT-signalling and oxidative phosphorylation pathways, during testis maturation. It also challenges traditional classifications of SSCs and highlights the need for more data on somatic cell lineages like Sertoli cells.

7.20. This review by (Aponte et al., 2024) aims to explore advanced reproductive technologies for male fertility preservation, underscoring the essential role that animal models to bridge the gap between current practices and next-generation technologies, aiming to address human male factor infertility and further refine fertility preservation strategies.

7.21. This scoping review of 72 studies by (von Rohden et al., 2024) evaluates current strategies for restoring fertility in prepubertal male cancer survivors and boys with cryptorchidism. It reviews different in vitro and in vivo approaches highlighting key barriers to clinical implementation including risks of reintroducing malignant cells and epigenetic alterations, with direct transplantation of cryopreserved testis tissue emerging as the most promising approach nearing clinical use.

Opinion articles – ethical and legal perspectives

7.22. An opinion article by (Pennings, 2023) discusses a range of applications for stem-cell derived in vitro gametes focusing on the arguments and counter arguments of the IVG potential to be used as an alternative method to obtain oocytes for infertility treatment.

7.23. An opinion article by (Adashi et al., 2024) explored the regulatory and ethical challenges posed in assisted same-sex conception using in vitro gametogenesis that could – in the future – enable same-sex couples have genetically related children. Among the discussed concerns are issues with embryo creation limits, unauthorised use of genetic material, and potential long-term effects on future generations. Furthermore, the widespread adoption of IVG could inadvertently reinforce the notion that only genetic parenthood is valid, potentially undermining the legal and social recognition of non-genetic parenthood in same-sex couples.

7.24. A social research study by (Le Goff et al., 2024) investigates the perspectives of stakeholders potentially most affected by IVG, focusing on individuals with infertility or LGBTQ+ family formation experiences. Through focus groups and interviews with 80 participants, the study found that respondents were hopeful about IVG's potential to enhance reproductive success than current ART, alleviate suffering associated with ART use, and foster social inclusion, while also expressing concerns related to equity and safety. The findings highlight the need for ongoing stakeholder engagement to guide IVG research and clinical application.

7.25. A review by (Villalba, 2024) discusses the current progress in generating artificial gametes from stem cells, highlighting their potential applications in addressing infertility and expanding reproductive options for various groups, such as same-sex couples, post-menopausal women and people with deceased partners. The study emphasises the significant bioethical questions raised by the potential use of human artificial gametes in these diverse reproductive scenarios. It proposes the development of a normative bioethical framework to guide consensus on ethically acceptable uses of artificial gametes in human reproduction.

7.26. An opinion article by (Horer et al., 2023) discusses the ethical implications and different scenarios under which IVG can be applied in clinical settings. The authors also call for an international ethical debate regarding the screening of many IVG-derived human embryos that may be required when deciding for an embryo transfer among those IVG-generated embryos.

7.27. In April 2023 the National Academies Board on Health Sciences Policy hosted a workshop the discussion of which were summarised in proceedings (National Academies of Sciences, 2023) to explore the scientific developments in IVG using pluripotent stem cells, as well as potential clinical implications, clinical pathways, and social, legal and ethical considerations. Participants discussed that rapid advancements in IVG necessitate careful consideration of ethical implications, societal norms, and the potential benefits and harms before clinical application. It was also argued that IVG offers possibilities for those facing infertility but raises concerns about equity, reproductive health policies, and generation of increased number of embryos. Rigorous

protocols for informed consent and long-term follow-up have also been discussed as essential before proceeding to clinical trials.

Animal studies:

Studies to recreate early stages of gametogenesis

7.28. A study by (Lin et al., 2024) explored the retention of epigenetic and transcriptomic memory in iPSCs derived from four different mouse cell types, and iPSC-derived primordial germ cell-like cells (PGCLCs). Authors found that while iPSCs retain residual epigenetic and transcriptomic features of their source cell types, these are largely erased during the transition to PGCLCs. The results suggest that in vitro reprogramming from iPSCs to PGCLCs effectively mimics the two-stage epigenetic reprogramming observed in vivo, supporting the potential of iPSC-derived germline cells for in vitro gametogenesis and infertility treatment.

7.29. A study by (Cooke et al., 2023) demonstrated that mouse gastruloids (ie a three-dimensional in vitro model of gastrulation) contain a population of gastruloid-derived PGCLCs (Gld-PGCLCs) that resemble early PGCs in vivo. Authors reported that the conserved organisation of mouse gastruloids led to coordinated spatial and temporal localisation of Gld-PGCLCs relative to surrounding somatic cells, even in the absence of specific exogenous PGC-specific signalling or extra-embryonic tissues. In gastruloids, self-organised interactions between cells and tissues, including the endodermal epithelium, enabled the specification and subsequent maturation of a pool of Gld-PGCLCs. They conclude that mouse gastruloids can serve as a novel model to study the dynamics of PGC development within integrated tissue environments.

In vitro oogenesis

7.30. A seminal study by (Mikhalchenko et al., 2024) developed a model of premature cell division, where nonreplicated, G0/G1 stage somatic cell nuclei are transplanted to the metaphase cytoplasm of mouse oocytes. They isolated mouse metaphase II (MII) spindles or first polar body (PB1) from hybrid B6/FVB (F1, C57BL--6/J, and FVB/NJ) oocytes, representing 1n2c ploidy, and transplanted into MII cytoplasts (ie an MII oocyte that has had its nucleus removed) derived from BDF1 females. Upon artificial activation, 100% (22 of 22) of transplanted MII spindles and 88.9% (5 of 6) of PB1 extruded the second polar body (PB2) and formed the female pronucleus, indicating that MII cytoplasts retain capacity for asymmetric cytokinesis. There was a high occurrence of somatic genome haploidization in nuclei from inbred genetic backgrounds but not in hybrids, emphasising the importance of sequence homology between homologs. Unlike meiosis, no evidence of recombination between somatic cell homologs was detected. Authors report that SCNT derived oocytes were successfully fertilised by in vivo sperm. Authors conclude that premature cell division relies on mechanisms similar to meiosis I, where genome haploidization is facilitated by homologous chromosome interactions, recognition, and pairing.

7.31. A study by (Gyobu-Motani et al., 2023) induced female cynomolgus embryonic stem cells (ESCs) into cynomolgus primordial germ cell-like cells (cyPGCLCs), and upon use of the xenogeneic reconstituted ovaries system (ie co-culture of cyPGCLCs with mouse embryonic ovarian somatic cells at E12.5), cyPGCLCs differentiated into oocyte-like cells at the zygotene stage of the meiotic prophase. The cynomolgus oogonia/oocyte-like cells underwent nearly proper epigenetic reprogramming, including genome-wide DNA demethylation and X-chromosome dosage compensation.

7.32. A study by (Jang et al., 2024) optimised a method for reconstituting the entire oogenesis process from rat embryonic gonads, successfully generating well-matured MII oocytes. Additionally, the

first in vitro reconstitution of xenogeneic follicles from mouse PGCs and rat somatic cells was achieved, with an appropriate culture medium and incubation period established, facilitating future research on xenogeneic follicular development and oocyte generation.

7.33. This study by (Aizawa et al., 2023) identifies epigenetic dysregulation, particularly the misregulation of Polycomb target genes, as a key factor limiting the developmental competence of in vitro-generated oocytes from mouse pluripotent cells. Systematic transcriptome analysis reveals that defects in oocyte differentiation and early oogenesis during in vitro culture are critical, with abnormalities in zygotic genome activation and 5-hydroxymethylcytosine acquisition further compromising developmental potential.

7.34. A study by (Di Berardino et al., 2024) validated the use of poly(ε-caprolactone) (PCL) electrospun fibrous scaffolds for supporting the in vitro development of multiple preantral follicles (PA) isolated from lamb ovaries. The results demonstrated that PCL scaffolds created a biomimetic ovarian microenvironment and supported the transition of PA towards early antral stage by supporting follicle growth and steroidogenic activation. The artificial ovaries containing ten PA significantly enhanced enhancing meiotic competence, large chromatin remodelling and parthenogenetic developmental competence.

In vitro spermatogenesis

7.35. A study by (Seita et al., 2023) established methods for culturing iPSCs in the common marmoset, enabling differentiation into PGCLCs with immunophenotypic and transcriptomic characteristics akin to in vivo pre-migratory PGCs. The study demonstrated that these common marmoset PGCLCs can be expanded and induced to acquire an early prospermatogonia-like phenotype when co-cultured with mouse testicular somatic cells.

7.36. A study by (Richer, Goyvaerts, et al., 2024) employed a biphasic culture approach to improve testicular organoid (TO) cultures. Authors used agarose microwells to generate TOs from prepubertal mouse C57BL/6 J testicular cells, with optimised media significantly enhancing germ cell survival and histological organisation during the initial cell reorganisation phase. During the differentiation phase, addition of alpha Minimum Essential Medium (α-MEM) plus 10% knock-out serum (KSR) medium and growth factors increased spermatid-like cell production, though these cells remained diploid with irregular nuclear maturation. Immune cell depletion of CD45+ cells negatively affected TO formation. The same authors described a protocol for three-dimensional (3D) bioprinting to mimic the in vivo testicular architecture in vitro (Richer, Vanhaecke, et al., 2024). Pneumatic microextrusion printers were used to create macroporous scaffolds resembling seminiferous tubules by layering hydrogel-encapsulated interstitial cells, followed by seeding epithelial cells into the macropores. The resulting structures aim to enhance cell organization and nutrient supply within the 3D constructs.

7.37. A study by (Tan et al., 2024) evaluates the efficacy of different hydrogels—Agarose, GelMA, AlgMA, DexMA, and their mixtures—for supporting in vitro spermatogenesis in adult mouse testicular tissues cultured using a gas-liquid interphase method. The results show that AlgMA hydrogel best preserved seminiferous tubule integrity and supported high spermatocyte density and SOX9-positive tubules, while DexMA hydrogel significantly enhanced testosterone production. The findings offer insights for optimising in vitro spermatogenesis protocols, potentially improving treatments for male infertility across species, including humans.

7.38. A study by (Shirasawa et al., 2024) developed a method for the efficient induction of primordial germ cell-like cells (PGCLCs) from newly established bovine embryonic stem cells (bESC). By optimising pluripotent culture conditions and using specific signalling modulators, the authors

improved the establishment of bESC and achieved robust differentiation of these cells into PGCLCs marked by expression of key germ cell genes (PRDM1/BLIMP1, TFAP2C, SOX17, and NANOS3), protein expression of KIT/CD117-positive and protein expression negativity for CD44.

7.39. A study by (Asgari et al., 2024) cultured neonatal mouse spermatogonial cells in a three-dimensional (3D) approach using decellularized human placental tissue as a natural scaffold. There was a significant increase in the expression of proliferation (Gfrα1) and differentiation (Sycp3, and protamine marker 1), compared to two-dimensional (2D) cultures, indicating that placental scaffolds could serve as effective bio-scaffolds for promoting spermatogenesis in vitro.

