Research Licence Renewal Inspection Report

Project Title	To Develop PGD for Mitochondrial DNA Disease
Centre Name	Oxford Fertility Unit
Centre Number	0035
Research licence Number	R0149
Centre Address	Level 4, Women's Centre, John Radcliffe Hospital, Oxford OX3 9DU
Treatment centres donating to this research project	0035 – Oxford Fertility Unit
Inspection date	21st May 2009
Licence Committee Date	TBA
Inspector(s)	Andrew Leonard Paula Nolan
Fee Paid - date	Fee paid
Person Responsible	Dr Karen Turner
Nominal Licensee	Mr Stephen Kennedy
Licence expiry date	31/08/2009

About the Inspection:
The purpose of the inspection is to ensure that research is carried out in compliance with the HF&E Act 1990, Code of Practice, licence conditions and directions and that progress is made towards achieving the stated aims of the project. The report is used to summarise the findings of the inspection highlighting areas of firm compliance and good practice, as well as areas where improvement may be required to meet regulatory standards. It is primarily written for the Licence Committee who makes the decision about the centre's licence renewal application. The report is also available to patients and the public following the Licence Committee meeting.

This report covers the period between 25th June 2008 and 20th May 2009.

Brief Description of the Project

Project R0149 is housed within a research laboratory in the Oxford Fertility Unit (Centre 0035), on level 4 of the Women's Centre, John Radcliffe Hospital. Genetic analysis of biopsied blastomeres, but no licensed activities with viable embryos, is carried out in research laboratories run by Reprogenetics, within an Oxford University Academic department 2 miles from Centre 0035. All embryos used in the research project between 1st March 2008 and 1st April 2009 were derived from Centre 0035 (90 fresh and 7 frozen embryos donated, 34 fresh and 2 frozen embryos were suitable for use on the project (ie biopsy and isolation of blastomeres for genetic analysis). The lay summary for the project states:

'Mitochondrial diseases affect about 1 in 10,000 people in the UK. Examples include Maternally inherited Leigh Syndrome (MILS), Myoclonic epilepsy and ragged red fibres (MERRF) syndrome, Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS), and Pearson syndrome. The diseases may involve any parts of the body that have a high energy demand (such as brain, heart muscle and liver), because mitochondria are the "power houses" in cells. Some women are carriers of defective mitochondrial genes. This means that they carry both normal and damaged mitochondrial genes, and can pass these devastating conditions on to any children they may have. It is particularly difficult to advise these women about the size of their individual risk, because the rules governing transmission of mitochondrial genes are not well understood.

The aim of this research is to establish the techniques for identifying defects in mitochondrial DNA in human embryos created by IVF to allow the selection of non-affected embryos for transfer to the mother. This technique is referred to as pre-implantation genetic diagnosis (PGD). We have demonstrated that we can successfully sample embryos and have started to develop our analysis of their mitochondrial genes. So far we have identified one woman in whom we were able to assess the way that the mitochondrial genes are transmitted. Preliminary results from this woman's embryos were generally encouraging. Further work is required to provide basic scientific information and enable us to determine whether PGD will be useful in certain mtDNA diseases.'

Summary for Licence Committee

The Centre has held a licence for project R0149 since 2004. The Centre also has research licences for two other projects (R0111 and R0143). The licensed research activities on licence R0149 include research on human embryos and storage of licensed material; the licence is due to expire on 31st August 2009. The licence renewal application states that the Centre do not wish to add further activities to licence R0149. The research licence renewal is requested for the same defined purposes as the existing licence: developing methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation.

All embryos used in the research project between 1st March 2008 and 1st April 2009 were derived from Centre 0035 (90 fresh and 7 frozen embryos donated, 34 fresh and 2 frozen embryos were suitable for use on the project (ie biopsy and isolation of blastomeres for genetic analysis). Embryo usage in the last year was as proposed in the progress report last year (100 fresh and 0 frozen embryos), except for the use of a small number of frozen embryos which became available.

The inspectorate were satisfied that research at centre 0035 is well organised and is carried out in a professional manner which complies in nearly all areas with the Code of Practice, 7th edition. The premises, donation processes, consenting and scientific practices were all compliant. The Person Responsible (PR) is appropriately experienced and has completed the research PR Entry Programme.

The inspectorate note the change in Nominal Licencee (NL) detailed in the renewal application and consider the proposed NL to be appropriately qualified and experienced. A copy of Section 13 of the application form signed by the PR and the proposed Nominal Licensee has been sent by the centre.

There were only two areas of concern:

1) Formal research group meetings should be held every six months, according to centre staff. Minutes were provided for meetings in January 2008, May 2008, then April 2009. It would appear that a meeting was missed in November 2008. It is recommended that the Centre maintain the 6 month periodicity of these meetings, to ensure continuing management and coordination between the research and clinical activities.

