Executive Licensing Panel - minutes

Centre 0017 (Newcastle Fertility Centre at Life)

Renewal Inspection Report – Research Project R0152

Tuesday, 5 May 2020

HFEA Teleconference Meeting

Panel members	Clare Ettinghausen Anna Coundley Yvonne Akinmodun	Director of Strategy and Corporate Affairs Policy Manager Head of Human Resources
Members of the Executive	Bernice Ash	Committee Secretary
External l Adviser		
Observers	Catherine Burwood	Licensing Manager

Declarations of interest:

- Members of 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 panel:

Papers enclosed:

- Inspection report
- Application form
- Consent forms
- Patient information forms
- List of publications
- Peer reviewer report
- Establishing research partnerships form
- Previous licensing minutes:
    - 2019-05-02, LC minutes, Executive Update – Variation of Aims & Objectives – Research Project R0152
    - 2019-03-07, LC minutes, Interim inspection report & variation of research aims and objectives – Research Project R0152
    - 2017-05-04, LC minutes, Renewal Inspection report

1. Background

1.1. The Newcastle Fertility Centre at Life (centre 0017) is a treatment and research centre, based within the International Centre for Life. The research laboratory is located within the same unit as the treatment and storage centre.

1.2. The panel noted that the current research project, entitled 'Towards improving assisted reproductive technologies for the treatment of infertility and prevention of disease' (R0152), was first licensed in 2004. 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).

1.3. The panel noted that the centre's current licence was granted by the Licence Committee (LC) on 4 May 2017 and is due to expire on 31 July 2020.

1.4. The panel noted that the research project has been varied once during the period of the current licence. No changes were made to the licensed activities. A further research objective was added, to allow the use of 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. These amendments were presented to the LC in March 2019, with the interim inspection report.

1.5. The panel noted that 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 projects.

2. Consideration of Application

2.1. The panel noted that an application was submitted to renew the research licence for project R0152.

2.2. The panel noted that the application to renew the research licence was made by the Person Responsible (PR) for a period of three years.

2.3. The panel noted that the centre has applied for the following activities:

- Creation of embryos in vitro
- Keeping embryos
- Use of embryos
- Storage of gametes
- Storage of embryos

2.4. The panel noted that the proposed activities are to be licensed 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 any other congenital disease or congenital medical condition
- 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

2.5. The panel noted that the desk based assessment for the renewal of the licence for project R0152 took place on 26 February 2020.

2.6. The panel noted that, at the time of the desk based assessment, there were no areas of non-compliance.

2.7. The panel noted that the Peer Reviewer was supportive of the project, stating that, 'The research questions addressed are very important. The applicants are focusing on mitochondrial DNA inheritance. When functioning incorrectly or mutated, mitochondrial DNA can cause serious and untreatable diseases. The fundamental problem is a lack of knowledge about eggs and early human embryos, and this is a broad enough project to address that too, through studies of ageing, chromosomal abnormalities, gene expression.'

2.8. The panel noted that the inspectorate recommends the renewal of the licence for research project R0152, for a period of three years, without additional conditions

3. Decision

3.1. The panel had regard to its decision tree. It was satisfied that the appropriate application and fee had been submitted and that the application contained supporting information required by General Directions 0008.

3.2. The panel noted that the premises to be licensed are suitable for the conduct of the licensed activities.

3.3. The panel was satisfied that the qualifications and character of the PR are such as is required for the supervision of licensed activities and the PR will discharge her duty under section 17 of the HFE Act 1990 (as amended).

3.4. The panel was satisfied that the research project has been approved by the Health Research Authority, Newcastle and North Tyneside 1, Ethics Committee. This approval remains active and covers the research activity described in the licence application.

