Licence Committee – minutes

Centre 0017 (Newcastle Fertility Centre at Life)
Interim Inspection Report and
Variation of Research Aims & Objectives - Research Project R0152

Thursday, 7 March 2019

HFEA, 10 Spring Gardens, London SW1A 2BU

Committee members	Kate Brian (Chair) Anita Bharucha (Deputy Chair) Ruth Wilde Jonathan Herring	
Members of the Executive	Dee Knoyle Moya Berry	Committee Secretary Committee Secretary (Observer)
Legal Adviser	Gerard Hanratty	Browne Jacobson LLP
Specialist Adviser		
Observers		

Declarations of interest

- Members of the panel declared that they had no conflicts of interest in relation to this item.

The panel had before it:

- 9th edition of the HFEA Code of Practice
- Standard licensing and approvals pack for committee members.

The following papers were considered by the committee:

Interim

- Inspection Report.

Variation

- Inspection report
- Application Form
- Supplement to application
- Patient information
    - PIS ART patients ARTI
    - PIS ARTI EDR
    - PIS sperm donor ARTI
- Consent forms
    - ARTI EDR ICF
    - ARTI sperm donor ICF
- Peer review

Previous licensing minutes for the last three years:

- Licence Committee Minutes - 4 May 2017 – Renewal Report
- Licence Committee Minutes - 12 January 2017 – Variation research objectives
- Licence Committee Minutes - 10 November 2016 – Variation research objectives
- Licence Committee Minutes - 8 September 2016 – Variation research objectives

1. Background

1.1. Newcastle Fertility Centre, centre 0017, is based within the International Centre for Life. The research laboratory is located within the same unit as the treatment and storage centre. Research project R0152, entitled 'Towards improving assisted reproductive technologies for the treatment of infertility and prevention of disease', has been licensed by the HFEA since August 2004. The committee noted that the current licence is due to expire on 31 July 2017.

1.2. The research project was amended twice during the previous renewal cycle, to reflect updates in the research objectives and the number of embryos expected to be created and/or used. These amendments were presented to Licence Committee and reflect the use of an additional nuclear transfer technique and the extension of embryo culture up to 14 days (or the appearance of the primitive streak). The potential use of TALENS (a 'gene editing' technique) was also described in the last renewal application.

1.3. Following the interim inspection in 2015, an additional licence for research project R0152 was granted to permit certain aspects of the research, particularly stem cell derivation and culture, to take place at the Francis Crick Institute, centre 0246.

Renewal Inspection - January 2017

1.4. A renewal inspection visit was carried out on 23 January 2017. The PR has also applied to vary the licence to change the research aims and objectives of the project and a desk-based assessment was completed on 29 January 2019, with further information provided during an interim inspection visit on 5 February 2019.

1.5. At its meeting on 4 May 2017, the Licence Committee granted the renewal of the centre's licence for research project R0152 with no additional conditions for a period of three years.

The following activities were approved:

- creation of embryos
- keeping embryos
- use of embryos
- storage of embryos
- storage of gametes

for the following purposes:

- increasing knowledge about serious disease or other serious medical conditions
- developing treatments for serious disease or other serious medical conditions
- increasing knowledge about the causes of congenital disease or congenital medical conditions
- promoting advances in the treatment of infertility
- increasing knowledge about the causes of miscarriage
- developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation
- increasing knowledge about the development of embryos.

1.6. The research project involves the derivation of human embryonic stem cell lines but not for human application. Research licence conditions R41-89 are therefore not applicable to this research project.

Summary of the research undertaken to date

1.7. During the 2014-2017 licensing period, the centre performed preclinical studies to test the efficacy of pronuclear transfer to prevent transmission of mtDNA disease. These findings have been published in Nature (Hyslop et al, 2016). They have also performed studies aimed at increasing knowledge of how pathogenic mtDNA mutations segregate during preimplantation development. This work is ongoing. In addition, they have conducted a study to determine the combined effect of trophectoderm biopsy and vitrification on blastocyst survival. Together, the findings from these studies will inform the development of their PGD service. The work on understanding the effects of age on female meiosis is expected to continue into the next licensing period.

Interim Inspection – February 2019

1.8. The inspectorate carried out an interim inspection in February 2019. The inspection report has been submitted for consideration by the Licence Committee.

Variation – Aims and objectives – Assessment January 2019 & Inspection February 2019

1.9. The Person Responsible (PR) has applied to vary the licence to reflect the revised aims and objectives of the research project. The inspection report has been submitted for consideration by the Licence Committee.

2. Consideration of application

Interim Inspection

2.1. The committee noted that the interim inspection took place on 5 February 2019.

2.2. The purpose of the inspection was to assess whether research using human embryos is carried out in compliance with the Human Fertilisation and Embryology (HF&E) Act 1990 (as amended) and the Code of Practice and that progress is made towards achieving the stated aims of the project. The inspection report summarises the findings of the inspection, highlighting areas of firm compliance and good practice, as well as areas where improvement may be required to meet regulatory standards.

2.3. The committee noted that at the time of the inspection there was one major area of non-compliance relating to implications counselling and one 'other' area of non-compliance relating to storage consent forms, identified. The committee noted that the PR has committed to fully implementing these recommendations.

Use of human embryos

2.4. The committee noted that at the last renewal, the Peer Reviewer agreed that the use of human embryos was necessary and justified for the proposed research project.

Ethics Approval

2.5. The committee noted that evidence that the research project has been approved by an ethics committee was provided to the HFEA in 2017 and this approval remains in place.

Recommendation

2.6. The committee noted that, in considering overall compliance, the inspectorate considers that it has sufficient information, drawn from documentation submitted by the centre prior to inspection and from observations and interviews conducted during the inspection visit, to draw a conclusion on the continuation of the centre's licence for research project R0152. The inspectorate recommends the continuation of this centre's licence without additional conditions, subject to the PR responding to the recommendations in the interim inspection report.

Variation of Licence to change the aims and objectives of the research project

2.7. The PR wishes to add research work which effectively extends how the approved licensed research activities, e.g. use of embryos in research, will be undertaken.

2.8. The PR proposes to revise the lay summary:

Proposed revision to the Lay Summary (proposed changes underlined):

Our over-arching goal is to improve outcomes of assisted reproductive technologies for the treatment of infertility and for the prevention of disease. Our research is focussed on genome inheritance, encompassing transmission of the nuclear and mitochondrial genomes. In relation the mitochondrial genome, we are particularly interested in transmission of mitochondrial DNA mutations from mother to child, and how this might be prevented using IVF based procedures. In relation to the nuclear genome we are investigating the mechanisms governing chromosome inheritance in eggs with the aim of better understanding the underlying causes of the decline in fertility as women get older. We also aim to investigate the underlying causes of the high incidence of chromosomal abnormalities during the embryonic cell divisions.

During the next three years, we propose to pursue the following specific aims.

(1) Further optimise “mitochondrial donation” procedures to reduce the risk of mtDNA disease in children of affected women

(2) Investigate mitochondrial turnover and segregation during formation of mature eggs and during early embryo development.

(3) Investigate the pathways leading to chromosomal abnormalities in eggs and embryos and test the feasibility of intervention strategies designed to reduce the risk of errors.

(4) Use gene editing to develop new tools for investigating chromosome and mitochondrial segregation during embryo development and for testing the effect of new IVF-related procedures.

We hope that these investigations will help to improve our basic knowledge of maternal inheritance and extend the scope of fertility treatments to preventing maternal transmission of disease and chromosomal abnormalities. In addition, by developing new tools to assess embryo development, we will extend the repertoire of assays for testing safety of new assisted reproductive technologies.

Activities & Purposes:

2.9. The committee noted that all of the activities and designated purposes remain the same.

2.10. The application includes a change to how the proposed research addresses the following research purposes:

- Promoting advances in the treatment of infertility
- Increasing knowledge about the causes of miscarriage
- Developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation
- Increasing knowledge about the development of embryos

2.11. The research amendments to address the research purposes are proposed with the following justifications:

- **Promoting advances in the treatment of infertility**

  Testing the effects of an intervention on embryo development is a basic tenet of developing new assisted reproductive technologies. The centre intends to add a fourth aim to their research objectives utilising gene editing technology to increase understanding of early human embryo development. This will enable them to gain insight into clinically relevant problems including the underlying causes of chromosomal abnormalities in human embryos. The proposed new aim will also provide new ways of assessing the effects of laboratory interventions on embryo development. The work is therefore also linked to the development of new treatments for infertility and disease prevention.

- **Increasing knowledge about the causes of miscarriage**

  The proposed new research aim utilising genome editing technology will help to:

  (i) better understand early embryo development;

  (ii) understand the causes for chromosome segregation errors resulting in pregnancy losses and infertility;

  (iii) develop new assays with which to assess the effects of laboratory interventions to underpin the safe development of the reproductive technologies of the future.