2) The information sheets 'research and training projects using surplus eggs and embryos, Pt Info 2 v.2' and 'Donating frozen embryos to research, v.1' are the only information/consent forms provided to patients consenting to donation of fresh and frozen embryos, respectively, to project R0149. Both sheets were reviewed and were found to not include several pieces of information required by the Code of Practice, (e.g. Standards S.8.2.1 and S.8.3.2, and Guidance G.5.13.1 (a,e,f), as listed in Section 4 in this report. This issue had been raised at the interim inspection in June 2008 and it was recommended that the PR should review these documents and the verbal information provided by the Research Nurse, to ensure patients receive verbally and/or in writing, all information required by the Code of Practice, 7th edition. It was noted on this inspection that these non-compliant documents were still in use.

Updated information sheets have not been prepared because Centre 0035 still have a considerable number of printed copies of the non-compliant versions. In addition, the PR

was expecting to have to update the information sheets further, since the Centre was due to move to new premises in late 2008, then early, then mid 2009. The delay in the move has delayed the update of the patient information provided. The move is now scheduled for late August 2009. Drafts of updated patient information/consent forms were provided on inspection. These drafts were reviewed by the inspectorate and were considered to be compliant with the requirements of the HFEA Code of Practice, 7th edition.

The inspectorate recognise that the revised information/consent forms will need further updating in the near future when the Centre moves to new premises. It is also noted that these revised versions are compliant with the requirements of the HFEA Code of Practice, 7th edition, whereas the versions currently in use at the Centre are not. To ensure patient information and consent forms are compliant, it is recommended that the PR immediately brings the revised versions of the patient information/consent forms into use at the Centre.

It was noted that embryos are biopsied in project R0149 and this occurs using facilities within the clinical embryology laboratory at Centre 0035, adjacent to the embryo culture laboratory. The embryologist associated with the research project who performs the research biopsies, also works in the clinical embryology laboratory. The PR was advised that separation between treatment and research activities should occur to comply with HFEA Code of Practice, 7th edition, Standard S.8.4.1. This issue was discussed with the PR on inspection and she considered that appropriate separation between research and treatment was achieved. For example, the nurses at the donating centre take all patient consents for research and the embryologist has no role in the process, nor advises patients regarding their clinical treatment. This is accepted by the inspectorate but it is recommended that the PR is vigilant in ensuring that working practices continue to maintain the separation between treatment and research activities.

Peer review of the project renewal application recommended acceptance of the application in a revised form.

A completed project renewal application form has been submitted to the HFEA and the licence renewal fee has been paid.

The inspectorate recommend renewal of research licence R0149 for a 3 year period.

Proposed licence variations

None

Report of Inspection findings

1. **Organisation**

Desired Outcome: The research is well-organised and managed and complies with the requirements of the HFE Act.

Summary of findings from inspection
Evidence of:

- Leadership and management
- Staffing
- Funding
- Organisation of the centre
- Resource management
- Research governance

Staff

Principal investigators	1
Scientists	4
Laboratory technicians	0
Support staff (receptionists, record managers, quality and risk managers etc)	0

Highlighted areas of firm compliance
The treatment and storage and research licences at Centre 0035 have different PRs and clinical and research practices are separated. There are two areas of close approach between clinical and research activities. One occurs because embryos in storage for treatment, for which consent for research use is then obtained, remain stored in the same dewar position until used in research. Removal of embryos from dewars for research use is appropriately witnessed and recorded in patient records. A second area of close approach is discussed below. The Person Responsible (PR) is a Consultant Embryologist and the Laboratory Manager at Centre 0035, while the Nominal Licensee is the Head of the Academic Obstetrics and Gynaecology Department, Oxford University, based on the floor below Centre 0035. The PR has been in post since before the current licence was issued, has completed the PR entry programme and is an external advisor to the HFEA. The inspectorate note the change in Nominal Licencee (NL) detailed in the renewal application and consider the proposed NL to be appropriately qualified and experienced. An academic professor (of mitochondrial genetics) is the effective research lead. Discussions with the PR and the research lead, and inspection of the premises, indicated that the project is appropriately led and managed. The project appeared to be appropriately staffed. An experienced molecular genetics lecturer (the proposed NL), a DPhil student molecular geneticist and an embryologist have joined the project. A senior embryologist researcher has left the project and the research nurse at Centre 0035 has also not been able to work on the project since September 2008 due to funding limitations. The research nurse briefed nursing staff regarding the project prior to leaving it, so that they are informed and able to provide information to patients about it. Furthermore, the research nurse is still employed at the Centre and can give advice and

information if she is available. The research nurse post on this project may be reactivated if funding becomes available.

The induction procedure for research staff follows the Oxford University requirements and was considered appropriate. Each element of induction requires sign-off such that a record of induction is prepared and placed in the staff record. The PR said that research staff training meets the requirements of Oxford University and involves attendance at conferences and internal and external training programmes. Weekly departmental seminars are also held and researchers are encouraged to attend.

Funding is in place from The British Research Council and from internal funds within Centre 0035. It does not cover all aspects of the research and further funds are being sought.

While a formal organisational chart is not present, nor is it a requirement, the PR described an appropriate organisation structure for research at the centre. Staff changes are listed in the renewal application and appropriate CVs for new staff were provided with the application. All research staff with access to licensed material and patient details are on the research licence and also on the treatment and storage licence.