7.40. A study by (Ogawa et al., 2024) explored an alternative approach to in vitro spermatogenesis without using bovine serum-derived albumin formulation (AlbuMAX) by identifying essential components for a synthetic culture medium. Key components include antioxidants (vitamin E, vitamin C, glutathione) and lysophospholipids. The authors made use of microfluidics devices method, by covering testicular tissue with a flat polydimethylsiloxane (PDMS) chip to mimic microfluidic device advantages, such as improved and uniform oxygen and nutrient supply through tissue flattening.

7.41. A study by (Mohammadi et al., 2024) focused on developing biocompatible tubular scaffolds to mimic the structural properties of seminiferous tubules for in vitro spermatogenesis. Scaffolds were synthesised using polycaprolactone (PCL), extracted extracellular matrix (ECM) from decellularized testicular tissue, and varying concentrations of graphene oxide (GO) via electrospinning. Among the formulations, the PCL/ECM scaffold with 0.5% GO demonstrated optimal fiber morphology, mechanical properties, and in vitro biocompatibility, suggesting its potential use in in vitro spermatogenesis for sperm production from spermatogonial cells.

7.42. A study by (Ibtisham et al., 2023) cultured testicular fragments from neonatal piglets under different media conditions and supplementation with different growth factors to assess the effects of growth factors on testicular tissue integrity, germ cell numbers, and the induction of in vitro spermatogenesis (IVS). The results demonstrated that Glial cell line-derived neurotrophic factor (GDNF) and basic fibroblast growth factor (bFGF) supplementation significantly enhanced seminiferous tubule integrity, reduced apoptosis, increased gonocyte counts, and promoted the maturation of spermatoginia to spermatocytes, indicating successful IVS induction.

7.43. A study by (Moutard et al., 2023) examined steroidogenesis and signalling in organotypic cultures of mouse prepubertal testicular tissue, revealing decreased transcript levels of Leydig cell and steroidogenic markers after 30 days of culture despite preserved Leydig cell density. The findings indicate disrupted steroid production and signalling, with increased progesterone and oestradiol, decreased androstenedione, together with decreased transcript levels of steroid metabolizing genes and steroid target genes. Human chorionic gonadotropin (hCG) was insufficient to facilitate Leydig cell differentiation, restore steroidogenesis, and improve sperm yield, highlighting the need for further refinement of the culture system before clinical application.

7.44. A study by (Ghaleno et al., 2024) investigated the effects of three extracellular matrix (ECM)-derived hydrogels – HA-alginate (HA-Alg), HA-alginate-collagen (HA-Alg-Col), and HA-alginate-decellularized ECM (HA-Alg-dECM), on mouse testicular cell culture (isolated at 5 days post-partum, dpp) and in vitro spermatogenesis in a 3D culture setting. After a 14-day culture, HA-Alg was identified as the most effective composite for organoid formation, although it did not show expected signs of testicular organoid formation and spermatogenesis induction. The analyses revealed features of hepatocyte-like cells (HLCs) and gene expression of angiogenesis markers, with extended 28-day culture period showing further differentiation into erythrocytes and renal

cells. Similarly, cell culture of 10 dpp mice for 14 days showed a wide range of cell lineages, including renal, glandular, chondrocyte, and hepatocyte-like cells in comparison to the 5 dpp mice.

Human studies on in vitro oogenesis and spermatogenesis

7.45. A key study by (Alves-Lopes et al., 2024) describes a protocol for generating and progressing human primordial germ cell-like cells (hPGCLCs) from human embryonic stem cells (hESCs) using a co-culture system with hindgut organoids derived from hESCs. Resetting hPGCLCs (rhPGCLCs) are specified from hESCs transitioning from the primed into the naive state of pluripotency. The rhPGCLC-hindgut co-culture system mimics in vivo developmental timing and allows for the subsequent 25-day progression of hPGCLCs within a developmental timing analogous to that observed in vivo, offering an alternative to mouse co-culture models. This method supports the study of hPGC specification, early development, and potential infertility or germ cell tumour aetiologies related to hPGC migration failures.

7.46. A study by (Hwang et al., 2023) utilised custom CRISPR screening in hPGCLCs to identify genes essential for primordial germ cell fate, revealing that TCL1A, an AKT coactivator, is crucial for later stages of hPGCLC development. Loss of TCL1A impaired AKT-mTOR signalling, reduced expression of genes related to translational control, and decreased global protein synthesis and cell proliferation, highlighting the role of TCL1A in hPGCLC development and the utility of CRISPR screening for studying human germ cell biology. The study's results also showed that some of the transcriptional and epigenetic properties of the oogonia and pro-spermatogonia were similar to their in vivo counterparts, whereas other aspects, such as reactivation of the X-chromosome in female gametes proceeded only partially and therefore did not fully replicate the expected in vivo situation. The authors argued that some of these epigenetic differences are due to defects in the pluripotent stem cells used as a starting material.

7.47. A breakthrough study by (Murase et al., 2024) developed a method for inducing epigenetic reprogramming and differentiating pluripotent stem cell-derived human PGCLCs (hPGCLCs) into mitotic pro-spermatogonia or oogonia, achieving over a 10^{10}-fold amplification. Bone morphogenetic protein (BMP) signalling was identified as a key driver, facilitating hPGCLC differentiation through attenuation of the MAPK (ERK) pathway and DNA methylation changes. TET1-deficient hPGCLCs differentiated into extraembryonic cells with aberrant gene activation, highlighting the critical role of TET1 in normal germ cell development.

7.48. A study by (Irie et al., 2023) used in vitro differentiation of human PGCLCs derived from human ESCs to examine the mechanisms of epigenetic reprogramming in human germline development. The authors showed that switching from BMP signalling for PGC specification to Activin A and retinoic acid resulted in DMRT1 and CDH5 expression, the indicators of migratory PGCs in vivo. They also found that DMRT1 promotes DNA methylation erasure at the point where the cells became committed to the germ line, likely mimicking the migration stage of germ cell development. The study results provide insight into the unique role of DMRT1 in germline development for advances in human germ cell biology and in vitro gametogenesis.

7.49. A study by (Silber et al., 2024) provides a review literature on in vitro maturation (IVM) of oocytes and describes an approach IVM of oocytes from human ovarian tissue, providing some findings that primordial follicle recruitment is triggered by tissue pressure gradients.

7.50. A comprehensive review by (Gilchrist et al., 2024) describes how recent advances in basic oocyte biology and their application in animal models has shown the way to increase the clinical efficacy

of IVM in human. It overviews current applications of different IVM methods, limitations and challenges with traditional IVM approaches, new directions and future indications of IVM. Authors conclude that clear goals for IVM are to improve the yield of cumulus oocyte complexes (COCs) collected per follicle aspirated in an IVM oocyte retrieval, and to further improve IVM culture systems to enhance oocyte developmental competence and equalise cumulative pregnancy rates with conventional IVF.

7.51. A study by (Khampang et al., 2024) investigates the role of YAP in human spermatogenesis using an in vitro model and gene editing approach to generate human spermatogonia stem cell-like cells (hSSLCs) from human embryonic stem cells (hESCs). It demonstrates that reducing YAP expression during early spermatogenic differentiation increases PLZF-positive hSSLCs and haploid spermatid-like cells, while up-regulation of YAP is crucial for cell survival in later stages.

7.52. A study by (Esfahani et al., 2024) describes the induction of human primordial germ cell-like cells (hPGCLCs) from human pluripotent stem cells (hPSCs) in a bioengineered culture system that mimics peri-implantation human development. The induction is mediated by paracrine signalling from amniotic ectoderm-like cells (AMLCs) derived from hPSCs, involving ISL1, as well as NODAL, WNT, and BMP signaling pathways. Using this biomimetic platform, hPGCLCs were derived from eight non-obstructive azoospermia (NOA) patient-derived hPSC lines, highlighting its potential for screening applications.

7.53. A study by (Akyash et al., 2024) attempted to differentiate hESCs) into PGCs and post-meiotic germ cells using two different media conditions, including testicular cell-derived conditioned medium (TCCM) and spontaneous differentiation (SD). Embryoid bodies (EBs) from hESCs were cultured and analysed over various time points. The results showed that TCCM significantly increased the expression of gametogenesis-related genes compared to SD groups, suggesting that TCCM serves as a suitable niche for in vitro germ cell development.

7.54. A study by (Salem M et al., 2024) investigated the effect of melatonin supplementation to decellularized testicular matrix (DTM) scaffolds culture setup on the viability and differentiation of spermatogonial stem cells (SSCs). SSCs isolated from deceased adult male patients were cultured on DTM scaffolds with and without melatonin, showing that melatonin significantly reduced germ cell apoptosis and improved SSC viability compared to non-melatonin supplemented controls.

8. Annex B: Definition of gametes and regulation of gamete use in the HFE Act

The HFE Act (1990) defines gametes under

(4) References in this Act to gametes, eggs or sperm, except where otherwise stated, are to live human gametes, eggs or sperm but references below in this Act to gametes or eggs do not include eggs in the process of fertilisation. (1990)

Amendments to the HFE Act were made in 2008:

In this Act (except in section (4A)
(a) references to eggs are to live human eggs, including cells of the female germ line at any stage of maturity, but (except in subsection (1)(b)) not including eggs that are in the process of fertilisation or are undergoing any other process capable of resulting in an embryo,
(b) references to sperm are to live human sperm, including cells of the male germ line at any stage of maturity, and
(c) references to gametes are to be read accordingly.] (2008)

(6) If it appears to the Secretary of State necessary or desirable to do so in the light of developments in science or medicine, regulations may provide that in this Act (except in section 4A) "embryo", "eggs", "sperm" or "gametes" includes things specified in the regulations which would not otherwise fall within the definition.

(7) Regulations made by virtue of subsection (6) may not provide for anything containing any nuclear or mitochondrial DNA that is not human to be treated as an embryo or as eggs, sperm or gametes.]

Prohibitions on the use of Gametes are defined under the HFE Act as follows:

(1) No person shall—
(a) store any gametes, or
(b) in the course of providing treatment services for any woman, use the sperm of any man unless the services are being provided for the woman and the man together or use the eggs of any other woman, or
(c) mix gametes with the live gametes of any animal, except in pursuance of a licence.

(2) A licence cannot authorise storing or using gametes in any circumstances in which regulations prohibit their storage or use.

(3) No person shall place sperm and eggs in a woman in any circumstances specified in regulations except in pursuance of a licence.

(4) Regulations made by virtue of subsection (3) above may provide that, in relation to licences only to place sperm and eggs in a woman in such circumstances, sections 12 to 22 of this Act shall have effect with such modifications as may be specified in the regulations.

(5) Activities regulated by this section or section 3 of this Act are referred to in this Act as "activities governed by this Act".

Section 4 Prohibitions in connection with gametes

4A Prohibitions in connection with genetic material not of human origin

(1) No person shall place in a woman—

(a) a human admixed embryo,

(b) any other embryo that is not a human embryo, or

(c) any gametes other than human gametes

Embryos for use in research may only be created under a research licence.

Schedule 2, point 6, paragraph 3

"Licences for research

3 (1) A licence under this paragraph may authorise any of the following—

(a) bringing about the creation of embryos in vitro, and

(b) keeping or using embryos,

for the purposes of a project of research specified in the licence.