- **Developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation**

  The centre will adapt the recently reported gene editing method (2C-HR-CRISPR) in mouse embryos (Gu et al., bioRxiv, 2017), to generate human embryos expressing fluorescent protein (FP) to label chromosomes. This will enable them to monitor chromosome segregation in real time. They will combine this approach with other techniques such as single cell genomics and transcriptomics to validate the reporter system and to understand the developmental fate of chromosomally abnormal cells. These experiments have the potential to greatly advance understanding of the causes and consequences of chromosome abnormalities in human embryos and will provide proof of concept for gene editing to generate human reporter embryos. The centre will also use the system to extend the investigation of mitochondrial homeostasis (current aim).

- **Increasing knowledge about the development of embryos**

  The centre's current licence covers use of mitochondrial genome editing techniques using mito-targeted TALENS. They now propose to adopt nuclear genome editing tools to generate reporter embryos and to knock out genes of interest. This will enable them to assess fundamental biological and developmental processes in the living embryo to advance knowledge of early human development.

Revised aims and objectives:

2.12. The major change to the project, described in the application, is that the PR wishes to extend the research objectives, beyond those contained in the last licence renewal application already approved by Licence Committee.

2.13. The committee considered the details in the report which outlined the proposed changes to aims 1 to 4 of the research project and objectives 3.1 to 3.3 and 4.1 to 4.4.

2.14. The committee noted that the research activities related to the additional aim and objective will initially be performed solely at centre 0017. The Francis Crick Institute, centre 0246, also holds a licence for R0152 and has submitted an application to vary the licence in the same way. Research activities involved in later stages of the experimental design will be performed at centre 0246.

Desk-Based Assessment & Inspection

2.15. The committee noted that a desk-based assessment took place on 29 January 2019, with further information provided during an interim inspection visit on 5 February 2019.

2.16. The committee noted that at the time of the inspection there were two 'other' area of non-compliance identified relating to storage consent forms and obtaining ethics approval for the additional research objectives. The committee noted that the PR has committed to fully implementing these recommendations.

Peer review

2.17. The Peer Reviewer was supportive of the research work described in the licence variation application.

2.18. The Peer Reviewer considered the project methodology is likely to answer the research objectives and that the research team have the necessary expertise and experience.

2.19. The Peer Reviewer also considered that the research objectives could not be addressed without the use of human embryos and that the type and number of embryos to be used in the project were justified.

Use of human embryos

2.20. The Peer Reviewer stated that since the work described for the project is largely directed towards development of tools to investigate/correct defects in mitochondrial or chromosome function it will require optimisation and extensive testing. The number of embryos predicted for use is therefore quite reasonable.

Ethics Approval

2.21. The Executive acknowledges the PR's commitment to provide evidence of ethics approval prior to commencing the new research aims.

Recommendation

2.22. In considering overall compliance, the inspectorate considers there is sufficient information drawn from the renewal inspection of the centre on 23 January 2017, the interim inspection on 5 February 2018 and documentation submitted by the centre, to conclude that the premises are suitable for the proposed research activities and the proposed practices are suitable.

2.23. The inspectorate recommends the approval of the licence variation application to include the additional research objectives within the project definition.

2.24. The PR should ensure that none of the additional research objectives commence until approval for them has been obtained from an appropriately constituted research ethics committee and evidence of this has been provided to and acknowledged by the HFEA Executive.

3. Decision

Interim

3.1. The committee was satisfied with the interim inspection report and endorsed the inspectorate's recommendation for the continuation of research licence R0152, without additional conditions, subject to the PR responding to the recommendations in this inspection report.

Variation

3.2. The committee considered that it had sufficient information, drawn from documentation submitted by the Executive to consider the application to vary research licence R0152 to reflect the revised aims and objectives of the research project. The committee endorsed the inspectorate's recommendation for the approval of the licence variation application.

3.3. The committee noted that the PR is in the process of seeking ethics approval for the variation of the aims and objectives of this project and agreed that the PR should ensure that none of the additional research objectives commence until approval for them has been obtained from an appropriately constituted research ethics committee and evidence of this has been provided to and acknowledged by the HFEA Executive.

3.4. The committee noted that in order to accommodate this variation the PR increased the forecasted number of fresh human embryos required for use in research project R0152 since the initial forecast in the renewal application. The committee queried under what circumstances embryos are classified as surplus to requirement for treatment. For the sake of transparency, the committee agreed that the PR should provide information on the circumstances in which fresh embryos are considered to be surplus to the requirements of treatment, for the Licence Committee to consider at its next meeting scheduled in May 2019.

4. Chair's signature

4.1. I confirm this is a true and accurate record of the meeting.

Signature

Name

Kate Brian

Date

4 April 2019

Research interim inspection report

Date of Inspection: 5 February 2019
Purpose of inspection: Interim inspection of research licence
Inspectors: Lesley Brown (Lead) and Karen Conyers.

Inspection details:
The report covers the pre-inspection analysis, the visit and information received from the centre.

Date of Licence Committee: 7 March 2019

Centre details

Project title	Towards improving assisted reproductive technologies for the treatment of infertility and prevention of disease
Centre name	Newcastle Fertility Centre at Life
Centre number	0017
Research licence number	R0152
Centre address	International Centre for Life, Bioscience Centre, Times Square, Newcastle upon Tyne, NE1 4EP, United Kingdom
Person Responsible	Meenakshi Choudhary
Licence Holder	Mary Herbert
Treatment centres donating to this research project	Newcastle Fertility Centre at LIFE, 0017
Date licence issued	1 August 2017
Licence expiry date	31 July 2020
Additional conditions applied to this licence	None

Contents

Purpose of the Inspection report

The purpose of the inspection is to assess whether research using human embryos is carried out in compliance with the Human Fertilisation and Embryology (HF&E) Act 1990 (as amended) and the Code of Practice and that progress is made towards achieving the stated aims of the project. The report summarises the findings of the inspection highlighting areas of firm compliance and good practice, as well as areas where improvement may be required to meet regulatory standards. It is primarily written for the Authority's Licence Committee which makes the decision about the centre's licence.

Report to Licence Committee

Brief description of the centre and its licensing history:

The Newcastle Fertility Centre is based within the International Centre for Life. The research laboratory is located within the same unit as the treatment and storage centre. Research project R0152 has been licensed since August 2004 and was last inspected in 2017. There are no additional conditions on the licence.

The research project was amended twice during the previous renewal cycle, to reflect updates in the research objectives and the number of embryos expected to be created and/or used. These amendments were presented to Licence Committee and reflect the use of an additional nuclear transfer technique and the extension of embryo culture up to 14 days (or the appearance of the primitive streak). The potential use of TALENS (a 'gene editing' technique) was also described in the last renewal application.

Following the interim inspection in 2015, an additional licence for R0152 was granted to permit certain aspects of the research, particularly stem cell derivation and culture, to take place at the Francis Crick Institute (centre 0246).

The Person Responsible (PR) has recently applied to amend the licence to include additional research aims and objectives. The licence variation is to be considered by Licence Committee alongside this interim report.

The research project involves the derivation of human embryonic stem cell lines but not for human application. Research licence conditions R41-89 are therefore not applicable to this research project.

Summary for licensing decision

In considering overall compliance, the inspection team considers that it has sufficient information drawn from documentation submitted by the centre prior to inspection and from observations and interviews conducted during the inspection visit to draw a conclusion on the continuation of the centre's licence.

The Licence Committee is asked to note that there is one area of major non compliance and one 'other' area of non compliance that require improvement.

The PR has given a commitment to fully implement the following recommendations:

Major area of non compliance:

- The PR should ensure that all patients donating gametes and embryos are offered the opportunity to receive counselling about the implications of their donation.

'Other' area of non compliance

- The PR should ensure that storage consent forms provide the option for donors to elect to consent to store for less than 10 years.

Recommendation to the Licence Committee:
The inspection team considers that overall there is sufficient information available to recommend the continuation of this centre's licence without additional conditions, subject to the PR responding to the recommendations in this inspection report.

Summary of project

Lay summary of the research project:

Our work encompasses maternal transmission of the mitochondrial and nuclear genomes.

Our ultimate aims are:
(i) To understand how the mitochondrial genome is sculpted during oogenesis and early embryogenesis and how this impacts on the transmission of mitochondrial DNA (mtDNA) disease

(ii) To uncover the mechanisms underlying the increased risk of chromosomal abnormalities in oocytes of older women.

(iii) To develop effective approaches to reducing reproductive risk for women of advanced reproductive age and for those who carry mtDNA mutations.

During the next three years, we propose to pursue the following specific aims:
(1) Further optimise “mitochondrial donation” procedures to reduce the risk of mtDNA disease in children of affected women

(2) Investigate mitochondrial turnover and segregation during formation of mature eggs and during early embryo development.

(3) Investigate the pathways leading to chromosomal abnormalities in eggs and embryos and test the feasibility of intervention strategies designed to reduce the risk of errors.