A formal research meeting between the PR and all researchers is held twice a year, at which research progress and all matters relating to the project are discussed and minuted. The inspection team were provided with minutes of these meetings in 2008/9 and noted that discussion of regulatory issues and action points had been minuted. The lead investigators and the researchers meet on a weekly basis. This provides an opportunity for cascading of HFEA Alerts and other essential information; e-mail is also used for cascading important information.

Contact between the researchers and clinical staff at Centre 0035 is as frequent as required for effective coordination of embryo supply and research activities. Communication is facilitated by the PR being the Head of Embryology at the Centre and licensed research taking place in a laboratory within Centre 0035. The researchers provide seminars at monthly all staff meetings, as a means to feedback research progress to clinical staff.

The Academic Department of Obstetrics and Gynaecology, is a component of Oxford University, which provides the management structure within which the research licence operates. The University supplies a full range of support services, e.g. health and safety, finance, personnel and facilities management. Thus the University ensures the licensed research premises are cleaned and maintained, and compliant with Health and Safety legislation.

Laboratory standard operating procedures (SOPs) related to embryo donation and culture were provided to the inspectorate and were considered fit for purpose. SOPs related to downstream laboratory analyses of biopsied blastomeres were not provided, as they are not used to work on viable embryos. The principal investigator and the PhD student discussed the project and made clear that the methods to analyse mitochondrial genetic analysis are in development.

At the last inspection in June 2008, it was recommended that a procedure for reporting serious adverse events to HFEA should be developed to ensure compliance with General

Licence Condition A.4.1. At this inspection, the inspectorate was advised that the research team now have this procedure, provided by the clinical staff at Centre 0035, and it was displayed on the culture laboratory wall. This subject was seen to be minuted in the last research group meeting minutes from April 2009.

Issues for consideration

1) Embryos are biopsied in project R0149 and this occurs using facilities within the clinical embryology laboratory at Centre 0035, adjacent to the embryo culture laboratory. The embryologist associated with the research project who performs the research biopsies, also works in the clinical embryology laboratory. The PR was advised that separation between treatment and research activities should occur to comply with HFEA Code of Practice, 7th edition, Standard S.8.4.1 'Where embryos are used in research, the Centre shall ensure that clinical and research roles are separated, so that individuals involved in advising patients regarding clinical decisions about their licensed treatment, are not involved in the research project to which patients are considering donating embryos'. This issue was discussed with the PR on inspection and she considered that separation between research and treatment was achieved. For example, the nurses at the donating centre take all patient consents for research and the embryologist has no role in the process, nor advises patients regarding their clinical treatment. This is accepted by the inspectorate but it is recommended that the PR is vigilant in ensuring that working practices within the clinical embryology laboratories continue to maintain the separation between treatment and research activities.

2) Formal research group meetings should be held every six months, according to centre staff. Minutes were provided for meetings in January 2008, May 2008, then April 2009. It would appear that a meeting was missed in November 2008. It is recommended that the Centre maintain the 6 month periodicity of these meetings, to ensure continuing management and coordination between the research and clinical activities.

Executive recommendations for Licence Committee

The Licence Committee is asked to endorse the recommendation made in relation to maintaining the six monthly periodicity of the formal research group meetings, discussed above.

Areas not covered in by this inspection

All covered

2. Premises and equipment

Desired Outcome: The premises and equipment are safe, secure and suitable for their purpose.

Summary of findings from inspection:

- Suitability of premises
- Storage facilities
- Safety of equipment
- Servicing and maintenance of equipment

Highlighted areas of firm compliance
The premises were well organised, clean and tidy on the day of inspection. The research premises comprise a research-dedicated laboratory within Centre 0035, accessed via the andrology laboratory, on Level 4 of the Women's Unit, John Radcliffe Hospital. This laboratory contains a class II air flow cabinet, incubator and inverted microscope, and is used for embryo culture. Embryo biopsies are performed in the adjacent clinical embryology laboratory. These facilities were seen to be appropriately maintained at the last renewal inspection in June 2008. The culture laboratory is secured by a numerical key pad lock, the code for which is restricted to licensed staff and changed when staff leave, thus at least annually. The clinical embryology laboratory is also securely locked. Both laboratories are risk assessed annually by Oxford University health and safety staff. Detailed health and safety documentation for research activities was provided to the inspectorate and all research procedures have been risk assessed. All licensed material and research records are confined to the research culture laboratory. There are no dedicated research embryo storage facilities, and all such embryos are stored in dewars used for clinical storage, until required on the research project. These storage facilities were considered fit for purpose during the inspection of the treatment and storage licence, and are secure and equipped with a low oxygen monitor, fan extractor with boost connection to the low oxygen monitor, and low level nitrogen alarms. A procedure for responding to activation of the low oxygen alarm is in place. Research staff must receive training before using liquid nitrogen and the storage facilities at Centre 0035. A documented system for equipment maintenance/servicing is in place; the laboratory head is the designated person responsible for the maintenance of equipment. Inspection of some items of equipment indicated they were all within servicing intervals and servicing documentation was provided. All electrical equipment inspected also evidence of portable electrical testing certification and the PR said that such testing was up to date. The centre ensures that appropriate training is provided to all staff using specialist equipment to enhance safety and prevent equipment damage.
Issues for consideration
There is an anticipated change to new premises in October 2009, these being in a renovated and refurbished building on the edge of Oxford. The Oxford Fertility Unit will move to the same premises.