9. References

Adashi, E. Y., Hayashi, K., & Cohen, I. G. (2024). Ethical and legal challenges in assisted same-sex conception through in vitro gametogenesis. In *Nature Medicine* (Vol. 30, Issue 2, pp. 322–323). Nature Research. https://doi.org/10.1038/s41591-023-02689-7

Aizawa, E., Ozonov, E. A., Kawamura, Y. K., Dumeau, C., Nagaoka, S., Kitajima, T. S., Saitou, M., Peters, A. H., & Wutz, A. (2023). Epigenetic regulation limits competence of pluripotent stem cell-derived oocytes. *The EMBO Journal*, *42*(23). https://doi.org/10.15252/embj.2023113955

Akyash, F., Aflatoonian, R., Farashahi-Yazd, E., Hajizadeh-Tafti, F., Golzadeh, J., Sadat Tahajjodi, S., & Aflatoonian, B. (2024). Testicular Cells Derived Conditioned Medium Supports Germ Cell Differentiation of Human Embryonic Stem Cells. *Royan Institute Cell Journal (Yakhteh*, *26*(6), 370–379. https://doi.org/10.22074/CELLJ.2024.2012768.1419

Alves-Lopes, J. P., Wong, F. C. K., & Surani, M. A. (2024). Human primordial germ cell-like cells specified from resetting precursors develop in human hindgut organoids. *Nature Protocols*, *19*(4), 1149–1182. https://doi.org/10.1038/s41596-023-00945-1

Aponte, P. M., Gutierrez-Reinoso, M. A., & Garcia-Herreros, M. (2024). Bridging the Gap: Animal Models in Next-Generation Reproductive Technologies for Male Fertility Preservation. In *Life* (Vol. 14, Issue 1). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/life14010017

Asgari, F., Asgari, H., Najafi, M., Hajiaghalou, S., Pirhajati-Mahabadi, V., Mohammadi, A., Gholipourmalekabadi, M., & Koruji, M. (2024). In vitro proliferation and differentiation of mouse spermatogonial stem cells in decellularized human placenta matrix. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *112*(5). https://doi.org/10.1002/jbm.b.35414

Bashiri, Z., Gholipourmalekabadi, M., Khadivi, F., Salem, M., Afzali, A., Cham, T. C., & Koruji, M. (2023). In vitro spermatogenesis in artificial testis: current knowledge and clinical implications for male infertility. In *Cell and Tissue Research* (Vol. 394, Issue 3, pp. 393–421). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00441-023-03824-z

Cooke, C. B., Barrington, C., Baillie-Benson, P., Nichols, J., & Moris, N. (2023). Gastruloid-derived primordial germ cell-like cells develop dynamically within integrated tissues. *Development (Cambridge, England)*, *150*(17). https://doi.org/10.1242/DEV.201790

Damyanova, K. B., Nixon, B., Johnston, S. D., Gambini, A., Benitez, P. P., & Lord, T. (2024). Spermatogonial stem cell technologies: applications from human medicine to wildlife conservation. *Biology of Reproduction*. https://doi.org/10.1093/biolre/ioae109

Di Berardino, C., Peserico, A., Camerano Spelta Rapini, C., Liverani, L., Capacchietti, G., Russo, V., Berardinelli, P., Unalan, I., Damian-Buda, A. I., Boccaccini, A. R., & Barboni, B. (2024). Bioengineered 3D ovarian model for long-term multiple development of preantral follicle: bridging the gap for poly(ε-caprolactone) (PCL)-based scaffold reproductive applications. *Reproductive Biology and Endocrinology*, *22*(1). https://doi.org/10.1186/s12958-024-01266-y

Esfahani, S. N., Zheng, Y., Arabpour, A., Irizarry, A. M. R., Kobayashi, N., Xue, X., Shao, Y., Zhao, C., Agranonik, N. L., Sparrow, M., Hunt, T. J., Faith, J., Lara, M. J., Wu, Q. Y., Silber, S., Petropoulos, S., Yang, R., Chien, K. R., Clark, A. T., & Fu, J. (2024). Derivation of human primordial germ cell-like cells in an embryonic-like culture. *Nature Communications*, *15*(1). https://doi.org/10.1038/s41467-023-43871-2

Fath-Bayati, L., Naserpour, L., Khoshandam, M., Jannatifar, R., & Fazaeli, H. (2023). Recent advances in developing 3D culture systems of spermatogonial stem cell preservation and differentiation: A

narrative review. In *International Journal of Reproductive BioMedicine* (Vol. 21, Issue 9, pp. 681–698). Research and Clinical Center for Infertitlity. https://doi.org/10.18502/ijrm.v21i9.14397

Frost, E. R., & Gilchrist, R. B. (2024). Making human eggs in a dish: are we close? In *Trends in Biotechnology* (Vol. 42, Issue 2, pp. 168–178). Elsevier Ltd. https://doi.org/10.1016/j.tibtech.2023.07.007

Ghaleno, L. R., Hajari, M. A., Choshali, M. A., Heidari, E. A., Shahverdi, A., Alipour, H., & Valojerdi, M. R. (2024). Hyaluronic acid-alginate hydrogel stimulates the differentiation of neonatal mouse testicular cells into hepatocyte-like and other cell lineages in three-dimensional culture. *Biology of the Cell*. https://doi.org/10.1111/BOC.202400049

Gilchrist, R. B., Ho, T. M., De Vos, M., Sanchez, F., Romero, S., Ledger, W. L., Anckaert, E., Vuong, L. N., & Smitz, J. (2024). A fresh start for IVM: capacitating the oocyte for development using pre-IVM. *Human Reproduction Update*, *30*(1), 3–25. https://doi.org/10.1093/HUMUPD/DMAD023

Gizer, M., Önen, S., & Korkusuz, P. (2024). The Evolutionary Route of in vitro Human Spermatogenesis: What is the Next Destination? *Stem Cell Reviews and Reports*. https://doi.org/10.1007/s12015-024-10726-2

Gyobu-Motani, S., Yabuta, Y., Mizuta, K., Katou, Y., Okamoto, I., Kawasaki, M., Kitamura, A., Tsukiyama, T., Iwatani, C., Tsuchiya, H., Tsujimura, T., Yamamoto, T., Nakamura, T., & Saitou, M. (2023). Induction of fetal meiotic oocytes from embryonic stem cells in cynomolgus monkeys. *The EMBO Journal*, *42*(9). https://doi.org/10.15252/embj.2022112962

Horer, S., Feichtinger, M., Rosner, M., & Hengstschläger, M. (2023). Pluripotent Stem Cell-Derived in Vitro Gametogenesis and Synthetic Embryos-It Is Never Too Early for an Ethical Debate. In *Stem Cells Translational Medicine* (Vol. 12, Issue 9, pp. 569–575). Oxford University Press. https://doi.org/10.1093/stcltm/szad042

Hwang, Y. S., Seita, Y., Blanco, M. A., & Sasaki, K. (2023). CRISPR loss of function screening to identify genes involved in human primordial germ cell-like cell development. *PLoS Genetics*, *19*(12). https://doi.org/10.1371/journal.pgen.1011080

Ibtisham, F., Cham, T. C., Fayaz, M. A., & Honaramooz, A. (2023). Effects of Growth Factors on In Vitro Culture of Neonatal Piglet Testicular Tissue Fragments. *Cells*, *12*(18). https://doi.org/10.3390/cells12182234

Irie, N., Lee, S. M., Lorenzi, V., Xu, H., Chen, J., Inoue, M., Kobayashi, T., Sancho-Serra, C., Drousioti, E., Dietmann, S., Vento-Tormo, R., Song, C. X., & Surani, M. A. (2023). DMRT1 regulates human germline commitment. *Nature Cell Biology*, *25*(10), 1439–1452. https://doi.org/10.1038/S41556-023-01224-7

Jang, S. W., Kim, Y. R., Han, J. H., Jang, H., & Choi, H. W. (2024). Generation of mouse and rat xenogeneic ovaries in vitro for production of mouse oocyte. *Animal Cells and Systems*, *28*(1), 303–314. https://doi.org/10.1080/19768354.2024.2363601

Jokar, J., Abdulabbas, H. T., Alipanah, H., Ghasemian, A., Ai, J., Rahimian, N., Mohammadisoleimani, E., & Najafipour, S. (2023). Tissue engineering studies in male infertility disorder. In *Human Fertility* (Vol. 26, Issue 6, pp. 1617–1635). Taylor and Francis Ltd. https://doi.org/10.1080/14647273.2023.2251678

Khampang, S., Lorthongpanich, C., Laowtammathron, C., Klaihmon, P., Meesa, S., Suksomboon, W., Jiamvoraphong, N., Kheolamai, P., Luanpitpong, S., Easley, C. A., Mahyari, E., & Issaragrisil, S. (2024). The dynamic expression of YAP is essential for the development of male germ cells derived from human embryonic stem cells. *Scientific Reports*, *14*(1). https://doi.org/10.1038/s41598-024-66852-x

Kwaspen, L., Kanbar, M., & Wyns, C. (2024). Mapping the Development of Human Spermatogenesis Using Transcriptomics-Based Data: A Scoping Review. In *International Journal of Molecular Sciences* (Vol. 25, Issue 13). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/ijms25136925

Le Goff, A., Jeffries Hein, R., Hart, A. N., Roberson, I., & Landecker, H. L. (2024). Anticipating in vitro gametogenesis: Hopes and concerns for IVG among diverse stakeholders. *Stem Cell Reports*, *19*(7), 933–945. https://doi.org/10.1016/j.stemcr.2024.05.002

Lin, Y. H., Lehle, J. D., & McCarrey, J. R. (2024). Source cell-type epigenetic memory persists in induced pluripotent cells but is lost in subsequently derived germline cells. *Frontiers in Cell and Developmental Biology*, *12*. https://doi.org/10.3389/fcell.2024.1306530

Lundin, K., Bentzen, J. G., Bozdag, G., Ebner, T., Harper, J., Le Clef, N., Moffett, A., Norcross, S., Polyzos, N. P., Rautakallio-Hokkanen, S., Sfontouris, I., Sermon, K., Vermeulen, N., & Pinborg, A. (2023). Good practice recommendations on add-ons in reproductive medicine. *Human Reproduction*, *38*(11), 2062–2104. https://doi.org/10.1093/HUMREP/DEAD184

Mikhalchenko, A., Marti Gutierrez, N., Frana, D., Safaei, Z., Dyken, C. Van, Li, Y., Ma, H., Koski, A., Liang, D., Lee, S.-G., Amato, P., & Mitalipov, S. (2024). Induction of somatic cell haploidy by premature cell division. In *Sci. Adv* (Vol. 10). https://www.science.org

Mizuta, K., & Saitou, M. (2023). Key mechanisms and in vitro reconstitution of fetal oocyte development in mammals. In *Current Opinion in Genetics and Development* (Vol. 82). Elsevier Ltd. https://doi.org/10.1016/j.gde.2023.102091