We hope that these investigations will help to improve our basic knowledge of maternal inheritance and extend the scope of fertility treatments to preventing maternal transmission of disease and chromosomal abnormalities.

Objectives of the research:

Aim 1: To develop and refine new clinical treatments to minimise transmission of mtDNA mutations.

Our first aim is to further refine nuclear genome transplantation techniques with the aim of preventing transmission of mtDNA disease

Objective 1.1 One hypothesis emerging from our recent work is that asynchrony between zygotes pairs may contribute to reduced blastocyst formation following ePNT. We therefore propose to characterise the timing and morphological correlates of key developmental events in human zygotes, including remodelling of the paternal genome, assembly of pre-replicative complexes, entry into S-phase, and duplication and migration of sperm centrioles. These investigations, which will play to our strengths in live cell imaging, will provide a biological basis for future clinical decisions related to optimal selection of patient and donor zygote pairs.

Objective 1.2 We will investigate the efficacy of MII spindle transfer (MST) and polar body transfer (PBT). Both techniques provide a naturally synchronised source of nuclear DNA and may reduce mtDNA carryover. In combination, they also offer the possibility of utilizing more than one maternal genome per oocyte, which has the potential to reduce the cost of mitochondrial donation treatment. However, evidence from others indicates that the incidence of abnormal fertilization and aneuploidy is increased after MII spindle transfer[4]. Moreover, while transplantation of the 1st PB appears to be successful in the mouse[5], a recent report indicates that assembly of a bipolar spindle and chromosome alignment following 1st PB transfer in human oocytes is defective, resulting in reduced blastocyst formation[3]. We will use our expertise in oocyte chromosome segregation and live cell imaging skills to address these problems.

Objective 1.3 Develop strategies to further minimize mtDNA carryover during nuclear genome transplantation. While our findings indicate that PNT blastocysts showed generally low levels of mtDNA carryover, we observed an upward drift in heteroplasmy in 2/9 ESC lines derived from ePNT blastocysts raising the theoretical possibility that karyoplast mtDNA might be amplified in the somatic and/or germ cell lineages. We propose to test a number of approaches to circumventing this problem. These will include manipulating a range of biological processes involved in mitochondrial homeostasis. We will also explore the feasibility of selective elimination of karyoplast mtDNA using targeted nucleases[6].

Aim 2: To improve the outcome of infertility treatments through gaining a better understanding of the cellular and molecular events occurring during pre-implantation development in vitro.

Our second aim is to investigate mitochondrial homeostasis and segregation of variant mtDNA during early human development.

Objective 2.1 Determine whether mitochondrial turnover occurs during oogenesis and early development. Work done under this objective will increase our knowledge of mitochondrial biology during early development and will inform experimental approaches in Objective 1.3 (above). We will also extend our investigations to explore the mechanisms and timing of destruction of paternal mitochondria in oocytes.

Objective 2.2 Determine mechanisms governing the segregation of variant mtDNA during embryonic development in vitro. This part of our work is relevant to inherited mtDNA mutations and to heteroplasmy arising from nuclear genome transfer. We will use embryos created by PGD and by nuclear genome transplantation. We will also derive and culture ESCs to investigate the basis for the stochastic increase in heteroplasmy due to mtDNA carryover in hESC lines created from ePNT and MST. The findings will advance our understanding of the reliability of PGD and “Mitochondrial Donation” treatments in reducing the risk of transmitting mtDNA disease for a variety of mutations.

Objective 2.3 Develop an in vitro model to investigate segregation of variant mtDNA in different cell lineages of the human blastocyst at a stage equivalent to implantation in vitro. Given the limitations of embryonic stem cells (ESC) as a model for post-implantation development and taking account of recent developments in techniques to promote self-

organisation of human embryos in vitro (Deglincerti et al, 2016, Nature:533; Shabazi et al, 2016, Nat Cell Biol.:18), we propose to culture up to 14 days or stop sooner if the primitive streak appears. If successful, we will use this system to measure heteroplasmy due to mtDNA carryover in different lineages. Compared with hESCs, we believe that this approach has the potential to provide a more realistic assessment of the risk of resurgence of karyoplast mtDNA during post-implantation development.

Aim 3: To investigate the molecular and genetic events leading to formation of normal oocytes and embryos.

Our third aim is to further advance our understanding of how the DNA is packaged into the chromosomes during oogenesis and look at the various molecular and genetic basis of chromosome segregation errors in oocytes from older women. The effect of female age on faithful transmission of the maternal nuclear genome has been a longstanding research interest at Newcastle Fertility Centre. Our previous findings using oocytes from naturally aged mice indicate that oocytes ovulated late in the reproductive lifespan show reduced levels of chromosomal cohesin and its protector Shugoshin (Sgo2)[7]. We propose that this provides a plausible molecular candidate for the age-related increase in the incidence of oocyte aneuploidy. This is supported by three independent lines of evidence: (i) From fetal life until shortly before ovulation, oocytes maintain their chromosomes in a rather precarious bivalent configuration in which cohesin stabilises physical linkages between maternal and paternal homologues formed during meiotic recombination. These linkages, and hence the cohesin complexes that stabilise them, are essential for accurate segregation of homologues during the first meiotic division. (ii) Protection of cohesin at centromeres by Sgo2 and the phosphatase PP2A followed by timely “deprotection” is essential for accurate segregation of chromatids during anaphase of meiosis II. (iii) According to our current understanding, chromosomal cohesion in mouse oocytes is dependent on cohesin loaded on chromosomes during the pre-meiotic round of DNA replication, which occurs in utero. Thus erosion of cohesin during the many decades of meiotic arrest could result in chronological “chromosomal ageing”. Indeed, our more recent work in mice indicates that cohesin depletion occurs predominantly in the non-growing stage when oocytes are enclosed in primordial follicles. We also find that while cohesin depletion occurs in parallel with depletion of the ovarian stock of primordial follicles, the two processes are governed by independent mechanisms (R. Ballesteros-Mejia, unpublished data). In light of our findings in mouse oocytes, we propose that the most realistic route to developing intervention strategies to reduce reproductive risk in older women is to minimise the impact of cohesin depletion on chromosome segregation during the meiotic divisions. Our research in this area over the next five years will therefore be focussed on gaining insights into the timing and molecular mechanisms underlying premature loss of centromeric cohesion, which, in humans and mice is highly correlated with female age[8]. In addition, depending on the outcome of experiments in the mouse, we will perform reciprocal nuclear transfer between oocytes from young and older women to explore the contribution of non-nuclear factors, including mitochondria, to age-related oocyte aneuploidy.

Objective 3.1. Determine the timing and mechanism of premature loss of oocyte centromeric cohesin.

Objective 3.2. Investigate the mechanisms by which centromeric cohesin is “deprotected” to enable chromatids to segregate during anaphase of meiosis II.

Objective 3.3. Perform nuclear genome transplantation to distinguish between nuclear and cytoplasmic contributions to chromosome segregation errors in female meiosis The work done under objectives 3.1-3.3 will largely involve unfertilised oocytes and will be outside the remit of HFEA, except for vitrification and storage, which will be important for some experiments. In addition, investigation of chromosome segregation during the second meiotic division will require oocyte activation to trigger anaphase of meiosis II. We propose to do this using a chemical stimulus, such as Ca2+ ionophore. Nuclear genome transfer will involve transplantation of GVs, MI/MII spindles and polar bodies and will be followed by activation to trigger the second meiotic division.

References:

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4 Tachibana, M. et al. Towards germline gene therapy of inherited mitochondrial diseases. Nature 493, 627-631, (2013).
5 Wang, T. et al. Polar Body Genome Transfer for Preventing the Transmission of Inherited Mitochondrial Diseases. Cell 157, 1591-1604, (2014).
6 Reddy, P. et al. Selective Elimination of Mitochondrial Mutations in the Germline by Genome Editing. Cell 161, 459-469.
7 Lister, L. M. et al. Age-Related Meiotic Segregation Errors in Mammalian Oocytes Are Preceded by Depletion of Cohesin and Sgo2. Current biology : CB 20, 1511-1521, (2010).
8 Herbert, M., Kalleas, D., Cooney, D., Lamb, M. & Lister, L. Meiosis and Maternal Aging: Insights from Aneuploid Oocytes and Trisomy Births. Cold Spring Harbor Perspectives in Biology 7, (2015).
9 Paull, D. et al. Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants. Nature 493, 632-637, (2013).
10 Deglincerti, A., Croft, G. & Brivanlou, A. A protocol for the growth and imaging of _in vitro_ attached human embryos. (2016).
11 Deglincerti, A. et al. Self-organization of the in vitro attached human embryo. Nature advance online publication, (2016).
12 Shahbazi, M. N. et al. Self-organization of the human embryo in the absence of maternal tissues. Nat Cell Biol 18, 700-708, (2016).