3.5. The panel was satisfied that the research licence would not apply to more than one research project.

3.6. The panel was satisfied with the suitability of the activities applied for:

- Creation of embryos in vitro
- Keeping embryos
- Use of embryos
- Storage of gametes
- Storage of embryos

3.7. The panel was satisfied that the activities to be licensed are necessary or desirable for the following purposes, specified in paragraphs 3A(1) and 3A(2) of Schedule 2 of the HF&E Act 1990 (as amended):

- 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 any other congenital disease or congenital medical condition
- 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

Prohibited Research Activities

3.8. The panel was satisfied that none of the proposed activities are prohibited by the HF&E Act 1990 (as amended).

3.9. The panel was satisfied that this is a research project and that no embryos used in the project would be implanted into a woman.

3.10. The panel was satisfied that the proposed research project does not involve the mixing of sperm with the egg of an animal.

Use of Human Embryos

3.11. The panel was satisfied that the use of human embryos is necessary for the purposes of the research.

3.12. The panel was satisfied that the proposed research project does not involve the derivation of human embryonic stem cell lines for human application or the genetic modification of embryos.

3.13. The panel was satisfied that no embryos would be used without obtaining proper consent for the use of embryos in research from patients.

3.14. The panel agreed to renew the research licence for project R0152 at centre 0017, entitled 'Towards improving assisted reproductive technologies for the treatment of infertility and prevention of disease', for a period of three years with no additional conditions. If no representations regarding the proposed decision or any other information is received within 28 days, the committee agreed for the final licence to be issued.

3.15. The panel agreed to the following activities and purposes:

Activities:

- Creation of embryos in vitro
- Keeping embryos
- Use of embryos
- Storage of gametes
- Storage of embryos

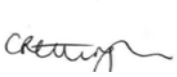
for the following purposes:

- 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 any other congenital disease or congenital medical condition
- 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

4. Chair's Signature

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

Signature



Name

Clare Ettinghausen

Date

11 May 2020

Research Renewal Report: Desk Based Assessment

Purpose of this inspection report

The HFEA licenses and monitors establishments undertaking human embryo research. This is a report of an inspection, carried out to assess whether this centre complies with essential requirements when carrying out such research. Licences for individual research projects can be granted for up to three years and this report provides information on the centre's application for a renewal of its existing licence. The Authority's Executive Licensing Panel (ELP) uses the application and this report to decide whether to grant a new licence and, if so, whether any additional conditions should be applied to the licence.

Date of assessment: 26 February 2020

Purpose of assessment: Renewal of a licence to carry out research

Inspection details: The report covers the performance of the centre since the last inspection, findings from the desk based evaluation, and communications received from the centre. For this assessment, an inspector completed a robust desk-based evaluation of appropriate documentation. There was no site visit.

Inspector: Lesley Brown

Date of Executive Licensing Panel: 5 May 2020

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	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

Section 1: Summary report

Brief description of the centre and its licensing history:

The Newcastle Fertility Centre at Life (centre 0017) is a treatment and research centre based within the International Centre for Life. The research laboratory is located within the same unit as the treatment and storage centre. The current research project, entitled "Towards improving assisted reproductive technologies for the treatment of infertility and prevention of disease" (R0152), was first licensed in 2004. 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 current licence is due to expire on 31 July 2020, having been renewed for three years by a Licence Committee on 4 May 2017.

The research project has been varied once during the period of the current licence. No changes were made to the licensed activities. A further research objective was added, to allow the use of 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. These amendments were presented to Licence Committee in March 2019, alongside the interim inspection 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.

There are no additional conditions on the licence.

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 inspection team 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 and patient information and consent forms
- the application has designated an individual to act as the Person Responsible (PR)
- the proposed licence applies to one project of research
- the centre has submitted fees to the HFEA in accordance with requirements

Research activities applied for:

An application has been made for the following activities for the purpose of research:

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

Purposes for which research activities may be licensed:

The activities specified above are required by the PR for the following purposes, as defined in Schedule 2 3A (1) and (2) of the HF&E Act 1990 (as amended):

- 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 any other congenital disease or congenital medical condition
- 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 PR and peer reviewer consider that the research project will meet the purposes defined in Schedule 2 3A (1) and (2) to the HF&E Act 1990 (as amended) as follows:

- Increasing knowledge about serious disease or other serious medical conditions

The PR has stated:

> 'The project is increasing our knowledge about the transmission of mitochondrial DNA and nuclear DNA during human preimplantation development.'

