Licence Committee - minutes

Centre 0017 (Newcastle Fertility Centre at LIFE),

Research Project R0152 - Renewal Inspection Report

Thursday, 4 May 2017

Church House Westminster, Dean's Yard, Westminster SW1P 3NZ

Committee members	Andy Greenfield (Chair) Lee Rayfield Ruth Wilde Kate Brian Anita Bharucha	
Members of the Executive	Dee Knoyle	Secretary
Legal Adviser	Ros Foster	Browne Jacobson LLP
Specialist Adviser		
Observers		

Declarations of interest:

- Members of the committee declared that they had no conflicts of interest in relation to this item, however, they did want to declare the following:
- Andy Greenfield – Chaired two previous Expert Panels on the safety and efficacy of mitochondrial donation
- Kate Brian – London Representative of Fertility Network UK and also Women's Voices Lead at the Royal College of Obstetricians and Gynaecologists
- Ruth Wilde – Fertility Counsellor for a Licensed Centre

The committee had before it:

- 8th edition of the HFEA Code of Practice

- Standard licensing and approvals pack for committee members

The following papers were considered by the committee:

- Research licence renewal inspection report
- Licence renewal application form
- Patient information and consent
- Peer Review
- Publications (page 31)

➢ Licensing minutes up to the previous licence renewal:

- 12 January 2017 – update to the research objectives
- 10 November 2016 – update to the research objectives
- 8 September 2016 – update to the research objectives
- 16 July 2015 – updates to the research objectives and lay summary (deferred from 22 May 2015)
- 22 May 2015 – research interim inspection
- 22 May 2015 – licence variation, and updates to the objectives and lay summary
- 31 October 2014 – change of PR
- 8 May 2014 – research licence renewal

1. Consideration of application

1.1. The committee noted that the 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 committee noted that 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). This collaboration continues. The licence for research project R0152 at centre 0246 will be considered for renewal separately in 2018.

1.3. The committee noted that research project R0152 has been amended twice during the current period to reflect updates in the research objectives and the number of embryos expected to be created and/or used. These amendments have been presented to the 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 team at Newcastle continue to develop and refine their research. Additions to the research project, compared with the last renewal, include the potential use of TALENS, a 'genome editing' technique.

1.4. The committee noted that the application was made by the Person Responsible (PR) for the licence for the research project to be renewed for a period of three years.

1.5. The committee noted that the centre has applied for the following activities:

- creation of embryos in vitro
- keeping embryos
- use of embryos
- storage of embryos.

1.6. The committee noted that storage of gametes was not included on the application form, however, this activity would be required.

1.7. The committee 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.

1.8. The committee noted that the treatment centres donating to this research project include:

- Newcastle Fertility Centre at LIFE, Centre 0017
- Queen Elizabeth Hospital, Gateshead, Centre 0170

1.9. The committee noted that the renewal inspection took place on 23 January 2017 and the report covers the performance of the centre since the last inspection, findings from the inspection and communications received from the centre. The committee noted that at the time of the renewal inspection no recommendations were made.

1.10. The committee noted that the research project has been approved by the North East - Newcastle & North Tyneside 1 Research Ethics Committee. Evidence was provided by the PR that this approval remains active and covers the research activity described in the application.

1.11. The committee noted that the inspectorate recommends the renewal of the centre's licence for research project R0152 for a period of three years with no additional conditions.

1.12. The committee had regard to its decision tree.

1.13. The committee was satisfied that the application was submitted in the form required and contained all the supporting information required by General Direction 0008. Furthermore, it was satisfied that the appropriate fees had been paid.

1.14. The committee 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 to the HFE Act 1990 (as amended):

- **increasing knowledge about serious disease or other serious medical conditions**

  The project is increasing knowledge about the transmission and propagation of abnormal mitochondria during pre-implantation human development and the contribution that aneuploidy may make to disease.

- **developing treatments for serious disease or other serious medical conditions**

  The research has led to the development of a procedure that has the potential to significantly reduce the risk of transmission of abnormal mitochondria to the embryo and subsequent child. This will be refined by further research. Related procedures will also be assessed.

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

  The proposed research will investigate the occurrence of aneuploidy in relation to age of the female donor by studying the processes of meiosis and embryo development.