Donation and use of embryos:

The centre has reported the use of 152 frozen embryos and 48 fresh created embryos in 2018.

The renewal application form proposed the use of 250 fresh embryos, 150 frozen embryos and 250 created embryos, each year for the three year term of the proposed licence. The difference between the estimated use and actual use is due to the research team optimising methods using mouse embryos, prior to using human embryos.

Details of inspection findings

Inspection findings

Ensure that all licensed research by the centre meets ethical standards, and is done only where there is both a clear scientific justification and no viable alternative to the use of embryos
(Guidance note 29, 30, 31)

What the centre does well.

The centre was granted a renewal of its research licence by Licence Committee in May 2017 for the following activities: creation of embryos in vitro, keeping embryos, use of embryos, storage of embryos and storage of gametes. None of these activities are prohibited by the HF&E Act 1990 (as amended).

The renewal of the licence was approved to allow research for the following designated purposes:

- increasing knowledge about serious disease or other serious medical conditions
- developing treatments for serious disease or other serious medical conditions
- increasing knowledge about the causes of congenital disease or congenital medical conditions
- promoting advances in the treatment of infertility
- increasing knowledge about the causes of miscarriage
- developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation
- increasing knowledge about the development of embryos.

At the last renewal, a peer reviewer agreed that the use of human embryos was necessary and justified for the proposed research project.

Evidence that the research project has been approved by an ethics committee was provided to the HFEA in 2017 and this approval remains in place.

What they could do better.

Nothing noted on this inspection.

Have respect for the special status of the embryo when conducting licensed activities
(Guidance note 15, 18, 22, 25, 26)

What the centre does well.

On inspection, a review of centre documentation and discussions with centre staff

demonstrated that:

- Proper records of the storage of gametes and embryos in the research project are maintained.
- Robust procedures are in place to ensure proper records of the use of embryos are maintained from donation to the project, use in research through to disposal at the end of the research process (RLC R13).
- The researchers have a documented procedure for ensuring that embryos do not develop beyond 14 days post-fertilisation or the appearance of the primitive streak, whichever is earlier (RLC R28).
- Discussions with the PR provided assurance that all embryos donated to the project will only be used for the objectives authorised by the licence to meet the defined statutory purposes (RLC R5 and R23). This is facilitated by restricted access to embryos during storage and use, and supervision of research staff by the PR.
- A storage log is maintained which records the storage consent expiry dates for any gametes and embryos in storage for research purposes. All frozen gametes and embryos in storage were within their consented storage period (RLC R39).

An audit of donor records showed that:

- Effective consent for the use of the embryos in the research project had been documented by the gamete providers recruited via the altruistic donation route (see description of this route, below) (RLC R18).
- Embryos are not allowed to develop after 14 days or the primitive streak has appeared (if earlier) (RLC R28).
- Embryos donated to the project have only been used in licensed activities to achieve the objectives authorised by the licence (RLC R5 and R23).

The PR has ensured that appropriate records of embryo use are maintained and that annual use is reported to the HFEA (General Direction 0002 and RLC R13, R14 and R15).

At the last renewal, Licence Committee was generally satisfied with the information provided to potential research participants. However, they queried the provision of information on creating stem cell lines following the generation of embryos using somatic nuclear transfer since this did not seem relevant to the project. The committee also noticed a lack of consistency between the individual information sheets, for example, concerning the amount of detail given on the disadvantages and risks of taking part in the research.

The centre has since revised the information sheets and these were reviewed on this inspection. The executive is satisfied that they provide sufficiently detailed, relevant and accurate information, with one exception noted below.

What they could do better.

There are two distinct routes to donation for this project. Altruistic gamete donors are recruited directly into the research programme, with all information sessions and consultations provided by dedicated research staff. Surplus gametes and embryos are also donated by patients who have completed treatment cycles at centre 0017. This second cohort of patients provide consent remotely. During an audit of records it was not clear that donors donating via the treatment pathway had been offered an opportunity to have counselling specific to the

implications of donating to research. The centre staff explained that the donor information does invite patients to contact the centre if they have further questions, however the inspection team felt that the offer of counselling should be more explicit (RLC R18, see recommendation 1).

For patients donating via the altruistic pathway, the HFEA does not provide a suitable consent form tailored to storage or donation solely for research purposes. The centre's practice of capturing consent on their own bespoke consent forms has been assessed as suitable. The centre's own information and consent forms allow donors to provide consent to the storage of gametes or embryos, for a period not exceeding the 10 year statutory storage period, but does not provide an option to indicate if they would like their gametes or embryos to be stored for less than 10 years (paragraph 2(2) of Schedule 3 of the Human Fertilisation and Embryology Act 1990 (as amended), see recommendation 2).

Changes and improvements since the last inspection

Following the renewal inspection in 2017, there were no recommendations for improvement.

Areas of practice that require the attention of the Person Responsible

The section sets out matters which the Inspection Team considers may constitute areas of non compliance. These have been classified into critical, major and others. Each area of non compliance is referenced to the relevant sections of the Act, Regulations, Standard Licence Conditions, Directions or the Code of Practice, and the recommended improvement actions required are given, as well as the timescales in which these improvements should be carried out.

Critical area of non compliance

A critical area of non compliance is an area of practice which poses a significant direct risk of causing harm to a patient, donor or to an embryo. A critical area of non compliance requires immediate action to be taken by the Person Responsible

Area of practice and reference	Action required and timescale for action	PR Response	Executive Review
None			

Major area of non compliance

A major area of non compliance is a non critical area of non compliance:

- which poses an indirect risk to the safety of a patient, donor or to an embryo through the procurement, use, storage or distribution of gametes and embryos, which do not comply with the centre's licence;
- which indicates a major shortcoming from the statutory requirements;
- which indicates a failure of the Person Responsible to carry out his/her legal duties
- a combination of several "other" area of non compliance, none of which on their own may be major but which together may represent a major area of non compliance.

Area of practice and reference	Action required and timescale for action	PR Response	Executive Review
1. The information provided to donors recruited via the treatment route does not	The PR should ensure that patients donating gametes and embryos are offered the	As part of our standard process for those undergoing ART treatments and considering	The executive acknowledges the PR's response, and the updated consent forms which

mention that patients can access counselling about the implications of their donation, nor is there any record that this has been offered. RLC R18.	opportunity to receive counselling about the implications of their donation. The PR has submitted amended information and consent forms as part of an application to vary the aims and objectives of this research project. The amended information and consent forms do make an explicit offer of counselling. However, these forms have not yet been fully approved by a research ethics committee. The PR should confirm, when responding to this report, the actions taken to ensure all donors to the research project are offered counselling, until such a time that the updated information and consent forms are fully approved and released.	participating in research, counselling provision is discussed. This was found to be acceptable in our previous HFEA research inspections. However, we note that we have not been very explicit in our documentation regarding this. Following the feedback at the interim inspection, we amended our information and consent forms (attached) pending ethics approval (submitted and under review). We confirm that in the interim, we will continue to ensure counselling provision is discussed with all research participants until the approval of updated PIS and ICF.	document the acknowledgement of an offer of counselling. Until the updated information and consent forms receive ethics approval, the PR is asked to ensure that the offer of counselling is documented in the donor record. No further action required.

Other areas of practice that requires improvement

Areas of practice that requires improvement is any area of practice, which cannot be classified as either a critical or major area of non compliance, but which indicates a departure from good practice.

Area of practice and reference	Action required and timescale for action	PR Response	Executive Review
2. The centre's own storage consent forms, do not provide the option for donors to elect to consent to store for less than the statutory maximum storage period of 10 years. Paragraph 2(2) of Schedule 3 of the Human Fertilisation and Embryology Act 1990.	The PR should ensure that storage consent forms provide the option for donors to elect to consent to store for less than 10 years. The PR should submit amended storage consent forms when responding to this report.	The egg donors for research are given the option to opt for a lesser storage duration than 10 years if so desired. Previous HFEA research inspections have not highlighted any concerns as regards this. However, we take your recommendation on board and are willing to be more explicit by submitting an amendment to our consent form to REC to state 'I consent to storage of my gametes/embryos created for research for a maximum of 10 years in accordance to HFEA regulations from date of storage If less than 10 years, please specify number of years……' We have informed REC	The executive acknowledges receipt of the updated consent form, and the PR's commitment to ensure that donors are able to consent to store gametes or embryos for less than the statutory storage period. No further action required.

		regarding this and will have to await the final outcome before submitting further amendment if required. If the donor elects to store the gametes and /or embryos for less than 10 years, this will be recorded on the egg donor record form (attached).	