- Developing treatments for serious disease or other serious medical conditions

The PR has stated:

> 'The research is leading to the development of a treatment that has the potential to significantly reduce the risk of transmission of abnormal mitochondrial DNA to the embryo and subsequent child.'

- Increasing knowledge about the causes of any other congenital disease or congenital medical condition

The PR has stated:

> 'We are investigating the origin of aneuploidy by studying chromosome segregation, including the effect of female age on chromosomal abnormalities in eggs and early embryos.'

- Promoting advances in the treatment of infertility

The PR has stated:

> '(1) By understanding how the nuclear DNA is packaged and transmitted during fertilisation and embryonic development we hope to advance knowledge on the causes of chromosomal abnormalities in eggs and embryos. (2) Our research may lead to improved fertility treatments for older women through optimisation of ovarian stimulation regimes and harvest of maturing oocytes. (3) By investigating how oocytes safeguard the integrity of the mitochondrial genome and how this is further shaped during preimplantation development, we will shed light on whether mitochondria contribute to infertility. (4)

Gaining greater insight into the process of epiblast specification will give foundation to a greater understanding of factors affecting blastocyst quality (5) By optimising conditions for generating reporter embryos, the research has the potential to transform the assessment of future developments in fertility treatments by enabling direct assessment of the effect of interventions on fundamental biological processes in living embryos.'

- Increasing knowledge about the causes of miscarriage

The PR has stated:

'Our studies of oocyte aneuploidy are relevant to understanding the causes of miscarriage.'

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

The PR has stated:

'The project requires the development of methods to detect mitochondria in single cells and the segregation of mitochondrial DNA within cells during the embryonic divisions. It also requires the development of methods to study gene expression and chromosome constitution in different parts of the embryo for comparative purposes as below.'

- Increasing knowledge about the development of embryos

The PR has stated:

'Prior to translation of the methods being developed in this project into clinical practice it is important that comparative studies with unmanipulated embryos created in vitro show similar outcomes. The information obtained in such comparisons will increase our knowledge about the development of embryos. We also aim to utilize 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.'

The Executive notes the use of the phrase "proposed new aim" in the paragraph above. The Executive has sought clarification from the PR and can confirm this relates to the recently approved additional aim.

The peer reviewer agrees the research is likely to contribute to knowledge in each of the above areas, and has stated:

'The research questions addressed are very important. The applicants are focusing on mitochondrial DNA inheritance. When functioning incorrectly or mutated, mitochondrial DNA can cause serious and untreatable diseases. The fundamental problem is a lack of knowledge about eggs and early human embryos, and this is a broad enough project to address that too, through studies of ageing, chromosomal abnormalities, gene expression.'

The reviewer concurs with the applicants that this research will make progress towards understanding and treating serious diseases, congenital medical conditions and developing diagnostic tools.

Prohibited research activities:
The activities to be licensed are not prohibited by the HF&E Act 1990 (as amended) including those activities specifically prohibited by Sections 3, 3ZA, 4 or 4A, or by Schedule 2, paragraph 3 of the Act.

Use of embryos:
The use of human embryos is considered necessary. This is based on the application and comments by the peer reviewer who states:

> 'The problems occurring in humans with mitochondrial DNA disorders and chromosomal disorders with female ageing do not arise in animal species. It is definitely necessary to study human embryos in order to answer the questions addressed by this research project.'