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

  The applicant is optimising procedures for vitrification and also developing trophectoderm biopsies for PGD (Pre-implantation Genetic Diagnosis). Their investigations into the problems encountered during meiosis and early embryo development associated with donor age should help older women to produce viable offspring.

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

  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 applicant is optimising methods for performing trophectoderm biopsy instead of blastomere biopsy as a means to diagnose genetic abnormality. This has the advantage that more cells are available for the tests, so are more likely to produce valid information, which may include detection of defective mtDNA.

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

  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 human embryos.

1.15. The committee was satisfied that none of the proposed activities are prohibited by the HFE Act 1990 (as amended).

1.16. The committee was satisfied that the research licence renewal would not apply to more than one research project.

1.17. The committee noted that the use of human embryos is necessary for this research project for the following reason:

- Use of human embryos

  The applicant has been using composite embryos for their research for some considerable time, and their use will address the purpose of identifying and eliminating problems associated with mitochondrial disease. Animal models are not suitable for such research.

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

1.19. The committee noted 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.

1.20. The committee was satisfied that the PR possesses the required qualifications and experience and that the character of the PR is such as is required for supervision of the licensed activities. It was further satisfied that the PR will discharge their duties under section 17 of the HFE Act 1990 (as amended). The committee noted that the inspectorate was satisfied that the PR had satisfactorily completed the PR entry programme.

1.21. The committee was satisfied that the premises and facilities are suitable for the conduct of the licensed activity applied for.

2. Decision

2.1. The committee agreed to renew the licence for research project R0152 at centre 0017, entitled 'Towards improving assisted reproductive technologies for the treatment of infertility and prevention of disease', with no additional conditions for a period of three years with the following activities:

- creation of embryos in vitro
- 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 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.

2.2. The committee was generally satisfied with the information provided to potential research participants . However, the committee queried the provision of information on creating stem cell lines following the generation of embryos using somatic nuclear transfer (e.g. from skin cells), since this did not seem to be relevant to this research project and might be confusing to potential participants. The committee also noticed a lack of consistency between the individual participant information sheets, for example, concerning the amount of detail given on the disadvantages and risks of taking part in the research. The committee encourage the centre to review the information sheets information provided to potential research participants to ensure all participants are provided with sufficiently detailed, relevant and accurate information.

3. Chair's signature

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

Signature

Name

Andy Greenfield

Date

23 May 2017

Research renewal inspection report

Purpose of this inspection report

We license and monitor 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. Our Licence Committee 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 inspection: 23 January 2017

Purpose of inspection: Renewal of a research licence

Inspection details: This report covers the performance of the centre since the last inspection, findings from the inspection, and communications received from the centre.

Inspectors: Dr Douglas Gray (lead), Mrs Lesley Brown, Mrs Gill Walsh

Date of Licence Committee: 4 May 2017

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	Dr Meena Choudhary
Licence holder	Prof Mary Herbert
Treatment centres donating to this research project	Queen Elizabeth Hospital Gateshead, 0170 Newcastle Fertility Centre at LIFE, 0017
Date licence issued	01/08/2014
Licence expiry date	31/07/2017
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 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. There are no additional conditions on the licence. The centre was last inspected for their research licence in 2015. In November 2014, Dr Alison Murdoch stood down as the PR and Dr Meena Choudhary was appointed.

The research project has been amended twice during the current period to reflect updates in the research objectives and the number of embryos expected to be created and/or used. These amendments have been presented to the 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 team at Newcastle continue to develop and refine their research, and additions to the research project compared with the last renewal include the potential use of TALENS (a 'gene editing' technique; described in the methodology section of the application form).

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). This collaboration continues. The licence for R0152 at centre 0246 will be renewed separately to this application in 2018.

Summary for licensing decision:

Taking into account the essential requirements set out in the Human Fertilisation and Embryology (HFE) Act 1990 (as amended), the HFE Act 2008 and our 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 us 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
- use of embryos
- storage of embryos
- storage of gametes[1]

The proposed 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.