Mohammadi, A., Koruji, M., Azami, M., Shabani, R., Mohandesnezhad, S., Bashiri, Z., & Asgari, H. (2024). Polycaprolactone/Testicular Extracellular Matrix/Graphene Oxide-Based Electrospun Tubular Scaffolds for Reproductive Medicine: Biomimetic Architecture of Seminiferous Tubules. *Macromolecular Bioscience*, *24*(2). https://doi.org/10.1002/mabi.202300342

Moutard, L., Goudin, C., Jaeger, C., Duparc, C., Louiset, E., Pereira, T., Fraissinet, F., Delessard, M., Saulnier, J., Rives-Feraille, A., Delalande, C., Lefebvre, H., Rives, N., Dumont, L., & Rondanino, C. (2023). Steroidogenesis and androgen/estrogen signaling pathways are altered in in vitro matured testicular tissues of prepubertal mice. *ELife*, *12*. https://doi.org/10.7554/eLife.85562

Murakami, K., Hamazaki, N., Hamada, N., Nagamatsu, G., Okamoto, I., Ohta, H., Nosaka, Y., Ishikura, Y., Kitajima, T. S., Semba, Y., Kunisaki, Y., Arai, F., Akashi, K., Saitou, M., Kato, K., & Hayashi, K. (2023). Generation of functional oocytes from male mice in vitro. *Nature*, *615*(7954), 900–906. https://doi.org/10.1038/s41586-023-05834-x

Murase, Y., Yokogawa, R., Yabuta, Y., Nagano, M., Katou, Y., Mizuyama, M., Kitamura, A., Puangsricharoen, P., Yamashiro, C., Hu, B., Mizuta, K., Tsujimura, T., Yamamoto, T., Ogata, K., Ishihama, Y., & Saitou, M. (2024). In vitro reconstitution of epigenetic reprogramming in the human germ line. *Nature*, *631*(8019), 170–178. https://doi.org/10.1038/s41586-024-07526-6

National Academies of Sciences, E. and M. (2023). In Vitro Derived Human Gametes as a Reproductive Technology: Scientific, Ethical, and Regulatory Implications: Proceedings of a Workshop (2023). *Washington, DC: The National Academies Press*. https://doi.org/10.17226/27259

Ogawa, T., Matsumura, T., Yao, T., Kimura, H., Hashimoto, K., Ishikawa-Yamauchi, Y., & Sato, T. (2024). Improvements in in vitro spermatogenesis: oxygen concentration, antioxidants, tissue-form design, and space control. In *Journal of Reproduction and Development* (Vol. 70, Issue 1).

Pennings, G. (2023). *Why we need stem-cell derived gametes*. https://doi.org/10.1016/j

Richer, G., Goyvaerts, C., Davida Marchandise, L., Vanhaecke, T., Goossens, E., & Baert, Y. (2024). Spermatogenesis in mouse testicular organoids with testis-specific architecture, improved germ cell survival and testosterone production. *Biofabrication*. https://doi.org/10.1088/1758-5090/ad618f

Richer, G., Vanhaecke, T., Rogiers, V., Goossens, E., & Baert, Y. (2024). Mouse In Vitro Spermatogenesis on 3D Bioprinted Scaffolds. In *Methods in Molecular Biology* (Vol. 2770, pp. 135–149). Humana Press Inc. https://doi.org/10.1007/978-1-0716-3698-5_11

Romualdez-Tan, M. V. (2023). Modelling in vitro gametogenesis using induced pluripotent stem cells: a review. In *Cell Regeneration* (Vol. 12, Issue 1). Springer. https://doi.org/10.1186/s13619-023-00176-5

Salem M, Khadivi F, Feizollahi N, Khodarahmian M, Saedi Marghmaleki M, Ayub S, Chegini R, Bashiri Z, Abbasi Y, Naji M, & Abbasi M. (2024). Melatonin Promotes Differentiation of Human Spermatogonial Stem Cells Cultured on Three-Dimensional Decellularized Human Testis Matrix. *Urology Journal*, *21*(4), 250–264.

Salem, M., Khadivi, F., Javanbakht, P., Mojaverrostami, S., Abbasi, M., Feizollahi, N., Abbasi, Y., Heidarian, E., & Rezaei Yazdi, F. (2023). Advances of three-dimensional (3D) culture systems for in vitro spermatogenesis. In *Stem Cell Research and Therapy* (Vol. 14, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13287-023-03466-6

Seita, Y., Cheng, K., McCarrey, J. R., Yadu, N., Cheeseman, I., Bagwell, A., Ross, C. N., Santana-Toro, I., Yen, L. H., Vargas, S., Navara, C. S., Hermann, B. P., & Sasaki, K. (2023). Efficient generation of marmoset primordial germ cell-like cells using induced pluripotent stem cells. *ELife*, *12*. https://doi.org/10.7554/eLife.82263

Shirasawa, A., Hayashi, M., Shono, M., Ideta, A., Yoshino, T., & Hayashi, K. (2024). Efficient derivation of embryonic stem cells and primordial germ cell-like cells in cattle. In *Journal of Reproduction and Development* (Vol. 70, Issue 2).

Silber, S. J., Goldsmith, S., Castleman, L., & Hayashi, K. (2024). In Vitro Maturation, In Vitro Oogenesis, and Ovarian Longevity. *Reproductive Sciences*, *31*(5), 1234–1245. https://doi.org/10.1007/s43032-023-01427-1

Tan, J., Li, J., Zhang, Y., Li, X., Han, S., Li, Z., & Zhou, X. (2024). Application of photocrosslinked gelatin, alginate and dextran hydrogels in the in vitro culture of testicular tissue. *International Journal of Biological Macromolecules*, *260*. https://doi.org/10.1016/j.ijbiomac.2024.129498

Villalba, A. (2024). Artificial Gametes and Human Reproduction in the 21st Century: An Ethical Analysis. In *Reproductive Sciences*. Springer Nature. https://doi.org/10.1007/s43032-024-01558-z

von Rohden, E., Jensen, C. F. S., Andersen, C. Y., Sønksen, J., Fedder, J., Thorup, J., Ohl, D. A., Fode, M., Hoffmann, E. R., & Mamsen, L. S. (2024). Male fertility restoration: In vivo and In vitro stem cell-based strategies using cryopreserved testis tissue – A scoping review. *Fertility and Sterility*. https://doi.org/10.1016/j.fertnstert.2024.07.010

Vuong, L. N., Ho, V. N. A., Ho, T. M., Dang, V. Q., Phung, T. H., Giang, N. H., Le, A. H., Pham, T. D., Wang, R., Smitz, J., Gilchrist, R. B., Norman, R. J., & Mol, B. W. (2020). In-vitro maturation of oocytes versus conventional IVF in women with infertility and a high antral follicle count: a randomized non-inferiority controlled trial. *Human Reproduction*, *35*(11), 2537–2547. https://doi.org/10.1093/humrep/deaa240

Scientific Considerations around 14-day rule for embryo research

Details about this paper

Area(s) of strategy this paper relates to:	Shaping the future
Meeting:	SCAAC
Agenda item:	8
Paper number:	HFEA (07/10/2024) 008
Meeting date:	7 October 2024
Author:	Rebecca Taylor, Scientific Policy Manager Molly Davies, Scientific Policy Officer
Annexes	Annex A: Scientific literature review Annex B: History of the 14-day rule

Output from this paper

For information or recommendation?	For recommendation
Recommendation:	Members are asked to advise on: • The scientific rationale for keeping vs extending the 14-day rule • The possible benefits and drawbacks of extending the 14-day rule • If an extension beyond 14 days is to be considered, what would the SCAAC recommend and why?
Resource implications:	TBC
Implementation date:	TBC
Communication(s):	TBC

Organisational risk	☐ Low	☒ Medium	☐ High

1. Introduction

1.1. The limit to which human embryos can be cultured is regulated by the **Human Fertilisation and Embryology Act 1990 (the HFE Act)**. This paper will consider whether it is necessary to recommend changes to the 14-day time limit (aka "14-day rule") for human embryo research and provide a summary of recent research developments in this area.

1.2. The SCAAC will be asked to give their advice and/or make recommendations concerning the 14-day rule, focusing on the scientific and technical aspects including potential benefits and drawbacks.

1.3. Any advice or recommendations received from the SCAAC will feed into the development of an Authority paper on the 14-day rule for the November 2024 Authority meeting. At that meeting the Authority will consider both the scientific/technical case as well as the wider ethical arguments to come to a consensus on proposals.

1.4. Any change in the Act is for Government and Parliament. However, it continues to be important for the HFEA to lead the discussions and understand the scientific, legal and ethical implications of extending the 14-day limit of in vitro embryo culture.

2. Background

2.1. The HFEA published **proposals for modernising the HFE Act** in November 2023. One of the four areas where recommendations were made was in future scientific developments and innovation. The recommendations made were:

- The Act should explicitly give the HFEA greater discretion to support innovation in treatment and research.
- The Act should be amended to 'future proof' it, so that it is better able to accommodate future scientific developments and new technologies.

2.2. One of the areas identified under future scientific developments was the 14-day rule for embryo research, which is set in law in the HFE Act. For further information on the history of the 14-day rule, please see annex B.

2.3. Research using human embryos and human admixed embryos requires a research licence from the HFEA. The Authority considers applications for research licences and may grant a licence if the research can be considered necessary and desirable to achieve at least one of the following principal purposes as defined in the **HFE Act (2008)**:

- (a) increasing knowledge about serious disease or other serious medical conditions,
- (b) developing treatments for serious disease or other serious medical conditions,
- (c) increasing knowledge about the causes of any congenital disease or congenital medical condition that does not fall within paragraph (a),
- (d) promoting advances in the treatment of infertility,
- (e) increasing knowledge about the causes of miscarriage,
- (f) developing more effective techniques of contraception,
- (g) developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation, or
- (h) increasing knowledge about the development of embryos.

2.4. With advancements in science enabling the culture of embryos beyond 14 days, some researchers have called for the 14-day limit to be extended, most commonly to 28 days.

2.5. The HFEA position is that if the '14-day rule' were to be reconsidered by the government in future, there is a need for public engagement and discussion of the implications of this, as the ISSCR guidelines, and many others have already recommended.

2.6. Concerns raised in the HFEA public consultation around any possible extension of the 14-day rule included:

- Respondents expressing the view that going beyond 14 days would mean research on embryos whose central nervous system (17 days), heart and brain (28 days) are beginning to develop, raising the question of sentience, (NB: scientific research is clear that embryos are not sentient at this stage of development).
- Respondents highlighting that if the 14-day limit were to be extended, it must be subject to strict conditions and clearly identifiable limits to ensure research remains safe and ethical.

2.7. If changes are to be made that could raise ethical and moral questions, such as extending the time limit for embryo research, this should be done with sufficient parliamentary scrutiny. Scientific considerations relevant to the 14-day rule were last discussed by the SCAAC in **October 2022** in which Professor Robin Lovell-Badge presented an **overview** of the arguments. Research on the extension of the 14-day rule has subsequently been considered as part of the annual literature search relevant to the SCAAC's horizon scanning function, with topic inclusion since **January 2022**.