Executive recommendations for Licence Committee
NONE
Areas not covered in by this inspection
All covered

3. Donation of material

Desired outcome: Donors are recruited appropriately and any research carried out on their embryos is in accordance with their consent.

Summary of findings from inspection:

- Recruitment of donors
- Ensuring prospective donors have access to further guidance
- Ensuring prospective donors have time to consider donation properly
- Ensuring patient consent is not breached
- Donor and patient records
- Prevention of coercion of prospective donors

Highlighted areas of firm compliance
Embryo donors are recruited to the Centre's three licensed research projects (R0111, R0143 and R0149) by a common research donor recruitment procedure. Embryos are obtained for research on project R0149 from within Centre 0035 only. All couples treated at Centre 0035 are provided research information with their treatment information when they first visit the Centre at an initial orientation open evening. When HFEA consent forms are signed at a consenting consultation some time afterwards, if patients indicate they wish to consent to research donation their consent is taken on the HFEA form. Research information is then discussed, if required, and research consents are taken by nursing staff. If further information or time to consider is required, a further consultation is arranged at which research is discussed and consent taken if patients so wish. The research consent form is detailed and allows them to specify the projects to which they consent to donate. Research consents are normally collected before the start of treatment and well before egg collection. Cryopreserved donated embryos from Centres 0035 are also used. Patients with frozen embryos are annually asked to confirm storage arrangements for the forthcoming year. If they express an interest in donating to research they are sent a 'donating frozen embryos to research' information sheet, which describes the project, and a consent form. The consent forms are signed by the patients and returned to the Centre. Defined processes are in place to prevent a breach of patients' research consent. Prior to egg collection, patient notes are reviewed for all consents including those for research and a note taken of research consented patients. After transfer of the best one or two embryos, the Centre has a specific procedure which defines the quality of embryos frozen for treatment; any remaining embryos are available for research. At this point research consents are verified in the patient notes, then checked again and witnessed by another clinical embryologist. Research consented embryos are then passed to the researchers with an affidavit from the clinical embryologists detailing the consents applying. The research donation is logged by the clinical embryologists in a book (detailing patient name; centre number; projects consented to; date of donation to research; developmental stage with length in culture post-fertilisation). Embryos are then taken to the research culture laboratory and placed in the incubator to equilibrate. Soon thereafter embryos are anonymised in that they are transferred to a dish labelled only with a unique research code. They are also allocated to a research project, depending on the consents provided and on which researchers are available according to the donation rota, and logged in the anonymisation book (detailing Centre identification number;

research number; projects consented to; date of arrival; project allocated; researcher responsible). The documenting of the Centre identification number allows back-tracking from research records to patient records; patient records remain in Centre 0035 at all times.

The researchers have not carried out a specific audit of stored research material as this is done in conjunction with the treatment and storage dewar audit. Cryopreserved embryos are also rapidly used after transfer to research, normally within days. Embryos remain catalogued within the treatment and storage dewar logs and subject to the bring-forward system used in the clinical embryology laboratories to prevent storage beyond the consented storage period. If research-consented embryos approach the end of their consented storage, the clinical embryologists inform the researchers who arrange for their thaw and use in research.

A research culture sheet labelled with the research number and culture dish location is maintained for each embryo, on which daily observations and culture activities are recorded. Culture sheets remain in the licensed culture laboratory at all times.

The donation procedures used by the centre would seem to prevent the possibility of coercion of research donors and no complaints have been received regarding this issue. No evidence was observed to indicate that the Centre offer inducements to donate.

At the last inspection it was noted that consent for research had in some cases been collected on the day of embryo transfer. On this inspection, the PR made clear that such consenting was not routine, and the consenting procedure ensures that patients normally sign research consent before treatment begins. The PR said however that if a patient couple on the day of embryo transfer said they wished to donate to research, then to concur with patient wishes, consents would be signed. This would though only occur if patients approached Centre staff about research donation, it was considered that the patients were competent to sign the consent form, and the patients had been in receipt of the patient research information for a reasonable period of time and understood what they were doing.

Issues for consideration
None
Executive recommendations for Licence Committee
None
Areas not covered in by this inspection
All covered

4. Patient information and consents

Desired outcome: Patients are provided with appropriate information which allows them to give informed consent.