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 HFE 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 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] The HFEA research licence renewal application form does not allow applicants to select the activity 'storage of gametes'; the PR has confirmed that this activity is however required and is not exempt under the HF&E (Special Exemption) Regulations, 2009 because stored gametes will be used to create 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 HFE Act 1990 (as amended) as follows:

- **increasing knowledge about serious disease or other serious medical conditions**

The PR states: 'The project is increasing our knowledge about the transmission of abnormal mitochondria during preimplantation human development.'

The peer reviewer agrees: 'The proposed research addresses the problems associated primarily with mitochondrial diseases and mitochondrial abnormalities arising with advanced maternal age. The applicants propose to continue their research in characterizing the problems that lead to mitochondrial disease in the following ways:
1. Studying the transmission of abnormal mitochondria during preimplantation development
2. Observing the origins of aneuploidy/chromosome misalignment in embryos from aged females
3. Investigating the process of DNA packaging and mitochondrial development during gametogenesis
4. Optimising methods of detection of mitochondria during cleavage of embryos
5. Profiling gene expression and chromosome constitution in various regions of the embryo.'

- **developing treatments for serious disease or other serious medical conditions**

The PR states: 'The research is leading to the development of a treatment that has the potential to significantly reduce the risk of transmission of abnormal mitochondria to the embryo and subsequent child.'

The peer reviewer agrees: 'The applicants plan to develop methods to reduce the carryover of karyoplast mtDNA in order to eliminate contamination of host oocytes with donor mitochondria that are defective. They propose to explore possibilities of transferring the second polar body or isolated spindles of affected donor eggs into enucleated normal host oocytes to reduce risk of diseased mtDNA transfer. Also, they will attempt to utilize methods of inhibiting expansion of contaminating mtDNA, which they have found in 20% of the ESC lines previously derived from embryos created using donor nuclei. More ambitious proposals are to recruit mitophagy to reduce the contribution of donor mtDNA, develop methods to induce mitochondrial clustering away from the chromosomes, or engineer a means to inhibit replication of donor mtDNA.'

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

The PR states: 'We are investigating the origin of aneuploidy by studying the ageing process during meiosis in eggs and during early embryo development.'

The peer reviewer agrees: 'The proposed research will investigate the occurrence of aneuploidy in relation to age of the donor female by observing the processes of meiosis and embryo development.'

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

The PR states: 'By understanding how the DNA is packaged within the chromosomes of the eggs and the basic mitochondrial biology of gametogenesis and early embryogenesis, we hope to look at ways to improve outcomes for older women in treatment of infertility. Further optimisation of vitrification techniques for in vitro maturation of immature eggs would also help in advancing this further.'

The peer reviewer agrees: ‘The applicants are optimising procedures for vitrification and also developing trophectoderm biopsies for PGD. Their investigations into the problems encountered during meiosis and early embryo development associated with donor age should help older women to produce viable offspring.’

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

The PR states: ‘Our studies of oocyte aneuploidy are relevant to understanding the causes of miscarriage.’

The peer reviewer agrees: ‘The proposed research will investigate the sources of aneuploidy.’

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

The PR states: ‘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.’

The peer reviewer agrees: ‘The applicants are developing methods for performing trophectoderm biopsy instead of blastomere biopsy as a means to diagnose genetic abnormality. This has the advantage that more cells are available for the tests, so more likely to produce valid information, which may include detection of defective mtDNA.’

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

The PR states: ‘Prior to translation of the methods being developed in this project into clinical practice is it 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.’

The peer reviewer agrees: ‘The control embryos to be used in this project will add information about normal embryonic development.’

Prohibited research activities:
The activities to be licensed are not prohibited by the HFE 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.

Creation and use of embryos:
The creation and/or use of human embryos is considered necessary. This is based on the application, and the following comments by the peer reviewer:

'The consequences of contamination of embryos with defective mtDNA cannot be investigated without allowing development of human embryos. Also, assessing the extent of aneuploidy requires whole embryos.' 'A major component of the proposed research is to develop and optimise methods for reducing contamination of embryos with defective donor mtDNA. This requires assembly of composite embryos from different individuals, including two female and one male donor.'

PR considerations:
The PR is suitable and has discharged their duty under Section 17 of the HFE Act 1990 (as amended).

Premises:
The premises are suitable.