3. Summary of research developments

3.1. Annex A provides a review of the literature published between January 1st 2024 and August 31st 2024 on scientific developments relevant to extending the '14-day rule'. Where relevant, seminal research presenting foundational insights or widely recognised paradigms are included.

3.2. There are arguments in favour of extending the 14-day limit for embryo research as well as arguments that do not favour such an extension. There is still much to learn from research on 7-14 day old embryos, leading to arguments that before extending beyond 14 days, further 7-14 day research is necessary (Blackshaw & Rodger, 2021). Such research could lead to further discoveries that are necessary or useful for researchers to know before they undertake research beyond 14 days. There also could be discoveries that reduce or remove the need to extend the embryo research time limit beyond 14 days.

3.3. On the 14-day rule it appears that science can already move beyond this limit (Cavaliere, 2017; Peng et al., 2022a) although some technical issues such as culture methods, are still not completely resolved (Pera, 2017).

3.4. Supporters of such research point to the potential benefit of expanding our understanding of developmental biology through study of early embryo development (Cavaliere, 2017; Chan, 2018; Peng et al., 2022a; Pera, 2017). However, given the concerns mentioned here as well as clarity for researchers, it remains important to have an identifiable time limit and other constraints on embryo research. It will also be important to reach a consensus on any new limit and/or constraints related to that limit, which requires a mechanism for doing so.

4. Current and future challenges to 14-day limit

4.1. When the '14-day rule' was proposed it was not possible to culture embryos in vitro beyond day five or six of development. However, advances over the last 40 years have shown that it could be technically feasible for scientists to culture embryos up or beyond to this 14-day limit. In (Deglincerti et al., 2016; Shahbazi et al., 2016), both reported culturing embryos in vitro past day seven, when implantation would usually occur, and had to terminate their experiments on day 13 due to the '14-day rule'.

4.2. Another scientific development which has brought the '14-day rule' into question is the creation of embryo models. These models involve developing human embryonic stem cells (ES cells) or induced pluripotent stems cells (IPS cells) into non-integrated and integrated stem cell-based embryo models (SCBEMs). Non-integrated SCBEMs recapitulate some, but not all aspects of the peri-implantation embryo, e.g. gastruloid embryo model. Integrated SCBEMs contain the relevant embryonic and extra-embryonic structures that could potentially achieve the complexity to further develop in vitro e.g. blastoid embryo model. However, SCBEMs do not explicitly fall within the definition of embryos in the HFE Act and therefore under current regulatory requirements for the culture of embryos. At the February 2023 meeting of the SCAAC (see **meeting minutes**), an external expert informed the Committee that in the UK, scientists undertaking research with integrated embryo models are currently following the rules that apply to embryo research including the 14-day time limit.

4.3. In 2021 the International Society for Stem Cell Research (ISSCR) published updated **guidance** for stem cell research and clinical translation. ISSCR guidance establishes categories of research:

- Category 1A – permissible without review
- Category 1B - permissible without review, but must be reported to designated body to monitor research in case issues arise
- Category 2 – permissible after review and approval through specialised scientific and ethical review
- Category 3a – prohibited despite scientific rationale as currently unsafe
- Category 3b – prohibited due to lack of scientific justification, being unsafe and/or considered unethical

Until 2021, research involving human embryos up to 14 days fell under category 2, while research beyond 14 days fell under category 3b. In its recommendation 2.2.2.1, ISSCR proposed moving embryo research beyond 14 days to category 2 as follows:

"*Should broad public support be achieved within a jurisdiction, and if local policies and regulations permit, a specialized scientific and ethical oversight process could weigh whether the scientific objectives necessitate and justify the time in culture beyond 14 days, ensuring that only a minimal number of embryos are used to achieve the research objectives*." (International Society for Stem Cell Research, 2021).

5. Reconsideration of the 14-day rule in other jurisdictions

5.1. Following a request from the Dutch Ministry of Health and Welfare, the Health Council of the Netherlands, an Advisory Body, convened a working group to examine the desirability of extending the 14 day rule and how to establish a developmental limit for embryo like structures (ELS). The working group produced a report in October 2023 (Health Council of the Netherlands, 2023), which recommended:

- Extending the time limit on human embryo research to 28 days
- Applying the 28-day time limit to non-conventional embryos/embryo like structures such as SCBEMs.
- Case-by-case approval from the Central Committee on Research involving Human Subjects (CCMO) required for any research project going beyond 14 days.

There has been a change of government in the Netherlands since the report was published and the new government has not given any indication that it wishes to consider or act on the report's recommendations.

5.2. In a statement published in May 2024, the Norwegian Biotechnology Advisory Board noted that its members were not united on their views around possible extension of the 14-day rule:

- Nine of the Board's 15 members believe current 14-day limit should be extended up to and including day 28 and that there should also be an opportunity to apply for an exemption from the 28-day limit in special cases, on a case-by-case basis.
- Five members want to keep the current 14-day time limit, but three of those members want a case-by-case dispensation up to day 28. (The Norwegian Biotechnology Advisory Board, 2024).

5.3. In France, the bioethics law, which covers embryo research, was amended in 2021. When proposals to amend the bioethics law were first discussed, a 21-day time limit for embryo research was suggested in place of the 7-day time limit which was already in place (Depadt & Hirsch, 2020). However, this was rejected in favour of a 14-day time limit which was adopted in the final legislation (LOI N° 2021-1017 Du 2 Août 2021 Relative à La Bioéthique, 2021). In 2023, the Conseil d'Orientation (advisory body of the French Biomedicine Agency) issued an opinion on stem cell based embryo models expressing the view that there should be no extension of the 14-day rule for human embryo research (Agence de Biomédecine, 2023). The Conseil's reasoning was the proposal to allow research on integrated embryo models such as blastoids until the equivalent of 28 days post fertilisation (Agence de Biomédecine, 2023).

5.4. Chinese researchers have suggested that China's Ethical Guidelines should be amended to set a new time limit for embryo research beyond 14 days (Peng et al., 2022a). No formal proposals have yet been developed.

6. Challenging the case for change

6.1. Research looking at the development of 7-14 day old embryos has not yet been fully explored (Blackshaw & Rodger, 2021). Most discoveries arising from embryo research have been made within the 7-day pre-implantation period (Williams & Johnson, 2020). There may be discoveries

to be made undertaking research during this stage of early human development that reduce or remove the need for undertaking research from 14-28 days. In addition, the fact there is still much to learn the 7-14 day period of embryo development may limit discoveries generated from research on 14-28 day embryos (Blackshaw & Rodger, 2021).

6.2. Post 14-day research is likely to expand knowledge of developmental biology (further details below), but unlikely to result in discoveries that lead to disease treatment (Pera, 2017). There are still questions about the reliability of culturing embryos in-vitro beyond 14 days. It has been observed that embryos cultured to 12 and 13 days become “disorganised” and “do not reflect the development of a 13-day old embryo” (Agence de Biomédecine, 2023) under current culture conditions (Pera, 2017).

6.3. Even with improved in-vitro culture, there is still a question as to how reliably this extended in-vitro culture could replicate embryogenesis in-vivo (Pera, 2017). replicate the post implantation environment of the womb. This could require approaches such as transcriptome or epigenome analysis, to assess whether cells in cultured embryos show a similar phenotype to those in in-vivo embryos using data from non-human primate studies (Pera, 2017).

6.4. The biological marker for 14 days, the primitive streak, is a clear and identifiable marker for researchers. It will be desirable and arguably necessary for both researchers and the regulator that any amended time limit would be similarly identifiable. However, this runs into what (Chan, 2018) calls the “14-day paradox”; embryos have not been cultured past 14 days, so the developmental processes they undergo after that point are not well known and need to be studied in order to understand embryo development that the 14-day rule is designed to avoid. This leads to the question as to whether a clear biological marker can currently be identified post 14 days, when embryo development of that stage has not yet been studied.

6.5. There is likely to be public uncertainty and concerns on ethical and moral grounds to extending the time limit, which has the potential to damage public confidence in fertility research and the HFEA. This was reflected in some of the responses to the HFEA consultation.

7. Making the case for change

7.1. The 7-28 day period of early human development is sometimes referred to as a “black box” due to the lack of understanding hampered by the fact that embryos are usually implanted in the womb (in-vivo) after day 7 and thus impossible to investigate in that in-vivo environment (Greely, 2022; Williams & Johnson, 2020). It is possible to study human embryos up to 14 days in-vitro and some research is possible after 28 days on tissues from aborted or miscarried embryos (Greely, 2022). Enabling research beyond 14 days has serious potential to expand knowledge of developmental biology in particular the gastrulation phase when neural plate and tube, major organs, body axes are established (Peng et al., 2022a). Days 14-28 are when embryological defects tend to occur, in particular during organogenesis (post 21 days) when the embryo is most sensitive to teratogens and heart and neural tube development begins (Peng et al., 2022).However, it will be necessary to define and justify any expanded time limit.

7.2. Studying early embryo development post 14 days could:

- Advance our knowledge of embryogenesis (Peng et al., 2022a).
- Enable investigation of what happens from around 7th day when implantation should begin to the point two to three weeks later when miscarriage occurs (Greely, 2022) including

improving understanding of how the complex signals of the intrauterine environment affect embryo development and subsequent early pregnancy loss (Chan, 2018).
- Provide a scientific foundation for preventing birth defects and teratogenesis(Peng et al., 2022a; Pera, 2017).
- Improve understanding of epigenetic programming that occurs during this stage of development that could impact disease progression in later life (Pera, 2017).

7.3. The above-mentioned advances in understanding of early embryo development have the potential to enable:

- Validation of stem cell based embryo models (SCBEMs), which could reduce the use of human embryos in research, although probably not replace it entirely (Pera, 2017).
- Refinement of the way pluripotent stem cell differentiation mimics embryogenesis (Pera, 2017).
- The identification of benchmarks for the safety and efficacy of human genome editing (CRISPR), mitochondrial replacement (MRT), and in-vitro derived gametes (IVGs).

7.4. The previous point raises an interesting conundrum for advocates of reducing human embryo research through the use of embryo models, namely the need to use human embryos to validate those models, including past the current 14-day limit.

7.5. Part of the case for extending the 14-day rule in a safe and ethical way is that it can contribute to the UK's reputation as a leading nation for enabling scientific innovation including well-regulated embryo research.

8. Questions for the committee

The Committee is asked to consider the following scientific questions related to the 14-day rule.

8.1. Do you know about any other research or recent developments related to the 14-day rule that our literature review has not covered?

8.2. Is there an argument that further research on 7-14 day embryos is required before extending the time limit beyond 14 days? Could such research lead to discoveries that would inform research beyond the 14-day time limit and/or reduce the need for research beyond 14 days?

8.3. If it is considered appropriate to set a new time limit for human embryo research extending beyond 14 days:

- Can the committee identify in any detail likely/desirable discoveries that may be established through allowing embryo research beyond 14 days?
- What new time limit should be placed on embryo research and on what basis would the SCAAC recommend it?
- How could any new limit be easy to determine for researchers and the Authority? E.g. via clear biological markers
- Should a new time limit be a general rule or part of the case-by-case consideration of the research licence application? The latter could require each research project wishing to go beyond 14 days to specify the desired time limit (up to any new fixed upper limit) and be granted permission (or not) based on an individual assessment of the application.