Additional information from the Person Responsible
We would like to express our sincere thanks to the HFEA inspection team for visiting us and for the feedback. There is an ongoing ethics approval in place but as mentioned to the HFEA, we have submitted an ethics application to REC for follow on study with revised research objectives (as per HFEA research licence variation application). The amended copies of the PIS and ICF submitted to REC following provisional opinion are attached. We will forward the final ethics approval to HFEA once received. As the REC review is in process for the submitted documents (version 2), the new HFEA recommendation to specify number of years of storage on the donor ICF (version 3) may need a minor amendment subsequent to this.

Licence variation inspection report

Purpose of this inspection report

The HFEA licenses and monitors establishments undertaking human embryo research. Licences for individual research projects can be granted for up to three years. This is a report of a desk-based assessment following an application to vary an existing research licence. The Authority's Licence Committee (LC) uses the application and this report to decide whether to vary the licence and, if so, whether any additional conditions should be applied to the licence.

Date of assessment: A desk based assessment took place on 29 January 2019, with further information provided during an interim inspection visit on 5 February 2019.

Inspector: Lesley Brown

Purpose of inspection: Variation to the purposes and objectives of a research licence

Inspection details: The report covers the findings from the desk-based assessment, communications received from the centre and discussions during the interim inspection visit.

Date of Licence Committee: 7 March 2019

Centre Details:

Project title	Towards improving assisted reproductive technologies for the treatment of infertility and prevention of disease
Centre name	Newcastle Fertility Centre At Life
Centre number	0017
Research project number	R0152
Centre address	International Centre for Life, Bioscience Centre, Times Square, Newcastle upon Tyne, NE1 4EP, United Kingdom.
Person Responsible (PR)	Meenakshi Choudhary
Licence Holder (LH)	Mary Herbert
Treatment centres donating to this research project	The Gateshead Fertility Unit, 0170 Newcastle Fertility Centre at LIFE, 0017
Date licence issued	1 August 2017
Licence expiry date	31 July 2020
Additional conditions applied to this licence	None

Summary report

Brief description of the centre and its licensing history:

The Newcastle Fertility Centre is based within the International Centre for Life. The research laboratory is located within the same unit as the treatment and storage centre. Research project R0152 has been licensed since August 2004 and was last inspected in 2017. There are no additional conditions on the licence.

The research project was amended twice during the previous renewal cycle, to reflect updates in the research objectives and the number of embryos expected to be created and/or used. These amendments were presented to Licence Committee, and reflect the use of an additional nuclear transfer technique and the extension of embryo culture up to 14 days (or the appearance of the primitive streak). The potential use of TALENS (a 'gene editing' technique) was also described in the last renewal application.

Following the interim inspection in 2015, an additional licence for R0152 was granted to permit certain aspects of the research, particularly stem cell derivation and culture, to take place at the Francis Crick Institute (centre 0246).

The research project involves the derivation of human embryonic stem cell lines but not for human application. Research licence conditions R41-89 are therefore not applicable to this research project.

The project is currently authorised for the following activities:

- Creation of embryos *in vitro*
- Storage of embryos
- Storage of gametes
- Keeping embryos
- Using embryos

These activities are authorised for the following purposes:

- Increasing knowledge about serious disease or other serious medical conditions
- Developing treatments for serious disease or other serious medical conditions
- Increasing knowledge about the cause of any congenital disease or congenital medical conditions
- Promoting advances in the treatment of infertility
- Increasing knowledge about the causes of miscarriage
- Developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation
- Increasing knowledge about the development of embryos

Application to vary the licence:

The PR applied to vary the licence on 8 November 2018. The application makes no changes to the activities to be licensed, which will remain as listed above.

The application includes a change to how the proposed research addresses several of the research purposes.

The research to address the following purposes remain unchanged:

- Increasing knowledge about serious disease or other serious medical conditions
- Developing treatments for serious disease or other serious medical conditions
- Increasing knowledge about the cause of any congenital disease or congenital medical conditions

The proposed research amendments are to address the following purposes:

- Promoting advances in the treatment of infertility
- Increasing knowledge about the causes of miscarriage
- Developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation
- Increasing knowledge about the development of embryos

The research amendments to address the research purposes are proposed with the following justifications from the PR.

- Promoting advances in the treatment of infertility

  The PR states:
  'Testing the effects of an intervention on embryo development is a basic tenet of developing new assisted reproductive technologies. We intend to add a fourth aim to our research objectives utilizing gene editing technology to increase our understanding of early human embryo development. This will enable us to gain insight into clinically relevant problems including the underlying causes of chromosomal abnormalities in human embryos. The proposed new aim will also provide new ways of assessing the effects of laboratory interventions on embryo development. The work is therefore also linked to the development of new treatments for infertility and disease prevention.'

- Increasing knowledge about the causes of miscarriage

  The PR states:
  'Our proposed new research aim utilizing genome
  editing technology will help us to (i) Better understand early embryo development (ii) Understand the causes for chromosome segregation errors resulting in pregnancy losses and infertility (iii) Develop new assays with which to assess the effects of laboratory interventions to underpin the safe development of the reproductive technologies of the future.'

- Developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation

  The PR states:
  'We will adapt the recently reported gene editing method (2C-HR-CRISPR) in mouse embryos (Gu et al., bioRxiv, 2017), to generate human embryos expressing

fluorescent protein (FP) to label chromosomes. This will enable us to monitor chromosome segregation in real time. We will combine this approach with other techniques such as single cell genomics and transcriptomics to validate the reporter system and to understand the developmental fate of chromosomally abnormal cells. These experiments have the potential to greatly advance our understanding of the causes and consequences of chromosome abnormalities in human embryos and will provide proof of concept for gene editing to generate human reporter embryos. We will also use the system to extend the investigation of mitochondrial homeostasis (our current Aim 2)'

- Increasing knowledge about the development of embryos

  The PR states:
  'Our current licence covers use of mitochondrial genome editing techniques using mito-targeted TALENS. We now propose to adopt nuclear genome editing tools to generate reporter embryos and to knock out genes of interest. This will enable us to assess fundamental biological and developmental processes in the living embryo to advance knowledge of early human development.'

The major change to the project, described in the application, is that the PR wishes to extend the research objectives, beyond those contained in the last licence renewal application approved by Licence Committee. The lay summary, research objectives and work undertaken to attain them at the last licence renewal in 2017 were described as:

'Lay summary of the research project:
'Our work encompasses maternal transmission of the mitochondrial and nuclear genomes.

Our ultimate aims are:
(i) To understand how the mitochondrial genome is sculpted during oogenesis and early embryogenesis and how this impacts on the transmission of mitochondrial DNA (mtDNA) disease

(ii) To uncover the mechanisms underlying the increased risk of chromosomal abnormalities in oocytes of older women.

(iii) To develop effective approaches to reducing reproductive risk for women of advanced reproductive age and for those who carry mtDNA mutations.

During the next three years, we propose to pursue the following specific aims:
(1) Further optimise "mitochondrial donation" procedures to reduce the risk of mtDNA disease in children of affected women

(2) Investigate mitochondrial turnover and segregation during formation of mature eggs and during early embryo development.

(3) Investigate the pathways leading to chromosomal abnormalities in eggs and embryos and test the feasibility of intervention strategies designed to reduce the risk of errors.

We hope that these investigations will help to improve our basic knowledge of maternal inheritance and extend the scope of fertility treatments to preventing maternal transmission of disease and chromosomal abnormalities.'

Objectives of the research:

'Aim 1: To develop and refine new clinical treatments to minimise transmission of mtDNA mutations.

Our first aim is to further refine nuclear genome transplantation techniques with the aim of preventing transmission of mtDNA disease

Objective 1.1 One hypothesis emerging from our recent work is that asynchrony between zygotes pairs may contribute to reduced blastocyst formation following ePNT. We therefore propose to characterise the timing and morphological correlates of key developmental events in human zygotes, including remodelling of the paternal genome, assembly of pre-replicative complexes, entry into S-phase, and duplication and migration of sperm centrioles. These investigations, which will play to our strengths in live cell imaging, will provide a biological basis for future clinical decisions related to optimal selection of patient and donor zygote pairs.

Objective 1.2 We will investigate the efficacy of MII spindle transfer (MST) and polar body transfer PBT. Both techniques provide a naturally synchronised source of nuclear DNA and may reduce mtDNA carryover. In combination, they also offer the possibility of utilizing more than one maternal genome per oocyte, which has the potential to reduce the cost of mitochondrial donation treatment. However, evidence from others indicates that the incidence of abnormal fertilization and aneuploidy is increased after MII spindle transfer 4. Moreover, while transplantation of the 1st PB appears to be successful in the mouse 5, a recent report indicates that assembly of a bipolar spindle and chromosome alignment following 1st PB transfer in human oocytes is defective, resulting in reduced blastocyst formation3. We will use our expertise in oocyte chromosome segregation and live cell imaging skills to address these problems.