The peer reviewer continues:

> 'The project will utilise spare embryos (surplus to treatment) and embryos newly created for research purposes, using eggs from different sources, including immature eggs collected prior to ICSI. The project will also involve the use of fresh and vitrified eggs from egg donors which are being used to generate research embryos. These eggs could potentially have been used for treatment instead. No information is provided regarding why the eggs are being donated, for example, are patients being offered the chance to share eggs as part of their treatment, or are these volunteer donors, all of whose eggs are being utilised? Given that the interval from hCG to egg collection is being varied, this is important to clarify, because varying that interval can affect treatment outcomes for patients.
> The ethics approval letters provided do not provide insight on the above point. Nevertheless, the use of fresh human oocytes for this research is necessary and important.'

The executive can confirm that altruistic egg donors are recruited directly into the research programme with all relevant information and consent. Copies of the patient information and consent forms provided by the PR are included in the papers.

PR considerations:
The PR is suitable and has discharged her duty under Section 17 of the HF&E Act 1990 (as amended).

Premises:
The premises are suitable. This is based on information submitted with this application, and the previous inspection visit in 2019.

Recommendation:
The ELP is asked to note that at the time of the desk based assessment, there were no areas of non-compliance.

The inspection team considers that, overall, there is sufficient information and evidence available to recommend the renewal of the centre's licence for a period of three years without additional conditions.

The inspection team recommends that the licence issued should include the following activities that the centre has applied for:

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

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 any other congenital disease or congenital medical condition
- 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

Section 2: Summary of the research project

This section summarises information submitted in the research licence application and from the Peer Reviewer.

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 encompasses transmission of the nuclear and mitochondrial genomes. In relation the mitochondrial genome, we are interested in transmission of mitochondrial DNA (mtDNA) 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 abnormalities in eggs with the aim of better understanding the underlying causes of the decline in fertility as women get older. We also aim to develop new approaches to studying segregation of the nuclear and mitochondrial genomes during embryonic development. We intend to use gene editing techniques to investigate mtDNA and nuclear DNA segregation during early embryo development and test 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.

Objectives of the research:

Aim 1: 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 remodeling 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 alternative approaches to pronuclear transfer including 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 minimise 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 in2/9 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: 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 during early embryo development. **Objective 2.2** 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: Investigate the molecular 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:
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.

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.

Summary of the research undertaken to date:

The pronuclear transfer (PNT) technique developed under our previous research licence has been translated to clinical treatment to prevent transmission of serious disease caused by mutations in mitochondrial DNA. We continue to perform experiments to optimise the PNT procedures and to understand why, in some cases, embryonic stem cell derived from PNT embryos revert to the original mitochondrial genome. We are also working towards developing strategies to prevent mitochondrial genome reversion. This research, which is ongoing, involved creation of 143

embryos, the use of 102 failed to fertiltise eggs and 34 donated frozen and 10 fresh embryos. We have also conducted experiments to better understand the mitochondrial biology of the early embryo with a focus on mitochondrial turnover during preimplantation development. Our findings so far reveal new layers of complexity in how the mitochondrial genome is shaped in the days before implantation. This work is ongoing and has so far involved the use of 206 embryos donated to research. To gain insight into the feasibility of reducing the very high incidence of chromosomal abnormalities in eggs of older women, we have established a pipeline for donation of immature oocytes from young and older women. We have also developed molecular tools to enable us to monitor oocyte chromosomes during the specialised cell divisions required to halve the nuclear genome in preparation for fertilisation. This work is ongoing and has so far involved artificial activation of 39 eggs to induce the final division of chromosomes. Together, the ground work carried out in the 2017-2020 period, will enable us to make progress during the next licencing period, towards improving reproductive outcomes for women who carry mitochondrial DNA mutations and for those at increased risk of transmitting the incorrect number of chromosomes during fertilisation.

Donation and use of embryos:

The renewal application proposes using 350 fresh eggs, 50 frozen eggs, 200 failed to fertilise embryos, 300 fresh embryos, 150 frozen embryos and 250 created embryos per year.