- If a case-by-case consideration, what grounds for approval or refusal should be used? E.g. research question may be answered by research on 7-14 day embryos or using stem cell based embryo models etc.
- Should human admixed embryos be included in any extension of the time limit?

8.4. When it comes to new types of cells such as in vitro gametes and stem cell-based embryo models (SCBEMs):

- How/under what circumstances could extending 14-day rule help validate alternative cells such as in-vitro gametes and SCBEMs?
- How could criteria be established to refuse a research licence for human embryo research (up to or beyond 14 days) on the grounds that the research proposed could be undertaken using new cells such as SCBEMs?

9. Annex A: Scientific Literature Review

9.1. This annex provides a review of the literature published between January 1st 2024 and August 31st 2024 on scientific developments relevant to extending the '14-day rule'. Where relevant, seminal research presenting foundational insights or widely recognised paradigms are included.

Scientific challenges to the 14-day rule

9.2. Since the introduction of the Warnock Report, knowledge of early human development has subsequently been refined. As laid out in this annex, the key arguments noted in support of the 14-day limit on embryo culture included that the limit should fall before the development of the nervous system, to accommodate ethical considerations relating to the sentience of the embryo, and that 14-days of development is the final stage in which twinning can occur, thus representing the beginning of individualisation.

9.3. Characterisation of human embryos and other models has provided insights into embryonic development up to the stages of gastrulation and early organogenesis:

9.4. In 2021, (Tyser et al., 2021) in the UK described the single-cell transcriptomic characterisation of a single human gastrulating embryo between 16-19 days post fertilisation, providing detailed descriptions of the cells present. The single embryo, derived from a termination of pregnancy, was completely intact and morphologically normal. Dorsal and ventral views of the embryonic disk showed the primitive streak extending approximately half the diameter of the disk along the long rostral–caudal axis with the primitive node visible at the rostral end. Single-cell RNA sequencing was performed across 1,195 cells, including 665 caudal, 340 rostral and 190 yolk sac cells. Findings indicated that markers of early neural induction (SOX1, SOX3 and PAX6) and differentiated neurones (TUBB3, OLIG2, NEUROD1) were undetectable or expressed at very low levels, with no cells expressing two or more of the markers SOX3, PAX6 or TUBB3. This led researchers to conclude, based on the investigations into the embryo obtained, that neural differentiation at 16-19 days has not yet commenced in vivo.

9.5. As no neural connections or sensory systems exist in the embryo it is therefore impossible for the embryo to experience sentience or suffering at this stage. Furthermore, studies into neural development have shown that synchronised impulses of neurons in the peripheral nervous system cannot be detected earlier than at least the second month of development (Greely, 2022; Sekulic et al., 2016).

9.6. The study of one embryo by (Tyser et al., 2021) additionally found a small population of putative primordial germ cells among the cells of the primitive streak, indicating that future sperm cells (from the male embryo) have been specified. This aligns with earlier findings of (Chen et al., 2019) who, using in vitro cultures of human embryos, showed that human primordial germ cells specification begins at 12-days post fertilisation.

9.7. (Zeng et al., 2023) at Beijing Normal University have subsequently analysed the transcriptional profiles of over 400,000 cells from 14 human samples collected between post conceptional weeks 3 to 12 (equivalent of Carnegie stages 10, 12 to 16, 18, and 20) characterising the molecular and cellular landscape of early gastrulation and nervous system development. Two of the embryos were post 3 weeks conception, two were post 4 weeks, and four embryos were post 5 weeks conception.

9.8. Despite studies on tissues from aborted or miscarried embryos, relatively little is known about the key biological markers occurring between 14- and 28-days post fertilisation (Chan, 2018). In

addition, this period represents a critical 'black box' in human development, where rapid cellular differentiation and organogenesis occur. Disruption of normal development at this stage is thought to underly embryological defects, but the lack of detailed knowledge about these mechanisms leaves a gap in our understanding of how genetic or environmental factors may contribute to congenital abnormalities[1].

9.9. Additional support for a 14-day limit was originally provided on the principle that there is substantial embryo loss from the time of fertilisation up to 14-days and, unless implantation is complete, there is no potential for further development of the embryo. As analysed by (Jarvis, 2016) under natural conditions, embryo loss is approximately 10-40% before implantation, with loss from fertilisation to birth accounting for loss of 40-60% of embryos.

Development of Embryo Culture Systems

9.10. When the 14-day rule was proposed it was not possible to culture embryos in vitro beyond day five or six of development. However, advances over the last 40 years have shown that it could be technically feasible for scientists to culture embryos up or beyond to this 14-day limit. In 2016, (Deglincerti et al., 2016) and (Shahbazi et al., 2016) first reported culturing human embryos in vitro past day seven, when implantation would usually occur.

9.11. Optimisation of culture systems through the use of polymer substrates, 3D scaffolds and microfluidic devices among others (Aguilera-Castrejon et al., 2021; Gu et al., 2022) have meant that further groups have now been able to demonstrate prolonged support of both human and non-human primate embryos in culture.

9.12. This includes the study by (Xiang et al., 2020) who demonstrated the specialisation of epiblast and hypoblast lineages, the emergency of the proamniotic cavity, bi-laminar disc and anterior–posterior polarity formation, amniotic and yolk sac cavitation, trophoblast diversification, and primitive streak formation of in vitro cultured day 14 human embryos.

9.13. Researchers in New York cultured 21 human embryos from days 5-6 to day 12, and in 6 of those embryos they observed markers of epiblast (OCT4) and early primitive streak cells (TBXT) (De Santis et al., 2024). Their results provide evidence that upon extended in vitro culture, some human embryos can self-organise their epiblast tissue and undergo very early steps towards primitive streak formation.

9.14. Although cells of the majority of embryonic and extraembryonic lineages found in vivo are detected in embryos in long-term culture, some disparities in the clustering of cell types in human embryos have been observed (Agence de Biomédecine, 2023). Discrepancies might be due to the appearance of transient and intermediate cell lineages, the possible presence of aberrant cells, or in vitro culture methods not reliably replicating the post-implantation womb.

9.15. Studies on non-human primate embryos are useful because of their close genetic and developmental similarities to humans, providing insights into early human embryogenesis that are ethically or technically challenging to obtain directly from human embryos (Yao et al., 2023). (Niu et al., 2019) and (Ma et al., 2019) were the first groups to successfully establish a culture

[1] It is important to note that studying 6 weeks post conception aborted or miscarried embryos cannot reveal information about placental development, whereas studying human embryos in vitro using endometrial co-culture systems would allow the study of early placental growth.

system that enabled the development of cynomolgus monkey embryos in vitro for up to 20 days.

9.16. Since, (Gong et al., 2023) have developed an embedded 3D culture system that allows for the extended ex utero culture of cynomolgus monkey embryos for up to 25-days post-fertilisation. Using this platform, researchers were able to delineate lineage trajectories and genetic programs involved in neural induction (Zhai et al., 2023), lateral plate mesoderm differentiation, yolk sac haematopoiesis, primitive gut formation, and primordial germ-cell-like cell development in monkeys. The 3D culture system additionally provides a reproducible platform for studying long-term primate embryogenesis ex utero (Sun et al., 2024).

9.17. The growth of human admixed embryos (also referred to as human-animal chimaeras) can also help researchers to elucidate the underlying mechanisms of early developmental biology. Human admixed embryos, broadly encompasses embryos created by combining human and animal genetic material or cells, such as introducing human cells into animal embryos. Like human embryos, under the HFE Act, human admixed embryos are subject to the 14-day rule in the UK, limiting research on these structures to 14 days post-fertilization or until the formation of the primitive streak.

9.18. However, the study by (Tan et al., 2021), conducted in China, reported on the in vitro growth of human-monkey chimeric embryos for up to 20 days post fertilisation, following human stem cell integration in cynomolgus monkey embryos. Despite the ethical issues with this study, as highlighted by (Greely & Farahany, 2021), it was able to demonstrate the ability to sustain human extended pluripotent stem cells inside monkey embryos for over 14-days. In this study, (Tan et al., 2021) modelled the proliferation of several peri- and early post-implantation cell lineages and uncovered signalling events that help researchers to understand the developmental trajectories of human cells during early embryogenesis.

9.19. Although research using non-humane primates is beneficial, species-specific differences between cynomolgus monkey and humans have been highlighted (Cui et al., 2022; Tyser et al., 2021). These include the timing of implantation and amniogenesis. Despite advancements in human and primate modelling, the mouse remains the predominant model used for research into mammalian gastrulation and organogenesis with many studies now having describe the molecular features of gastrulation and organogenesis during early mouse development (Argelaguet et al., 2019; Cao et al., 2019; Pijuan-Sala et al., 2019; Wang et al., 2023).

9.20. Despite the ISSCR revising the guidelines on the 14-day limit in 2021, there are no published studies reporting the culture of human embryos beyond 14 days post fertilisation to date.

Challenges of SCBEM, IVGs and Genome Editing

9.21. Advancements in the generation of SCBEM, IVGs and genome editing have indirectly raised another challenge for the 14-day rule, centred around the need to characterise and validate models, and to establish the safety and efficacy of new technologies.

9.22. As summarised by (Iltis et al., 2023) embryo models raise numerous ethical, legal, regulatory and policy considerations, including those associated with the 14-day rule. One key scientific challenge regarding its application to SCBEM includes the non-canonical development of these models, whereby the model may mimic later developmental stages within the permitted timeframe.

9.23. To ensure SCBEM are faithfully recapitulating the processes of in vivo development, SCBEM require validation against natural human embryos (Peng et al., 2022b). Without being able to

culture SCEBM, IVGs and natural embryos past 14-days, it is difficult to identify biological markers that verify the accuracy and functionality of these models and can be used to define a later limit (Chan, 2018).

9.24. In addition, utilising models in possible toxicology testing and drug modelling or, in the case of IVGs, for fertilisation of gametes will put pressure on researchers to understand the long-term risks of these applications, crucial ahead of any clinical applications.

9.25. Different definitions of embryos and gametes have also been highlighted as a challenge when regulating such models in line with the 14-day rule. Broadening of definitions has been argued to have potential implications for wider stem cell research which could unintentionally be restricted by the terminology used (Hengstschläger & Rosner, 2021; Iltis et al., 2023).

9.26. With genome editing, the effects of modifications may only manifest in later stages of development. The 14-day restriction limits the ability to assess long-term outcomes and the full implications of gene edits, which is critical before applying these techniques to human embryos used in a clinical setting.

9.27. Optimisation of in vitro culture systems and embryo models together with refinements in genome editing technologies and testing/validation platforms will further allow us to improve our understanding of early human development up to 14-days and beyond.

Review articles and ethical arguments

9.28. As scientific techniques have advanced, numerous articles have considered whether the 14-day rule needs to be reevaluated and whether the ethical arguments with respect to the developments in our understanding of early human development are still valid.