Summary of findings from inspection:

- Patient information
- Consent forms
- Patient information for projects deriving embryonic stem cells
- Consent forms for projects deriving embryonic stem cells

Results of consent audit
Six sets of patient records were reviewed for research consents. All but one contained appropriately completed specific research consent forms, as well as HFEA research consents. In one case, the female partner but not the male partner had signed to consent for project R0143, whereas both had consented to projects R0111 and R0149. This discrepancy had been detected by the embryologists reviewing consents before egg collection, and noted in the patient record. The embryos were subsequently used for project R0111, which was consented to by both patients. This indicates that the Centre's procedure for reviewing patient consents, and using embryos in research according to those consents, is robust. No consents were seen to have been taken on the day of embryo transfer. Indeed all the consents appeared to have been taken before the start of stimulation in the cycle of embryo donation. There was no evidence that patient consents had been breached in any way.
Highlighted areas of firm compliance
The patient research information/consent form, which describes and collects patient consent for project R0149, as well as the other research projects, was reviewed (Research and training projects using surplus eggs and embryos, Pt Info 2 v.2), as was the information/consent form provided to patients with frozen embryos (Donating frozen embryos to research, v.1). These forms were well presented and broadly compliant with Code of Practice requirements regarding information provided to research donors, however some issues of content are raised below. Variable consents are possible as the consent form allows the provision of consent for each research project individually. Procedures within Centre 0035 prevent the use of embryos in research without patient consent.
Issues for consideration
The document 'research and training projects using surplus eggs and embryos, Pt Info 2 v.2' is the only information/consent sheet provided to patients consenting in Centre 0035 to the use of fresh embryos in project R0149. Likewise, the form 'Donating frozen embryos to research, v.1' is the only information/consent sheet provided to patients consenting in the Centre 0035 to the use of frozen embryos in project R0149. At the last inspection it was noted that these information sheets were non-compliant with the HFEA Code of Practice (e.g. Standards S.8.2.1 and S.8.3.2, and Guidance G.5.13.1 (a,e,f). It was accepted in the last inspection report that these information requirements may be satisfied verbally by the Research Nurse, albeit this was unlikely to happen for patients donating frozen embryo and the Research Nurse is now not employed on the project.

At this inspection it was observed that the same versions of the patient information/consent forms were still being used, despite them being non-compliant with the HFEA Code of Practice, 7th edition. This matter was discussed with the PR. Updated information sheets have not been printed because Centre 0035 still have a considerable number of printed copies of the non-compliant versions of the information sheets. In addition, the PR was expecting to have to update the documents further, since the Centre was due to move to new premises in late 2008, then early, then mid 2009. The delay in the move has delayed the update of the patient information/consent form documents. The move is now scheduled for late August 2009 and the inspectorate were provided with drafts of updated patient information/consent forms. These drafts were reviewed by the inspectorate and were considered to be compliant with the requirements of the HFEA Code of Practice, 7th edition.

The inspectorate recognise that the updated information/consent forms will need further updating in the near future when the Centre moves to new premises. It is also noted that the revised versions are compliant with the requirements of the HFEA Code of Practice, 7th edition, whereas the versions currently in use at the Centre are not. To ensure patient information and consent forms are compliant, it is recommended that the PR immediately brings the revised versions of the patient information/consent forms into use at the Centre.

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Executive recommendations for Licence Committee

The Licence Committee is asked to endorse the recommendation that the Centre immediately brings into use the revised versions of the patient research/consenting information forms, the contents of which are compliant with the research information requirements of the HFEA Code of Practice, 7th edition.

Areas not covered in by this inspection

All covered

5. Scientific practice R0149, To Develop PGD for Mitochondrial DNA Disease

Desired outcome: Research is carried out in accordance with licence conditions and makes progress towards achieving stated aims

Summary of:

- Peer review

Summary
The current licensed research activities include research on human embryos and storage of licensed material. The Centre do not wish to add further activities but state that downstream analytical methods used include: PCR analysis or research involving staining of chromosomes or specific proteins. The research licence has been granted for the following defined purposes: developing methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation. When embryos are accepted into project R0149, they are cultured in the research culture laboratory until day 6 post-fertilisation, at which point they are discarded if non-viable or used in research. If allocated to project R0149, they are subjected to embryo biopsy to isolate blastomeres for molecular analysis of mitochondrial genetics. These blastomeres are currently being stored within Centre 0035 until molecular analytical techniques have been developed. This is currently in progress using cell line models of mitochondrial disease, as was evidenced by a seminar provided to the inspectorate by the lead researcher and the DPhil student. Normal working practices mean that no embryos are cultured for more than 8 days post-fertilisation before being rendered non-viable. The centre also has a procedure which states that no embryos should be cultured for 14 days post-fertilisation. This is also discussed in patient information *Research project lay summary:* 'Mitochondrial diseases affect about 1 in 10,000 people in the UK. Examples include Maternally inherited Leigh Syndrome (MILS), Myoclonic epilepsy and ragged red fibres (MERRF) syndrome, Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS), and Pearson syndrome. The diseases may involve any parts of the body that have a high energy demand (such as brain, heart muscle and liver), because mitochondria are the "power houses" in cells. Some women are carriers of defective mitochondrial genes. This means that they carry both normal and damaged mitochondrial genes, and can pass these devastating conditions on to any children they may have. It is particularly difficult to advise these women about the size of their individual risk, because the rules governing transmission of mitochondrial genes are not well understood. The aim of this research is to establish the techniques for identifying defects in mitochondrial DNA in human embryos created by IVF to allow the selection of non-affected embryos for transfer to the mother. This technique is referred to as pre-implantation genetic diagnosis (PGD). We have demonstrated that we can successfully sample embryos and have started to develop our analysis of their mitochondrial genes. So far we have identified one woman in whom we were able to assess the way that the mitochondrial genes are transmitted. Preliminary results from this woman's embryos were generally encouraging. Further work is

required to provide basic scientific information and enable us to determine whether PGD will be useful in certain mtDNA diseases.'