Objective 1.3 Develop strategies to further minimize mtDNA carryover during nuclear genome transplantation. While our findings indicate that PNT blastocysts showed generally low levels of mtDNA carryover, we observed an upward drift in heteroplasmy in 2/9 ESC lines derived from ePNT blastocysts raising the theoretical possibility that karyoplast mtDNA might be amplified in the somatic and/or germ cell lineages. We propose to test a number of approaches to circumventing this problem. These will include manipulating a range of biological processes involved in mitochondrial homeostasis. We will also explore the feasibility of selective elimination of karyoplast mtDNA using targeted nucleases 6.

Aim 2: To improve the outcome of infertility treatments through gaining a better understanding of the cellular and molecular events occurring during pre-implantation development in vitro.

Our second aim is to investigate mitochondrial homeostasis and segregation of variant mtDNA during early human development.

Objective 2.1 Determine whether mitochondrial turnover occurs during oogenesis and early development. Work done under this objective will increase our knowledge of mitochondrial biology during early development and will inform experimental approaches in Objective 1.3 (above). We will also extend our investigations to explore the mechanisms and timing of destruction of paternal mitochondria in oocytes.

Objective 2.2 Determine mechanisms governing the segregation of variant mtDNA during embryonic development in vitro. This part of our work is relevant to inherited mtDNA mutations and to heteroplasmy arising from nuclear genome transfer. We will use embryos created by PGD and by nuclear genome transplantation. We will also derive and culture ESCs to investigate the basis for the stochastic increase in heteroplasmy due to mtDNA carryover in hESC lines created from ePNT and MST. The findings will advance our understanding of the reliability of PGD and "Mitochondrial Donation" treatments in reducing the risk of transmitting mtDNA disease for a variety of mutations.

Objective 2.3 Develop an in vitro model to investigate segregation of variant mtDNA in different cell lineages of the human blastocyst at a stage equivalent to implantation in vitro. Given the limitations of embryonic stem cells (ESC) as a model for post-implantation development and taking account of recent developments in techniques to promote self-organisation of human embryos in vitro (Deglincerti et al, 2016, Nature:533; Shabazi et al, 2016, Nat Cell Biol.:18), we propose to culture up to 14 days or stop sooner if the primitive streak appears. If successful, we will use this system to measure heteroplasmy due to mtDNA carryover in different lineages. Compared with hESCs, we believe that this approach has the potential to provide a more realistic assessment of the risk of resurgence of karyoplast mtDNA during post-implantation development.

Aim 3: To investigate the molecular and genetic events leading to formation of normal oocytes and embryos.

Our third aim is to further advance our understanding of how the DNA is packaged into the chromosomes during oogenesis and look at the various molecular and genetic basis of chromosome segregation errors in oocytes from older women. The effect of female age on faithful transmission of the maternal nuclear genome has been a longstanding research interest at Newcastle Fertility Centre. Our previous findings using oocytes from naturally aged mice indicate that oocytes ovulated late in the reproductive lifespan show reduced levels of chromosomal cohesin and its protector Shugoshin (Sgo2)7. We propose that this provides a plausible molecular candidate for the age-related increase in the incidence of oocyte aneuploidy. This is supported by three independent lines of evidence: (i) From fetal life until shortly before ovulation, oocytes maintain their chromosomes in a rather precarious bivalent configuration in which cohesin stabilises physical linkages between maternal and paternal homologues formed during meiotic recombination. These linkages, and hence the cohesin complexes that stabilise them, are essential for accurate segregation of homologues during the first meiotic division. (ii) Protection of cohesin at centromeres by Sgo2 and the phosphatase PP2A followed by timely "deprotection" is essential for accurate segregation of chromatids during anaphase of meiosis II. (iii) According to our current understanding, chromosomal cohesion in

mouse oocytes is dependent on cohesin loaded on chromosomes during the pre-meiotic round of DNA replication, which occurs in utero. Thus erosion of cohesin during the many decades of meiotic arrest could result in chronological "chromosomal ageing". Indeed, our more recent work in mice indicates that cohesin depletion occurs predominantly in the non-growing stage when oocytes are enclosed in primordial follicles. We also find that while cohesin depletion occurs in parallel with depletion of the ovarian stock of primordial follicles, the two processes are governed by independent mechanisms (R. Ballesteros-Mejia, unpublished data). In light of our findings in mouse oocytes, we propose that the most realistic route to developing intervention strategies to reduce reproductive risk in older women is to minimise the impact of cohesin depletion on chromosome segregation during the meiotic divisions. Our research in this area over the next five years will therefore be focussed on gaining insights into the timing and molecular mechanisms underlying premature loss of centromeric cohesion, which, in humans and mice is highly correlated with female age 8. In addition, depending on the outcome of experiments in the mouse, we will perform reciprocal nuclear transfer between oocytes from young and older women to explore the contribution of non-nuclear factors, including mitochondria, to age-related oocyte aneuploidy.

Objective 3.1. Determine the timing and mechanism of premature loss of oocyte centromeric cohesin.

Objective 3.2. Investigate the mechanisms by which centromeric cohesin is "deprotected" to enable chromatids to segregate during anaphase of meiosis II.

Objective 3.3. Perform nuclear genome transplantation to distinguish between nuclear and cytoplasmic contributions to chromosome segregation errors in female meiosis The work done under

Objectives 3.1-3.3 will largely involve unfertilised oocytes and will be outside the remit of HFEA, except for vitrification and storage, which will be important for some experiments. In addition, investigation of chromosome segregation during the second meiotic division will require oocyte activation to trigger anaphase of meiosis II. We propose to do this using a chemical stimulus, such as Ca2+ ionophore. Nuclear genome transfer will involve transplantation of GVs, MI/MII spindles and polar bodies and will be followed by activation to trigger the second meiotic division.

References:

1 Craven, L. et al. Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. Nature 465, 82-85, (2010).
2 Hyslop, L. A. et al. Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease. Nature 534, 383-386, (2016).
3 Ma, H. et al. Functional Human Oocytes Generated by Transfer of Polar Body Genomes. Cell Stem Cell.
4 Tachibana, M. et al. Towards germline gene therapy of inherited mitochondrial diseases. Nature 493, 627-631, (2013).
5 Wang, T. et al. Polar Body Genome Transfer for Preventing the Transmission of Inherited Mitochondrial Diseases. Cell 157, 1591-1604, (2014).
6 Reddy, P. et al. Selective Elimination of Mitochondrial Mutations in the Germline by Genome Editing. Cell 161, 459-469.

7 Lister, L. M. et al. Age-Related Meiotic Segregation Errors in Mammalian Oocytes Are Preceded by Depletion of Cohesin and Sgo2. Current biology : CB 20, 1511-1521, (2010). 8 Herbert, M., Kalleas, D., Cooney, D., Lamb, M. & Lister, L. Meiosis and Maternal Aging: Insights from Aneuploid Oocytes and Trisomy Births. Cold Spring Harbor Perspectives in Biology 7, (2015).
9 Paull, D. et al. Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants. Nature 493, 632-637, (2013).
10 Deglincerti, A., Croft, G. & Brivanlou, A. A protocol for the growth and imaging of _in vitro_ attached human embryos. (2016).
11 Deglincerti, A. et al. Self-organization of the in vitro attached human embryo. Nature advance online publication, (2016).
12 Shahbazi, M. N. et al. Self-organization of the human embryo in the absence of maternal tissues. Nat Cell Biol 18, 700-708, (2016).'

Summary of the research undertaken to date

'During the 2014-2017 licensing period, we have performed preclinical studies to test the efficacy of pronuclear transfer to prevent transmission of mtDNA disease. These findings have been published in Nature (Hyslop et al, 2016). We have also performed studies aimed at increasing knowledge of how pathogenic mtDNA mutations segregate during preimplantation development. This work is ongoing. In addition, we have conducted a study to determine the combined effect of trophectoderm biopsy and vitrification on blastocyst survival. Together, the findings from these studies will inform the development of our PGD service. Our work on understanding the effects of age on female meiosis has been boosted by a substantial EU grant and will be continued in the next licensing period.'

The PR has updated the lay summary of the research to reflect the proposed changes:

Lay summary of the research project:

Our over-arching goal is to improve outcomes of assisted reproductive technologies for the treatment of infertility and for the prevention of disease. Our research is focussed on genome inheritance, encompassing transmission of the nuclear and mitochondrial genomes. In relation the mitochondrial genome, we are particularly interested in transmission of mitochondrial DNA mutations from mother to child, and how this might be prevented using IVF based procedures. In relation to the nuclear genome we are investigating the mechanisms governing chromosome inheritance in eggs with the aim of better understanding the underlying causes of the decline in fertility as women get older. We also aim to investigate the underlying causes of the high incidence of chromosomal abnormalities during the embryonic cell divisions.

During the next three years, we propose to pursue the following specific aims.
(1) Further optimise "mitochondrial donation" procedures to reduce the risk of mtDNA disease in children of affected women
(2) Investigate mitochondrial turnover and segregation during formation of mature eggs and during early embryo development.