The peer reviewer states:

> 'The numbers of eggs and embryos used and created, as reported in the text of the application, are reasonable. The projected utilisation rates are also suitable given the nature and scale of the project.'

Section 3: Details of the inspection findings

Principle:

3. Have respect for the special status of the embryo when conducting licensed activities.

What we inspected against:

Research Licence Conditions (RLC) R23, R24, R26, R27, R28, R29, CoP Guidance Note 22.

What the centre does well.

The SAQ and other documents provided by the PR in support of the application, and observations during the inspection in February 2019, provided assurance that the special status of the human embryo will be respected:

- processes, documented in standard operating procedures (SOPs), are in place to ensure that no embryo obtained for the purposes of any research project is kept or used for any purpose other than the purposes of that research project (RLC R23). Staff training and their close supervision ensure procedures are adhered to, preventing the use of donated embryos in unlicensed activities.
- recruitment practices ensure that no money or other benefit is given to those donating gametes or embryos to research unless authorised by directions (RLC R24).
- each embryo used in the research project is uniquely labelled (RLC R26)
- documented procedures have been established, implemented and complied with to ensure that clinical and research roles are separated (RLC R27).
- procedures ensure that embryos do not develop after 14 days or the primitive streak has appeared (if earlier) (RLC R28). The culture and manipulation of each embryo is recorded in the laboratory records, which are regularly reviewed.
- when human embryonic stem cell lines are derived, a sample of all stem cell lines is deposited in the UK Stem Cell Bank (RLC R30).

What they could do better.

Nothing noted.

Principle:

5. Provide prospective and current patients and donors with sufficient, accessible and up-to-date information in order to allow them to make informed decisions.

6. Ensure that patients and donors have provided all relevant consents, before any licensed activity is undertaken.

What we inspected against:

Information, counselling and consent; CoP Guidance Note 22, RLC R18, R19, R20, R21,

R22. Consent for storage; CoP Guidance Note 22, RLC R31, R32, R33, R34, R35, R36, R37, R38, R39.

What the centre does well.

Provision of information and counselling to those consenting to donate to research

Prior to giving consent, those donating to research should be provided with relevant information, and given a suitable opportunity to receive counselling about the implications of their donation. The PR has provided assurance that:

- prior to giving consent, those donating to research are given a suitable opportunity to receive proper counselling about the implications of their donation (RLC R18).
- necessary information is provided to patients prior to giving their consent (RLC R19 and R20).
- information is provided to patients by trained personnel in a manner and using terms that are easily understood (RLC R21). The competence of staff at the recruiting centres to provide information in this way, and to seek consent, has been assessed.
- a designated individual, who is not directly involved in the patient's treatment, is available to discuss with the patient the project of research and the possibility of donating material to the project (RLC R22). Contact details for this designated individual are provided in the patient information.

Consent for storage

The SAQ and risk assessment of the project, provided by the PR in support of the application, and findings during previous inspections at the centre, provide good evidence that:

- Stored embryos are obtained only from HFEA licensed centres (RLC R32 and R33).
- The centre has effective processes for monitoring stored gametes and embryos and ensuring they remain within their consented storage period. All frozen gametes and embryos used in the research project have been used within their consented storage period (RLC R36, and R37).

What they could do better.

Nothing noted.

Principle:

8. Ensure that all premises, equipment, processes and procedures used in the conduct of licensed activities are safe, secure and suitable for the purpose.

What we inspected against:

Premises and facilities; RLC R10.

What the centre does well.

Premises and facilities

Based on the centre's SAQ and the last inspection visit in February 2019, the inspector is assured that the premises and facilities are secure, clean, well maintained and are suitable

for carrying out the licensed activities (RLC R10). In addition, all of the equipment and materials used in licensed research activity are designated for the purpose and are appropriately maintained in order to minimise any hazard to patients and/or staff.

What they could do better.
Nothing noted.

Principle:
10. Maintain proper and accurate records and information about all licensed activities

What we inspected against:
Information and record keeping; RLC R13, R14, R15, R16, R17, General Direction 0002.