Recommendation:
The Licence Committee is asked to note that at the time of the inspection there were no recommendations. 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
- 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 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 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 progress:

'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.'

On the progress made by the centre, the peer reviewer states: 'The applicants have already made progress in addressing the purpose of the current proposal. They have published several important papers.'

Donation and use of embryos:

Since the last inspection, the centre reported the donation, creation and use of the following:

Embryos donated:

Date (Jan to Dec)	Remaining from previous year (frozen)	Received (fresh/frozen)	Not suitable for use (fresh/frozen)	Used (fresh/frozen)
2015	279	284/83	15/0	269/141
2016	221	119/116	10/4	109/49

Embryos created:

Date (Jan to Dec)	Remaining from previous year (frozen)	Created (fresh)	Not suitable for use (fresh/frozen)	Used (fresh/frozen)
2015	0	183	0/0	183/0
2016	0	8	0/0	8/0

The peer reviewer agrees that the number of embryos used is justified, stating: 'The applicants have made a major contribution to the field and have disseminated their findings in high profile publications.'

In their application, the PR proposes to use:

	Year 1 (2017-18)	Year 2 (2018-19)	Year 3 (2019-20)
Fresh eggs	300	300	300
Frozen eggs	50	50	50
Failed to fertilise	200	200	200
Fresh embryos	250	250	250
Frozen embryos	150	150	150
Created embryos	250	250	250

The peer reviewer agrees that the number of embryos to be used is justified, stating: 'This group leads the field in human embryo research, particularly the construction of 3 parent embryos. They have the ideal expertise to produce beneficial knowledge and technology.'

Peer reviewer comments:

A peer review was sought and extracts are presented throughout this report. Overall, the peer reviewer was supportive of the application and considered it would be appropriate to carry out the proposed research.

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.

Observations during the inspection provided assurance that the special status of the human embryo 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 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 beyond 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). During 2015, 12 stem cells lines were derived all using nuclear transfer techniques which are all in the process of being banked. No stem cells were created during 2016.

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

Before 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. Observations and discussion during the inspection provided assurance that:

- Before 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 before 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.

Information and consent forms used in the recruitment of donors has been submitted to the committee alongside this report.

Consent for storage

Stored gametes and embryos are obtained only from centres to which a HFEA licence or third party agreement applies (RLC R31, R32, R33).

No gametes or embryos are kept in storage for longer than the statutory storage period (RLC R35, R36, R38 and R39), or the period specified in a patients' consent if less than the statutory storage period. This was assessed by reviewing the centre's record of stored gametes and embryos. A bring-forward system is maintained, ensuring gametes and embryos are stored only within the statutory storage period or the patients' consent.

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

The premises and facilities are secure, clean, well maintained and are suitable for carrying out the licensed activities (RLC R10).

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.

A review of embryo storage and usage records indicate that proper records are maintained (RLC R13 and R15). These records are in a form that prevents the removal of data (RLC R16). If embryos are to be transferred to another centre, the required information is also sent with them (RLC R17).

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

What they could do better.

Nothing noted.

Principle:

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

What we inspected against:

Incidents; RLC R40.

What the centre does well.

Processes are in place to detect, report to us 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:

HFE Act 1990 (as amended), Schedule 2 (3(5) and 3A).

What the centre does well.

The research project has been approved by the North East - Newcastle & North Tyneside 1 Research 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 HFE 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 us 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; HFE Act 1990 (as amended) Section 16 & 17, RLC R8, R9.

What the centre does well.

Licensing

Inspection of the licensed premises indicated that all licensed research activities are performed only on the premises specified on the licence and 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 HFE 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 HFE 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 (HFE Act 1990 (as amended), Section 16 (2)(ca)). The PR has successfully completed our PR entry programme. The Inspection team considered that the PR has fulfilled her responsibilities under Section 17 of the HFE Act 1990 (as amended).

What they could do better.

Nothing noted.

Section 4: Monitoring of the centre's performance

Following an interim inspection in 2015, recommendations for improvement were made in relation to one major and one 'other' area or practice. The PR provided evidence that both recommendations were 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 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 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 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' 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 require improvement

‘Other’ areas of practice that require improvement are any areas 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
None			

The PR has confirmed they agree with the report and have no further comments.

Additional information from the person responsible