(3) Investigate the pathways leading to chromosomal abnormalities in eggs and embryos and test the feasibility of intervention strategies designed to reduce the risk of errors.
(4) Use gene editing to develop new tools for investigating chromosome and mitochondrial segregation during embryo development and for testing the effect of new IVF-related procedures.

We hope that these investigations will help to improve our basic knowledge of maternal inheritance and extend the scope of fertility treatments to preventing maternal transmission of disease and chromosomal abnormalities. In addition, by developing new tools to assess embryo development, we will extend the repertoire of assays for testing safety of new assisted reproductive technologies.

The PR states regarding revised research objectives in this licence variation application:

'Recent evidence from mouse embryos indicates that it is possible to introduce fluorescent proteins into endogenous loci using a modified CRISPR/Cas9 (2C-HR-CRISPR; Gu et al., 2018, Nat. Biotechnol.). This makes it possible to study proteins and processes of interest in living embryos. The ability to do this in human embryos would transform research on early human development. The techniques developed under this new aim will greatly strengthen research done under the existing aims. It will also enable us to directly address the long standing question of why human embryos are so remarkably prone to chromosomal abnormalities. This will greatly complement the work done under Aim 3.'

The PR has proposed the following expansion to the aims and objectives:

Aim 4: Expand the repertoire of assays for determining whether embryos are developing normally

Testing the effects of an intervention on embryo development is a basic tenet of developing new assisted reproductive technologies. Despite this, the current repertoire of assays is limited, and is generally confined to single time point analysis. We propose to tackle this problem using genome editing tools such as CRISPR/Cas9 to generate embryos expressing fluorescent reporters and to knock out genes of interest. This will enable us to assess fundamental biological processes in the living embryo by timelapse microscopy.

Objective 4.1. To generate reporter and knock-out embryos for functional analysis and monitoring cellular processes in real time.
The advent of gene editing technologies such as CRISPR/Cas9 has made is possible to accurately and efficiently generate point mutations and small insertions in early embryos of a number of species, including human (Tang et al., 2018 Mol. Reprod. Dev). Recent evidence from mouse embryos indicates that it is also possible to introduce large DNA fragments by homologous recombination using a modified CRISPR/Cas9 (2C-HR-CRISPR; Gu et al., 2018, Nat. Biotechnol.). We propose to adapt this system to generate human embryos expressing fluorescent

reporters for proteins of interest. This would provide an *in vivo* system for deciphering fundamental cellular and developmental processes by direct visualisation in real time in human embryos. We propose to optimise this approach and will perform proof of concept experiments using an FP-tagged chromosome marker, such as histone H2, to enable us to visualise chromosomes in human embryos.
Objective 4.3 Optimise conditions for long term live cell fluorescence imaging of preimplantation embryos and for tracking individual cells during development.
Objective 4.4 Develop a panel of markers to monitor a variety of processes including cell state transitions and mitochondrial homeostasis during early development.

The PR has stated that the proposed aim and objective will be achieved by:

Aim 4: Expand the repertoire of assays for determining whether embryos are developing normally

Objective 4.1. To optimise techniques to knock out genes for functional analysis of selected proteins and to knock in large DNA fragments such as fluorescent proteins (FPs) at specific loci to enable us to track cellular processes in real time.
Recent evidence from mouse embryos indicates that it is possible [to introduce large DNA fragments by homologous recombination using a modified CRISPR/Cas9 (2C-HR-CRISPR; Gu et al., 2018, Nat. Biotechnol.)]. We are currently performing experiments in mouse embryos to determine whether this approach works well in our hands. Once we are satisfied with the efficiency and absence of off-target effects, we will commence experiments to generate human embryos expressing fluorescent reporters for proteins of interest. Our initial approach will be to adapt the recently reported method 2C-HR-CRISPR for introducing large DNA fragments by homologous recombination in mouse embryos (Gu et al., bioRxiv, 2017).

Objective 4.2 Optimise conditions for long term live cell fluorescence imaging of preimplantation embryos. We propose to perform live cell imaging of human embryos expressing fluorescent markers for proteins and processes of interest. We will use mouse embryos to optimise imaging and environmental conditions before moving to human.

Objective 4.3 Generate reporter embryos to monitor cell cycle transitions and chromosome segregation in human embryos. We will generate embryos expressing FP-tagged histone using a combination of mRNA injection and gene editing to insert the FP at the desired location. This will enable us to visualise chromosomes throughout the early and late preimplantation divisions. Once we have optimised the chromosome markers, the next step will be to combine this with a spindle marker to monitor spindle dynamics during the mitotic divisions. We will combine these approaches with single cell genomics and transcriptomics to validate

the reporter system and to understand the developmental fate of aneuploid cells. These experiments have the potential to greatly advance our understanding of the causes and consequences of aneuploidy in human embryos and will provide proof of concept for gene editing to generate human reporter embryos.

Objective 4.4 Develop a panel of markers to monitor cellular processes including cell state transitions and mitochondrial homeostasis during early development.
Once we have optimised procedures for efficient gene editing and live cell imaging, we will create human reporter embryos to map cell fate and cell state transitions. We will also use the system to extend the investigation of mitochondrial homeostasis (Aim 2).

In an email to accompany the variation application the PR has stated they expect to use 350 fresh eggs, 50 frozen eggs, 200 failed to fertilise embryos, 300 fresh embryos, 150 frozen embryos and 250 created embryos per year for the remainder of the licence.

The executive considered it necessary and prudent to seek the approval of Licence Committee for this licence variation application, because it seeks to add research work which effectively extends how the approved licensed research activities, e.g. use of embryos in research, will be undertaken.

Peer review

The executive considered it necessary and prudent to seek the opinion of a peer reviewer regarding the application and the response is provided in the committee papers. The peer review was supportive of the research work described in the licence variation application.

Summary for licensing decision:

Taking into account the essential requirements set out in the Human Fertilisation and Embryology (HF&E) Act 1990 (as amended), the HF&E Act 2008 and the HFEA Code of Practice (CoP), the inspector considers that it has sufficient information to conclude that:

Administrative requirements:

- The centre has submitted an appropriately completed application form
- The centre has submitted the supporting information required by General Direction 0008, including evidence of ethics approval albeit the PR is in the process of obtaining ethics approval for 'new' additional elements of the research project.
- The application has designated an individual to act as the PR
- The proposed licence applies to one project of research
- A fee is not required for this type of licence variation application, and so no fee was payable in this instance.

In considering overall compliance, the inspector considers that she has sufficient information drawn from the renewal inspection of the centre on 23 January 2017, the interim inspection on 5 February 2018 and documentation submitted by the centre, to conclude that:

- the premises are suitable for the proposed research activities
- the proposed practices are suitable

The Licence Committee is asked to note that at the time of the assessment there were two 'Other' areas of practice that required improvement.

The PR has given a commitment to fully implement the following recommendations:

- Other' area of non-compliance The PR should ensure that storage consent forms provide the option for donors to elect to consent to store for less than 10 years.

- The PR should ensure that none of the additional research objectives are to commence until approval for them has been obtained from an appropriately constituted research ethics committee and evidence of this has been provided to and acknowledged by the HFEA executive.

Recommendation:

The inspector considers that there is sufficient information available to recommend the Licence Committee approves the licence variation application, to include the additional research objectives within the project definition.

Details of assessment findings

The licence variation application

An application has been received from the PR of project R0152 at centre 0017 to vary the research licence. There are no changes to the licensed research activities but additional research aims and objectives will be undertaken as part of the 'use of embryos' in research, these being:

> Aim 4: Expand the repertoire of assays for determining whether embryos are developing normally
>
> Objective 4.1. To optimise techniques to knock out genes for functional analysis of selected proteins and to knock in large DNA fragments such as fluorescent proteins (FPs) at specific loci to enable us to track cellular processes in real time.
>
> Objective 4.2 Optimise conditions for long term live cell fluorescence imaging of preimplantation embryos.
>
> Objective 4.3 Generate reporter embryos to monitor cell cycle transitions and chromosome segregation in human embryos.
>
> Objective 4.4 Develop a panel of markers to monitor cellular processes including cell state transitions and mitochondrial homeostasis during early development.

In an email to accompany the variation application the PR has stated they expect to use 350 fresh eggs, 50 frozen eggs, 200 failed to fertilise embryos, 300 fresh embryos, 150 frozen embryos and 250 created embryos per year for the remainder of the licence. This is an increase from the embryo use forecasted in the renewal application, where expected embryo use was stated as being; 300 fresh eggs, 50 frozen eggs, 200 failed to fertilise embryos, 250 fresh embryos, 150 frozen embryos and 250 created embryos

Desk based assessment of the application

The inspector notes that research project R0152 does not involve the derivation of stem cells for human application, therefore the project only needs to comply with research licence conditions (RLCs) 1 – 40. Evidence provided by the centre was reviewed against the relevant requirements of the Human Fertilisation and Embryology Act 1990 (as amended), RLCs and the Code of Practice (CoP), with the following findings:

1. A desk based assessment was completed following the PR's request to add further aims and objectives to research project R0152. Further information was sought during the interim research inspection.
2. The PR, Dr Choudhary has requested an amendment to further broaden their research. The application has been peer reviewed. The peer review was supportive of the research work described in the licence variation application.