Usage and expected usage in next year:
All embryos used in the research project between 1st March 2008 and 1st April 2009 were derived from Centre 0035 (90 fresh and 7 frozen embryos donated, 34 fresh and 2 frozen embryos were suitable for use on the project (ie biopsy and isolation of blastomeres for genetic analysis). Embryo usage in the last year was as proposed in the progress report last year (100 fresh and 0 frozen embryos), except for the use of a small number of frozen embryos which became available. Usage next year is estimated at 100 fresh embryos only.

Summary of audit of stored and biopsied material

No licensed materials were in store on the day of inspection so no audit was performed.

Renewed project objectives

No changes to project aims and objectives

Summary of research undertaken

A) How the work undertaken relates to the objectives.
We have designed and begun optimisation of a number of methods intended to quantify levels of mutant mitochondria in single cells. These methods could potentially be used for clinical embryo testing in the future, however, at present we are still accumulating validation data using cell lines from affected individuals. We anticipate beginning analysis of the embryo cells collected as part of this project in the near future. An application for permission to perform clinical testing of embryos will be submitted to the HFEA once sufficient preclinical validation has been completed.

To provide more detail on the methods being used; there are several strategies for the diagnosis of mtDNA mutations being developed in parallel. At this point, it is not yet clear which method will prove to be the most reliable for this purpose.

The first strategy under development involves multiplex nested PCR. Single cells are placed in 0.2 ml microcentrifuge tubes using conventional methods of micromanipulation and lysed using proteinase K. A first round of PCR facilitates the simultaneous amplification of three distinct genetic loci. The amplified DNA fragments include the following: a fragment encompassing the mutation site in the mtDNA; a fragment containing a hypervariable polymorphism on chromosome 21. A limited number of PCR cycles are undertaken, providing a modest amplification of each DNA fragment.

After completion of the initial amplification a second 'nested' round of PCR is undertaken, in which each of the fragments is amplified independently. Three aliquots (0.5 ul) are taken from the first PCR. One aliquot is used to initiate the amplification of the hypervariable chromosome 21 polymorphism using a fluorescent (FAM) labeled primer. The products of this reaction are analysed using capillary electrophoresis. This assists in the detection of DNA contaminants and some forms of aneuploidy. Embryos are expected to have a combination of alleles derived from the parents (one allele from the father and one from the mother). If non-parental alleles are detected the most likely explanation is the presence of a contaminant. If extra parental alleles are detected (e.g. 2 alleles from one parent plus one allele from the

other), this may be explaned by contamination with parental DNA or aneuploidy for the chromosome upon which the polymorphism is situated, in this case chromosome 21. Not all aneuploidies affecting chromosome 21 can be detected in this way, but a high proportion of meiosis I errors affecting chromosome 21 are detectable.

The other two aliquots taken from the first PCR are use to amplify the fragment of mtDNA containing the mutation in two separate reactions using real-time PCR. One reaction utilises mutation specific primers, which only generate a product in the presence of the mutation, while the second reaction employs primers specific to the normal mitochondial DNA sequence, only producing products when normal mtDNAs are present. The quantity of DNA produced in each reaction is compared to standards containing known quantities of mutant or normal DNA fragments, allowing precise quantification of the number of mutant and normal mtDNAs. We are investigating the use of SYBR green and sequence specific TaqMan probes for this purpose.

We have already completed optimisation of the first round of PCR at the single cell level and are close to completing optimisation of the nested amplifications too. The next step in the development of this method will be validation of the protocol using single cells from cell lines carrying known mutation loads and assessment of amplification efficiencies in the type of material used for PGD (polar bodies, blastomeres and trophectoderm biopsies).

The second protocol is similar to the first, but utilizes whole genome amplification prior to mutation specific real-time PCR testing. We are investigating several whole genome amplification (WGA) methods for this purpose, including multiple displacement amplification, Genomeplex, Picoplex and degenerate oligonucleotide primed PCR. We think it less likely that we will obtain accurate mitochondrial DNA quantification using this method. Nonetheless, we believe this is an approach worth exploring, as if it can be made to work it would allow mitochondrial mutation screening to be combined with comprehensive aneuploidy screening via microarray or comparative genomic hybridization (CGH). This would allow problems such as Down syndrome to be detected prior to embryo transfer, a significant issue since a significant number of PGD patients are of advanced reproductive age. We have already developed reliable methods for CGH and array-CGH using WGA products from single polar bodies, blastomeres and trophectoderm biopsies.