What the centre does well.

The PR has provided all necessary information requested during this assessment within the required timescales (RLC R3).

Since the last inspection, the centre has submitted the annual research information and data sheet to the HFEA within the required timeframes (RLC R14 & General Direction 0002).

What they could do better.
Nothing noted.

Principle:
11. Report all adverse incidents (including serious adverse events and reactions) to the HFEA, investigate all complaints properly, and share lessons learned appropriately

What we inspected against:
Incidents; RLC R40.

What the centre does well.

The SAQ and risk assessment of the project, provided by the PR in support of the application, and findings during previous inspections at the centre, provide good evidence that processes are in place to detect, report to the HFEA and investigate adverse incidents (RLC R40).

What they could do better.
Nothing noted.

Principle:

12. 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.

What we inspected against:

HF&E Act 1990 (as amended), Schedule 2 (3(5) and 3A).

What the centre does well.

The research project has been approved by the Health Research Authority, Newcastle and North Tyneside 1, Ethics Committee. Evidence was provided by the PR that this approval remains active and covers the research activity described in the licence application.

The research project does not include any activities that have been prohibited by the HF&E Act 1990 (as amended).

A peer review was obtained for this renewal application and it is supportive of the licence renewal. Justifications that the activities to be licensed are necessary or desirable to meet the statutory purposes, have been provided by the PR and the peer reviewer, as discussed in detail in the 'Summary for Licensing Decision'. The PR and peer reviewer have also provided reasons why the use of human embryos is necessary and the proposed number of embryos to be used is justified.

What they could do better.

Nothing noted.

Principle:

13. Conduct all licensed activities with regard for the regulatory framework governing treatment and research involving gametes or embryos within the UK, including:

- maintaining up-to-date awareness and understanding of legal obligations;
- responding promptly to requests for information and documents;
- co-operating fully with inspections and investigations by the HFEA or other agencies responsible for law enforcement or regulation of healthcare.

What we inspected against:

Licensing; RLC R1, R2, R3, R5, R6. The Person Responsible; HF&E Act 1990 (as amended) Section 16 & 17, RLC R8, R9.

What the centre does well.

Licensing

Information obtained at the last inspection in February 2019 and a review of the SAQ provided by the PR in support of the application, confirm that all licensed research activities will be performed only at the licensed premises under the supervision of the PR (RLC R1,

R2).

The Person Responsible

The PR has a key role to play in implementing the requirements of the HF&E Act 1990 (as amended) and is the person under whose supervision the licensed activities are authorised. The PR has the primary legal responsibility under Section 17 of the HF&E Act 1990 (as amended) to secure:

- that suitable practices are used in undertaking the licensed activities;
- that other persons working under the licence are suitable and;
- that the conditions of the licence are complied with.

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. The inspection team considered that the PR has fulfilled her responsibilities under Section 17 of the HF&E Act 1990 (as amended).

What they could do better.

Nothing noted.

Section 4: Monitoring of the centre's performance

Following an interim inspection in 2019, recommendations for improvement were made in relation to, one major and one 'other' area of non-compliance or practice that required improvement.

The PR provided information and evidence that these recommendations were fully implemented within the agreed timescales.

Following a research variation assessment, considered alongside the interim inspection report in 2019, recommendations for improvement were made in relation to two 'other' areas of non-compliance or practices that required improvement.

The PR provided information and evidence that these recommendations were also fully implemented within the agreed timescales.

Section 5: 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 areas 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
Nothing noted.			

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 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
Nothing noted.			

‘Other’ areas of practice that require improvement

‘Other’ areas of practice that require 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	PR Response	Executive Review
Nothing noted.			

Additional information from the Person Responsible
We would like to express our sincere thanks to our HFEA inspector, Lesley Brown and the HFEA for their constant support and guidance to ensure we remain compliant with the regulations in delivery of our research licence.