3. The additional aim and objective will require no changes to the licensed activities or purposes for which the licence was granted.
4. The PR has suitable qualifications and experience for the activity authorised by the licence (HF&E Act 1990 (as amended), section 16 (2) (ca)). The PR has successfully completed the HFEA PR Entry Programme.
5. The PR has confirmed that suitable practices are used in undertaking the licensed activities and recruitment processes ensure that persons working under the licence are suitably qualified and experienced and are of suitable character.
6. Research nurses, responsible for delivering patient information and taking consent, have been provided with training in regards to the additional aim and objective of the research project (RLC R21, RLC R22).
7. The centre has developed documented processes for research activities to ensure compliance with HFEA requirements, including the prevention of: gamete and embryo use in research without consent; gamete and embryo storage without consent; embryo culture beyond 14 days or the appearance of the primitive streak (RLC R28, R36, R38 and R39).
8. Processes also ensure that gametes and embryos received for use in project R0152 are obtained only from centres to which a HFEA licence applies (RLC R32, R33). At the time of renewal, embryos were received directly to the research project under the research licence held at centre 0017, via the treatment and storage licence, under supervisor of PR Dr Jane Stewart at centre 0017 and from centre 0170. The PR has confirmed that centre 0170 are no longer donating to this project.
9. Information and consent forms have been updated to reflect the proposed variation, and increase clarity of the genetic manipulation of gametes and embryos, necessary for this research (RLC R19, RLC R20). Information and consent forms ensure gamete and embryo donors are aware that counselling is available (RLC R18). The centre's own information and consent forms allow donors to provide consent to the storage gametes or embryos, for a period not exceeding the 10 year statutory storage period, but does not provide an option to indicate if they would like their gametes or embryos to be stored for less than ten years (paragraph 2(2) of Schedule 3 of the Human Fertilisation and Embryology Act 1990 (as amended), see recommendation 1).
10. This study recruits altruistic donors, donating exclusively for research purposes. This cohort of donors consent to the storage of their gametes for research, and the storage of any embryos created using their gametes on the centre's own research consent form, as there is no HFEA consent form, listed in the schedule of general direction 0002, appropriate for capturing storage consent not related to treatment purposes.
11. The premises and facilities were considered at the interim inspection, on 5 February 2019, to be secure, clean, well maintained and suitable for carrying out licensed activities (RLC R10), including those necessary to undertake the research work described in the variation application, including facilities for molecular analysis and embryo microscopy.
12. The Francis Crick Institute, centre 0246, also holds a licence for R0152. The PR of the licence for R0152 held there, Dr Niakan, has also submitted an application to vary that licence in the same way.
13. Dr Choudhary has confirmed that the research activities related to the additional aim and objective will initially be performed solely at centre 0017. Research activities involved in later stages of the experimental design will be performed at centre 0246.

The research work at centre 0246 is not anticipated to begin within the next 12 months.

14. The PR is in the process of seeking ethics approval for the additional aim and objective. The research ethics committee has requested that the PR submits evidence of HFEA approval of the amended research, before ethics approval can be considered. Evidence of approval by a suitably constituted ethics committee is required by General Direction 0008 upon application for a new research licence, to renew an existing licence, or to vary the purposes for which a project is licensed. There is no direct requirement in General Direction 0008 for evidence of ethics approval to be submitted alongside a request to amend the research objectives, but the inspector considers it reasonable to require confirmation that either the project's existing ethics approval remains valid, or that an amended ethics approval has been granted, before commencing research described in the revised objective.

Peer review

The executive considered it necessary and prudent to seek the opinion of a peer reviewer regarding the application.

The peer reviewer agreed that the purposes defined by the PR are relevant: i.e.

- Increasing knowledge about serious diseases or other serious medical conditions
- Developing treatments for serious disease or other serious medical conditions
- Increasing the knowledge about the causes of congenital disease or other congenital medical conditions
- Promoting advances in the treatment of infertility
- increasing knowledge about the causes of miscarriage
- Developing methods for detecting the presence of gene, chromosome or mitochondrial abnormalities in embryos before implantation
- increasing knowledge about the development of embryos

When commenting on the importance of the research questions/objectives, the peer reviewer stated:
'The research questions are directly related to finding ways to improve assisted conception protocols generally, and also specifically (i) for patients at risk of mitochondrial disease or (ii) to help reduce the occurrence of aneuploidy in aging females. Among others the proposed genetic modifications will target proteins associated with chromosome/centromere segregation during meiosis.'

The peer reviewer considered the project methodology is likely to answer the research objectives and that the research team have the necessary expertise and experience, commenting;
'The research team has a long history and proven record (multiple high profile publications) of successful research into the areas proposed in the licence amendment. They are extremely well placed in terms of expertise and experience.'

The peer reviewer considered that the research objectives could not be addressed without the use of human embryos and that the type and number of embryos to be used in the project were justified and important.

Areas of practice that require the attention of the Person Responsible

The section sets out matters which the inspection team considers may constitute areas of non compliance. These have been classified into critical, major and others. Each area of non-compliance is referenced to the relevant sections of the Acts, Regulations, Standard Licence Conditions, Directions or the Code of Practice, and the recommended improvement actions required are given, as well as the timescales in which these improvements should be carried out.

Critical areas of non compliance

A critical area of non compliance is an area of practice which poses a significant risk of causing harm to a patient, donor, embryo or to a child who may be born as a result of treatment services. A 'critical' area of non-compliance requires immediate action to be taken by the Person Responsible.

Area of practice and reference	Action required and timescale for action	PR Response	Executive Review
None			

Major areas of non compliance

A major area of non compliance is a non critical area of non compliance:

- which poses an indirect risk to the safety of a patient, donor, embryo or to a child who may be born as a result of treatment services
- which indicates a major shortcoming from the statutory requirements;
- which indicates a failure of the Person Responsible to carry out his/her legal duties
    - a combination of several “other” areas of non-compliance, none of which on their own may be major but which together may represent a major area of non-compliance.

Area of practice and reference	Action required and timescale for action	PR Response	Executive Review
None			

‘Other’ areas of practice that requires improvement

Areas of practice that requires improvement is any area of practice, which cannot be classified as either a critical or major area of non compliance, but which indicates a departure from statutory requirements or good practice.

Area of practice and reference	Action required and timescale for action	PR Response	Executive Review
1. The centre’s own storage consent forms, do not provide the option for donors to elect to consent to store for less than 10 years. Paragraph 2(2) of Schedule 3 of the Human Fertilisation and Embryology Act 1990.	The PR should ensure that storage consent forms provide the option for donors to elect to consent to store for less than 10 years. The PR should submit amended storage consent forms when responding to this report.	The egg donors for research are given the option to opt for a lesser storage duration than 10 years if so desired. Previous HFEA research inspections have not highlighted any concerns as regards this. However, we take your recommendation on board and are willing to be more explicit by submitting an amendment to our consent form to REC to state 'I consent to storage of my gametes/embryos created for research for a maximum of 10 years in accordance to HFEA regulations from date of storage If less than 10 years, please specify number of years……' We have informed REC regarding this and will have to	The executive acknowledges receipt of the updated consent form, and the PR’s commitment to ensure that donors are able to consent to store gametes or embryos for less than the statutory storage period. No further action required.

		await the final outcome before submitting further amendment if required. If the donor elects to store the gametes and /or embryos for less than 10 years, this will be recorded on the egg donor record form (attached).	
2. Ethics committee approval for the research project may not adequately cover the proposed research amendment. GD 0008.	The research ethics committee has requested that the PR submits evidence of HFEA approval of the amended research, before ethics approval can be considered. The PR should ensure that none of the additional research objectives are commenced until approval for them has been obtained from an appropriately constituted research ethics committee and evidence of this has been provided to and acknowledged by the HFEA executive.	We are awaiting final outcome from the Research Ethics Committee (REC) pending approval of submitted PIS/ICF documents and HFEA approval of the revised objectives. We would like to provide the assurance that the additional research objectives relating to nuclear gene editing will be commenced only after receiving approvals from both HFEA and REC. We would submit the evidence of the approvals to the respective bodies (HFEA and REC) prior to commencement of the new research aims.	The executive acknowledges the PR's commitment to provide evidence of ethics approval prior to commencing the new research aims. Further action required.

Additional information from the Person Responsible
Current ethics approval in place but as mentioned we are awaiting final outcome from REC for our submitted revised documents and research objectives. We would like to take this opportunity and thank our HFEA inspector, Lesley Brown for the feedback and recommendations on the research licence variation..