9.29. (Anifandis et al., 2022) discuss the ethical aspects of research into the use of assisted reproductive technologies, concentrating on constraints arising from the perceived ‘unnaturalness’ of many of the procedures and ethical concerns raised with improving its success.

9.30. (Ghaly & Abdelalim, 2024) discuss the bioethical perspective on the reconsideration of the 14-day rule in modern Islam, advocating for the extension of the limit to at least 40-days with specified conditions.

9.31. (Yui et al., 2023) discuss findings from a survey of Japanese researchers and the public regarding the culture of human embryos in vitro beyond 14 days. Among researchers, 46.2% agreed that embryos could be cultured beyond 14 days, a result that was slightly lower among the public 37.9%. The same survey reported that 24% of researchers and 19.2% of public respondents opposed embryo culture beyond 14 days. Support for embryo culture beyond 14 days was therefore nearly twice as high as opposition for both groups.

9.32. Further research into public perspectives on the 14-day rule in the UK has been considered by the Human Development Biology Initiative (HDBI) dialogue project. In which most of the dialogue’s participants (n = 70) supported some form of extension or modification of the 14-day rule, provided it is guided by society’s expectations about respect for the embryo and robustly regulated.

9.33. (Gyngell et al., 2024) focus on the ethical justifications for treating SCBEM differently to embryos and the moral status of models when considering an extension to the 14-day rule.

9.34. (Hyun et al., 2021) urged policy makers and the International Society for Stem Cell Research (ISSCR), the latter then finalising its updated guidelines on stem cell and embryo research, to consider a cautious stepwise approach to scientific exploration beyond the 14-day time limit.

9.35. The review article by (Loseva & Gladyshev, 2024) further discuss ways to define life with regards to the 14-day rule in their review article, including the use of a definition of biological ageing.

9.36. Following an exploration of recent advances in embryonic research, (Peng et al., 2022b) evaluate the ethical controversies and recent public debate on the 14-day rule with a specific lens on the Chinese regulatory framework. Learnings from the Chinese legislative model of human embryo and embryoid research, with relation to the 14-day rule, is further discussed by (Xue & Shang, 2022).

10. Annex B: History of the 14-day rule

The 14-day rule was incorporated into UK legislation by the **Human Fertilisation and Embryology (HFE) Act 1990**, which states in sub-section 3:

"(3) A license cannot authorise –

(a) keeping or using of an embryo after the appearance of the primitive streak."

And further stipulates:

"For the purposes of subsection (3)(a) above, the primitive streak is to be taken to have appeared in an embryo not later than the end of the period of 14 days beginning with [the day on which the process of creating the embryo began], not counting any time during which the embryo is stored."

This legal limitation of not culturing embryos in vitro beyond 14 days originated from recommendations in the 1984 Warnock committee report in the UK, with other commissions with similar recommendations being published around this time. These included the Ethics Advisory Board of the US Department of Health, Education, and Welfare in 1979 and and US National Institutes of Health's Human Embryo Research Panel in 1994.

The Warnock committee was tasked with producing an advisory report on the moral, legal and social issues raised by IVF, embryo research and other practices (Cavaliere, 2017). When it came to embryo research, they sought a compromise between those who opposed embryo research and those who wanted it to go ahead (Cavaliere, 2017; Chan, 2018).

The compromise was to allow research, but to recognise the "special status" of the embryo (Chan, 2018) by restricting such research with a clear cut-off corresponding to a certain level of embryo development (Cavaliere, 2017; Chan, 2017). The committee discussed several time limits before settling on the proposal from biologist Anne McClaren for 14 days (Cavaliere, 2017). McClaren proposed 14 days because this is when the primitive streak, a precursor of the brain and spinal cord, and the last moment an embryo could cleave into twins (Cavaliere, 2017) and thus the beginning of individual development (Chan, 2018).

This cut-off had an ethical and moral dimension in relation to the question of sentience (ability to feel pain) as neural development begins at around 17 days (Chan, 2018). At the time of the Warnock Committee discussions, little was known about when neural cells first develop in human embryos or when those cells might connect to transmit signals.

The committee recognised that the establishment of a clear limit was important but did not see the specific cut-off chosen as a moral or scientific principle; rather it was seen as an embryo research policy decision (Chan, 2018).

The '14-day rule' has since been legally implemented in more than a dozen countries (Hyun et al., 2016; Matthews & Moralí, 2020) including:

- Australia – Research Involving Human Embryos Act 2002
- Belgium – Law on research on in-vitro embryos 2003
- Canada – Assisted Reproduction Act 2004
- China – Ethical Guidelines for Human Embryonic Stem Cell Research 2003
- France – Bioethics Act 1994 (revised in 2004, 2011 and 2021)
- India – National Guidelines for Stem Cell Research 2017
- Israel – Public Health Regulations (extra corporeal fertilisation), 1987
- Japan – Guidelines on the Derivation and Distribution of Human Embryonic Stem Cells 2009
- USA (federal) – Advisory Board of the Department of Health Education and Welfare 1979
- Netherlands – Dutch Embryo Act 2002 (revised 2016)
- Norway – Biotechnology Act 1994 (revised 2021)
- South Korea – Bioethics and Biosafety Act 2005
- Spain – Biomedical Research Law 2007
- Sweden – Activities Involving Human Eggs for Research or Treatment Act 1991 (revised 2005)
- Taiwan – Assisted Reproduction Act 2007 (amended 2018)

11. References

Agence de Biomédecine. (2023). *Opinion on stem cell based embryo models*. https://www.agence-biomedecine.fr/IMG/pdf/22-06_avis_du_co_embryoi_des_eng-2.pdf

Aguilera-Castrejon, A., Oldak, B., Shani, T., Ghanem, N., Itzkovich, C., Slomovich, S., Tarazi, S., Bayerl, J., Chugaeva, V., Ayyash, M., Ashouokhi, S., Sheban, D., Livnat, N., Lasman, L., Viukov, S., Zerbib, M., Addadi, Y., Rais, Y., Cheng, S., … Hanna, J. H. (2021). Ex utero mouse embryogenesis from pre-gastrulation to late organogenesis. *Nature*, *593*(7857), 119–124. https://doi.org/10.1038/S41586-021-03416-3

Anifandis, G., Sutovsky, P., Turek, P. J., Chavez, S. L., Kunej, T., Messini, C. I., Schon, S. B., Mavroforou, A., Adashi, E. Y., & Krawetz, S. A. (2022). Bioethics in human embryology: the double-edged sword of embryo research. *Systems Biology in Reproductive Medicine*, *68*(3), 169–179. https://doi.org/10.1080/19396368.2022.2052771

Argelaguet, R., Clark, S. J., Mohammed, H., Stapel, L. C., Krueger, C., Kapourani, C. A., Imaz-Rosshandler, I., Lohoff, T., Xiang, Y., Hanna, C. W., Smallwood, S., Ibarra-Soria, X., Buettner, F., Sanguinetti, G., Xie, W., Krueger, F., Göttgens, B., Rugg-Gunn, P. J., Kelsey, G., … Reik, W. (2019). Multi-omics profiling of mouse gastrulation at single-cell resolution. *Nature*, *576*(7787), 487–491. https://doi.org/10.1038/S41586-019-1825-8

Blackshaw, B. P., & Rodger, D. (2021). Why we should not extend the 14-day rule. *Journal of Medical Ethics*, *47*(10), 712–714. https://doi.org/10.1136/medethics-2021-107317

Cao, J., Spielmann, M., Qiu, X., Huang, X., Ibrahim, D. M., Hill, A. J., Zhang, F., Mundlos, S., Christiansen, L., Steemers, F. J., Trapnell, C., & Shendure, J. (2019). The single-cell transcriptional landscape of mammalian organogenesis. *Nature*, *566*(7745), 496–502. https://doi.org/10.1038/S41586-019-0969-X

Cavaliere, G. (2017). A 14-day limit for bioethics: the debate over human embryo research. *BMC Medical Ethics*, *18*(1). https://doi.org/10.1186/s12910-017-0198-5

Chan, S. (2017). How to Rethink the Fourteen-Day Rule. *Hastings Center Report*, *47*(3), 5–6. https://doi.org/10.1002/hast.698

Chan, S. (2018). How and Why to Replace the 14-Day Rule. *Current Stem Cell Reports*, *4*(3), 228–234. https://doi.org/10.1007/s40778-018-0135-7

Chen, D., Sun, N., Hou, L., Kim, R., Faith, J., Aslanyan, M., Tao, Y., Zheng, Y., Fu, J., Liu, W., Kellis, M., & Clark, A. (2019). Human Primordial Germ Cells Are Specified from Lineage-Primed Progenitors. *Cell Reports*, *29*(13), 4568-4582.e5. https://doi.org/10.1016/J.CELREP.2019.11.083

Cui, G., Feng, S., Yan, Y., Wang, L., He, X., Li, X., Duan, Y., Chen, J., Tang, K., Zheng, P., Tam, P. P. L., Si, W., Jing, N., & Peng, G. (2022). Spatial molecular anatomy of germ layers in the gastrulating cynomolgus monkey embryo. *Cell Reports*, *40*(9). https://doi.org/10.1016/J.CELREP.2022.111285

De Santis, R., Rice, E., Croft, G., Yang, M., Rosado-Olivieri, E. A., & Brivanlou, A. H. (2024). The emergence of human gastrulation upon in vitro attachment. *Stem Cell Reports*, *19*(1), 41–53. https://doi.org/10.1016/j.stemcr.2023.11.005

Deglincerti, A., Croft, G. F., Pietila, L. N., Zernicka-Goetz, M., Siggia, E. D., & Brivanlou, A. H. (2016). Self-organization of the in vitro attached human embryo. *Nature*, *533*(7602), 251–254. https://doi.org/10.1038/NATURE17948

Depadt, V., & Hirsch, E. (2020, September 6). *Loi relative à la bioéthique : entre avancées, renoncements et désillusions, ce qu'il faut retenir*. The Conversation. https://theconversation.com/loi-relative-a-la-bioethique-entre-avancees-renoncements-et-desillusions-ce-quil-faut-retenir-145283

Ghaly, M., & Abdelalim, E. M. (2024). Advanced human embryo research beyond the 14-day limit: A bioethical perspective from the Muslim world. *Stem Cell Reports*, *19*(8), 1048–1052. https://doi.org/10.1016/J.STEMCR.2024.06.008

Gong, Y., Bai, B., Sun, N., Ci, B., Shao, H., Zhang, T., Yao, H., Zhang, Y., Niu, Y., Liu, L., Zhao, H., Wu, H., Zhang, L., Wang, T., Li, S., Wei, Y., Yu, Y., Ribeiro Orsi, A. E., Liu, B., … Tan, T. (2023). Ex utero monkey embryogenesis from blastocyst to early organogenesis. *Cell*, *186*(10), 2092-2110.e23. https://doi.org/10.1016/J.CELL.2023.04.020

Greely, H. T. (2022). The 14-Day Embryo Rule: A Modest Proposal. *Houston Journal of Health Law and Policy*, *22*(1). https://houstonhealthlaw.scholasticahq.com/article/38532-the-14-day-embryo-rule-a-modest-proposal