B) Research undertaken under the term of the current licence.
For reasons explained below, no genetic analysis has been undertaken using human material regulated by HFEA. However, methodologies have been designed and validation work has commenced using single cells isolated from cell lines. Work using the cells collected from the embryos will be initiated in the near future

C) Results during the term of the current licence
We anticipate that we will be using the first blastomeres in our assay towards the end of April 2009. For the time being analysis of single cells isolated from cell lines have yielded promising results.

The biopsied material will be used for critical aspects of PGD protocol development over the coming weeks and months. A preliminary version of the mitochondrial PGD protocol has already been designed and is currently undergoing validation using adult somatic cells in order not to waste the biopsied cells. It is anticipated that this protocol will have been

optimised in the next two or three weeks, after which we plan to move directly to the collected embryo material.

D) If progress was slower than anticipated, the reasons for this.
Blastomere analysis has not yet proceeded at present, because until October 2008 we did not have funding for suitable research staff. The mutation-specific techniques required have now been further developed for specific families who have been recruited. These techniques will be piloted on the control material stored under this license in the very near future. Initiation of the PGD programme has been aided by the appointment Dr Dagan Wells from Yale University to the Nuffield Department of Obstetrics & Gynaecology.

The unforeseen staffing and funding problems that hampered the initiation of this research have now been overcome. A DPhil student has been assigned full-time to achieving the aims of the project under supervision of the research leader and the NL. Support has been obtained from the BRC to cover consumables costs. Samples previously collected for this project have been carefully stored and will facilitate the progression of the study. The project will greatly benefit from having access to this bank of material.

The DPhil student, has experience with molecular biological methods, but nonetheless she will be closely supervised as discussed above. The other new member of staff involved in the project is an embryologist charged with the biopsy of cells for this project and who forms a vital link between the embryology laboratory and the scientists involved in the development of the PGD protocol. She will not be performing any unsupervised research and will not be responsible for directing any of the scientific studies. It is not anticipated that she will be involved in any bench work.

E) Publications which have arisen from work under the licence.
Poulton J, Kennedy S, Oakeshott P, Wells D. Preventing transmission of maternally inherited mitochondrial DNA diseases. British Medical Journal. 2009;338:345-9.

F. Future work

Renewed objectives
The objectives remain as originally outlined, with the addition that some forms of chromosome abnormality may also be detected in oocytes or cells biopsied from embryos.

Methods
In the main the methods remain as originally outlined. Evaluation of the proportion of abnormal mitochondria affected by mutation will be assessed using PCR-based methodologies, principally real-time quantitative PCR. Methods employing whole genome amplification and microarray analysis may also be evaluated for potential diagnostic use. Some of these methods are also likely to provide data on chromosome copy number. Thus far, cells collected for the purposes of this study have been fixed or prepared for DNA amplification. However, it is intended that in the future a subset of cells will be vitrified with the aim of preserving their viability. The collection of viable cells provides an opportunity to measure mitochondrial function and assess other diagnostically relevant aspects of mitochondrial biology.

Discussion (with particular reference to how the proposed studies relate to the objectives

stated fro this project). All of the planned studies are directly relevant to diagnosis of mitochondrial disease in human reproductive material (gametes and embryos), either via quantification of the proportion of mitochondrial DNAs affected by mutation or via analysis of mitochondrial function.
Peer review comments (if applicable)
The peer reviewer initially expressed reservations regarding the application. The application was subsequently re-drafted and represented to the HFEA. The peer reviewer considered the revised application acceptable and recommended renewal of research licence R0149 for three years.
Issues for consideration
NONE
Executive recommendations for Licence Committee
NONE
Areas not covered on this inspection
All covered

Report compiled by:

Name	Andrew Leonard
Designation	HFEA inspector
Date	2nd July 2009

Appendix A: Centre Staff interviewed

PR, research lead and PhD student

Appendix B: Licence history

Licence	Active From	Expiry Date	Changes
R0149/1/a	Expired	03/06/2004	30/04/2006
R0149/2/a	Active	01/09/2006	31/08/2009

Appendix C:

RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number 0035

Name of PR Dr Karen Turner

Research project RO149

Date of Inspection 20th May 2009

Date of Response 9th July 2009

Please state any comments regarding the inspection and actions you have taken or are planning to take following the inspection with time scales

The PR recognizes the comprehensive Report and thorough manner in which the Inspection was conducted. Both the Inspection and the Report are helpful and constructive. The Inspectorate recommends that the Centre i) organizes six monthly meetings of the research committee, and ii) immediately brings into use the revised versions of the patient research information compliant with the requirements of the HFEA Code of Practice, 7th edition.

i. We currently plan six monthly research meetings, but one meeting was missed in 2008. We will continue to plan these meetings every six months and ensure that we do indeed meet on this regular basis.
ii. We are in the process of finalizing revised patient information sheets and consent forms in accordance with the inspectorate's recommendations. The Inspectorate recommends in the Report that the documents are prepared in-house rather than commercially since they will need revising when the OFU moves to new premises. This will avoid delays and updated versions will be used as soon as possible.

In addition to the above, please note that we wish to change the Nominal Licensee on this project to Dr Dagan Wells, given the extent of his likely future involvement in this. The signed Declaration document from the application (Section 13) from both the PR and NL has been sent to the centres inspector.

2. Correction of factual inaccuracies

Please let us know of any factual corrections that you believe need to be made (NB we will make any alterations to the report where there are factual inaccuracies. Any other comments about the inspection report will be appended to the report).

None

HFEA Research Licence Committee Meeting

15 July 2009

21 Bloomsbury Street London WC1B 3HF

Minutes – Item 6

Oxford Fertility Unit (0035; R0149) – Renewal

Members of the Committee:

Emily Jackson (lay) – Chair
Richard Harries (lay)
David Archard (lay)
Lesley Regan (clinician)
Hossam Abdalla (clinician)

Committee Secretary:
Kristen Veblen

Legal Adviser:
Sarah Ellson, Field Fisher Waterhouse

Declarations of Interest: members of the Committee declared that they had no conflicts of interest in relation to this item.

The following papers were considered by the Committee:

- papers for licence committee (68 pages)
- tabled papers (3 pages).

The Committee also had before it:

- HFEA Protocol for the Conduct of Licence Committee Meetings and Hearings
- 7th edition of the HFEA Code of Practice
- Human Fertilisation and Embryology Act 1990 (as amended)
- HFEA (Licence Committees and Appeals) Regulations 1991 (SI 1991/1889)
- Decision Trees for Granting and Renewing Licences and Considering Requests to Vary a Licence; and
- Guidance for members of Authority and Committees on the handling of conflicts of interest approved by the Authority on 21 January 2009.

1. The Committee noted that the research project had commenced on 20 May 2004, that the current licence would expire on 31 August 2009, and that the renewal application had been made for 3 years.
2. The Committee considered the papers, which included the renewal inspection report, peer review and Licence Committee minutes from 16 September 2008, 25 July 2007 and 26 July 2006. Additionally, the Committee noted the tabled papers, an email from the Person Responsible (PR) covering the application form, the form declaration signed by the Person Responsible and proposed new Nominal Licensee and a letter concerning Ethics Committee approval.
3. The Committee noted that the renewal inspection had taken place on 21 May 2009 and that the PR had responded to the report on 9 July 2009.
4. It was noted by the Committee that at the time of the inspection the patient information sheet and consent form, although under revision, were not yet updated. Further, the Committee noted that in the PR's response, it was indicated that the Centre were in the process of finalising revised patient information sheets and consent forms.
5. The report also observed that that one of the scheduled meetings, to be held every six months had been missed. Further to this concern, the Committee noted the response of the PR, indicating that these meetings would continue to take place regularly every six months and be recorded appropriately.
6. The Committee also noted that in the PR's response to the report he indicated that the Centre wished to change the Nominal Licensee for this project to Dr Dagan Wells and that the information provided with the application included Dr Wells' CV.
7. When considering the Peer Review, the Committee noted that the reviewer made a comment about the slow progress of one aspect of the research and the Committee expressed hope that progress would be made on this aspect over the course of the next licence period.

<u>The Committee's Decision</u>

8. The Committee identified the activity to be authorised by a licence as the use of donated embryos for research. The Committee agreed that they were satisfied that this activity was not prohibited under the HFE Act 1990 (as amended).
9. The Committee decided that the activity was necessary and desirable for the following purpose:
    - developing methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation

HFE Act 1990 (as amended) 2 3(2)(e)

10. The Committee decided that it was satisfied that the proposed use of human embryos was necessary for the purpose of research. In making this decision the Committee took into account that the purpose of the project was to identify defects in human mitochondrial DNA. The Committee agreed that in order to understand these defects in human DNA, it was necessary to use human embryonic cells.
11. The Committee noted that the patient information and consent forms were due to be updated, as explained in the response of the PR and request that the Centre submit these forms for Executive approval of their suitability by 28 August 2009. This approval would be sufficient for the Committee to be satisfied of the suitability of the patient information and consent forms.
12. The Committee considered itself satisfied that it was appropriate to grant a licence, noting that it was in possession of an application from the Centre, indication of ethics committee approval and that the appropriate fee had been paid.
13. The Legal Adviser clarified that the Committee could treat the request in the PR's response as an application for a change to the NL for the new licence (this was not a variation of an existing licence) and could determine the matter if it was satisfied it had sufficient information.
14. The Committee agreed that it was satisfied as to the character, qualifications and experience of the proposed Nominal Licensee, Dr Dagan Wells. The Committee agreed to recognise Dr Wells as the Nominal Licensee for this research project.
15. The Committee noted that the PR had completed the PR Entry Programme and agreed that, it also continued to be satisfied as to the character, qualifications and experience of the PR as required for the supervision of the activities to be discharged under Section 17 of the HFE Act 1990 (as amended).
16. Further, the Committee agreed that it continued to be satisfied that the premises for which the Licence was to be renewed, as described by the report, were suitable for the activities.
17. The Committee, in accordance with the recommendation of the Executive, decided to grant a licence for a period of 3 years, with no additional conditions.

Signed..... Date 30.7.09.
Emily Jackson (Chair)