Greely, H. T., & Farahany, N. A. (2021). Advancing the ethical dialogue about monkey/human chimeric embryos. *Cell*, *184*(8), 1962–1963. https://doi.org/10.1016/J.CELL.2021.03.044

Gu, Z., Guo, J., Zhai, J., Feng, G., Wang, X., Gao, Z., Li, K., Ji, S., Wang, L., Xu, Y., Chen, X., Wang, Y., Guo, S., Yang, M., Li, L., Han, H., Jiang, L., Wen, Y., Wang, L., … Gu, Q. (2022). A Uterus-Inspired Niche Drives Blastocyst Development to the Early Organogenesis. *Advanced Science (Weinheim, Baden-Wurttemberg, Germany)*, *9*(28). https://doi.org/10.1002/ADVS.202202282

Gyngell, C., Lynch, F., Sawai, T., & Savulescu, J. (2024). Stem cell-derived embryo models: moral advance or moral obfuscation? *Journal of Medical Ethics*. https://doi.org/10.1136/JME-2023-109605

Health Council of the Netherlands. (2023). *The 14-day rule in the Dutch Embryo Act*. https://www.healthcouncil.nl/binaries/healthcouncil/documenten/advisory-reports/2023/10/31/the-14-day-rule-in-the-dutch-embryo-act/16e_The-14-day-rule-in-the-Dutch-Embryo-Act_advisory-report.pdf

Hengstschläger, M., & Rosner, M. (2021). Embryoid research calls for reassessment of legal regulations. *Stem Cell Research & Therapy*, *12*(1). https://doi.org/10.1186/S13287-021-02442-2

Hyun, I., Bredenoord, A. L., Briscoe, J., Klipstein, S., & Tan, T. (2021). Human embryo research beyond the primitive streak. *Science*, *371*(6533), 998–1000. https://doi.org/10.1126/science.abf3751

Hyun, I., Wilkerson, A., & Johnston, J. (2016). Embryology policy: Revisit the 14-day rule. *Nature*, *533*(7602), 169–171. https://doi.org/10.1038/533169a

Iltis, A. S., Koster, G., Reeves, E., & Matthews, K. R. W. (2023). Ethical, legal, regulatory, and policy issues concerning embryoids: a systematic review of the literature. *Stem Cell Research & Therapy*, *14*(1). https://doi.org/10.1186/S13287-023-03448-8

International Society for Stem Cell Research, T. (2021). *ISSCR Guidelines for Stem Cell Research and Clinical Translation*. https://www.isscr.org/policy/guidelines-for-stem-cell-research-and-clinical-translation

Jarvis, G. E. (2016). Estimating limits for natural human embryo mortality. *F1000Research*, *5*, 2083. https://doi.org/10.12688/F1000RESEARCH.9479.1

LOI N° 2021-1017 Du 2 Août 2021 Relative à La Bioéthique, Pub. L. No. 2021–1017, Journal officiel de la République Française (2021). https://www.legifrance.gouv.fr/jorf/id/JORFTEXT000043884384/

Loseva, P. A., & Gladyshev, V. N. (2024). The beginning of becoming a human. *Aging (Albany NY)*, *16*(9), 8378. https://doi.org/10.18632/AGING.205824

Ma, H., Zhai, J., Wan, H., Jiang, X., Wang, X., Wang, L., Xiang, Y., He, X., Zhao, Z. A., Zhao, B., Zheng, P., Li, L., & Wang, H. (2019). In vitro culture of cynomolgus monkey embryos beyond early gastrulation. *Science*, *366*(6467). https://doi.org/10.1126/SCIENCE.AAX7890/SUPPL_FILE/PAPV2.PDF

Matthews, K. R., & Moralí, D. (2020). National Human Embryo and Embryoid Research Policies: A Survey of 22 Top Research-Intensive Countries. *Regenerative Medicine*, *15*(7), 1905–1917. https://doi.org/10.2217/rme-2019-0138

Niu, Y., Sun, N., Li, C., Lei, Y., Huang, Z., Wu, J., Si, C., Dai, X., Liu, C., Wei, J., Liu, L., Feng, S., Kang, Y., Si, W., Wang, H., Zhang, E., Zhao, L., Li, Z., Luo, X., … Tan, T. (2019). Dissecting primate early post-implantation development using long-term in vitro embryo culture. *Science (New York, N.Y.)*, *366*(6467). https://doi.org/10.1126/SCIENCE.AAW5754

Peng, Y., Lv, J., Xiao, Z., Ding, L., & Zhou, Q. (2022a). A framework for the responsible reform of the 14-day rule in human embryo research. *Protein & Cell*, *13*(8), 552–558. https://doi.org/10.1007/S13238-022-00907-5

Peng, Y., Lv, J., Xiao, Z., Ding, L., & Zhou, Q. (2022b). A framework for the responsible reform of the 14-day rule in human embryo research. *Protein & Cell*, *13*(8), 552–558. https://doi.org/10.1007/S13238-022-00907-5

Pera, M. F. (2017). Human embryo research and the 14-day rule. *Development (Cambridge)*, *144*(11), 1923–1925. https://doi.org/10.1242/dev.151191

Pijuan-Sala, B., Griffiths, J. A., Guibentif, C., Hiscock, T. W., Jawaid, W., Calero-Nieto, F. J., Mulas, C., Ibarra-Soria, X., Tyser, R. C. V., Ho, D. L. L., Reik, W., Srinivas, S., Simons, B. D., Nichols, J., Marioni, J. C., & Göttgens, B. (2019). A single-cell molecular map of mouse gastrulation and early organogenesis. *Nature*, *566*(7745), 490–495. https://doi.org/10.1038/S41586-019-0933-9

Sekulic, S., Bukurov, K. G., Cvijanovic, M., Kopitovic, A., Ilic, D., Petrovic, D., Capo, I., Pericin-Starcevic, I., Christ, O., & Topalidou, A. (2016). Appearance of fetal pain could be associated with maturation of the mesodiencephalic structures. *Journal of Pain Research*, *9*, 1031–1038. https://doi.org/10.2147/JPR.S117959

Shahbazi, M. N., Jedrusik, A., Vuoristo, S., Recher, G., Hupalowska, A., Bolton, V., Fogarty, N. M. E., Campbell, A., Devito, L. G., Ilic, D., Khalaf, Y., Niakan, K. K., Fishel, S., & Zernicka-Goetz, M. (2016). Self-organization of the human embryo in the absence of maternal tissues. *Nature Cell Biology*, *18*(6), 700–708. https://doi.org/10.1038/NCB3347

Sun, N., Shao, H., Zhang, Y., Ci, B., Yao, H., Bai, B., & Tan, T. (2024). Establishing a 3D culture system for early organogenesis of monkey embryos ex vivo and single-cell transcriptome analysis of cultured embryos. *STAR Protocols*, *5*(1). https://doi.org/10.1016/J.XPRO.2023.102835

Tan, T., Wu, J., Si, C., Dai, S., Zhang, Y., Sun, N., Zhang, E., Shao, H., Si, W., Yang, P., Wang, H., Chen, Z., Zhu, R., Kang, Y., Hernandez-Benitez, R., Martinez Martinez, L., Nuñez Delicado, E., Berggren, W. T., Schwarz, M., … Izpisua Belmonte, J. C. (2021). Chimeric contribution of human extended pluripotent stem cells to monkey embryos ex vivo. *Cell*, *184*(8), 2020-2032.e14. https://doi.org/10.1016/J.CELL.2021.03.020

The Norwegian Biotechnology Advisory Board. (2024). *Uttalelse om embryoforskning (statement on embryo research)*. https://www.bioteknologiradet.no/filarkiv/2024/05/Bioteknologiradets-uttalelse-om-embryoforskning-mai-2024.UU_.pdf

Tyser, R. C. V., Mahammadov, E., Nakanoh, S., Vallier, L., Scialdone, A., & Srinivas, S. (2021). Single-cell transcriptomic characterization of a gastrulating human embryo. *Nature 2021 600:7888*, *600*(7888), 285–289. https://doi.org/10.1038/s41586-021-04158-y

Wang, R., Yang, X., Chen, J., Zhang, L., Griffiths, J. A., Cui, G., Chen, Y., Qian, Y., Peng, G., Li, J., Wang, L., Marioni, J. C., Tam, P. P. L., & Jing, N. (2023). Time space and single-cell resolved tissue lineage trajectories and laterality of body plan at gastrulation. *Nature Communications*, *14*(1). https://doi.org/10.1038/S41467-023-41482-5

Williams, K., & Johnson, M. H. (2020). Adapting the 14-day rule for embryo research to encompass evolving technologies: 14 day rule. *Reproductive Biomedicine and Society Online*, *10*, 1–9. https://doi.org/10.1016/J.RBMS.2019.12.002

Xiang, L., Yin, Y., Zheng, Y., Ma, Y., Li, Y., Zhao, Z., Guo, J., Ai, Z., Niu, Y., Duan, K., He, J., Ren, S., Wu, D., Bai, Y., Shang, Z., Dai, X., Ji, W., & Li, T. (2020). A developmental landscape of 3D-cultured human pre-gastrulation embryos. *Nature*, *577*(7791), 537–542. https://doi.org/10.1038/S41586-019-1875-Y

Xue, Y., & Shang, L. (2022). Are we ready for the revision of the 14-day rule? Implications from Chinese legislations guiding human embryo and embryoid research. *Frontiers in Cell and Developmental Biology*, *10*. https://doi.org/10.3389/FCELL.2022.1016988

Yao, H., Sun, N., Shao, H., Wang, T., & Tan, T. (2023). Ex utero embryogenesis of non-human primate embryos and beyond. *Current Opinion in Genetics & Development*, *82*. https://doi.org/10.1016/J.GDE.2023.102093

Yui, H., Muto, K., Yashiro, Y., Watanabe, S., Kiya, Y., Fujisawa, K., Harada, K., Inoue, Y., & Yamagata, Z. (2023). Survey of Japanese researchers and the public regarding the culture of human embryos in vitro beyond 14 days. *Stem Cell Reports*, *18*(4), 799–806. https://doi.org/10.1016/J.STEMCR.2023.02.005

Zeng, B., Liu, Z., Lu, Y., Zhong, S., Qin, S., Huang, L., Zeng, Y., Li, Z., Dong, H., Shi, Y., Yang, J., Dai, Y., Ma, Q., Sun, L., Bian, L., Han, D., Chen, Y., Qiu, X., Wang, W., … Wang, X. (2023). The single-cell and spatial transcriptional landscape of human gastrulation and early brain development. *Cell Stem Cell*, *30*(6), 851-866.e7. https://doi.org/10.1016/J.STEM.2023.04.016

Zhai, J., Xu, Y., Wan, H., Yan, R., Guo, J., Skory, R., Yan, L., Wu, X., Sun, F., Chen, G., Zhao, W., Yu, K., Li, W., Guo, F., Plachta, N., & Wang, H. (2023). Neurulation of the cynomolgus monkey embryo achieved from 3D blastocyst culture. *Cell*, *186*(10), 2078-2091.e18. https://doi.org/10.1016/J.CELL.2023.04.019