Research Licence Renewal Inspection Report

Project Title	To derive human embryonic stem cells and trophoblast cell lines
Centre Name	Oxford Fertility Unit
Centre Number	0035
Research licence Number	R0143
Centre Address	Level 4, Women's Centre, John Radcliffe Hospital, Oxford OX3 9DU
Treatment centres donating to this research project	0035 – Oxford Fertility Unit 0139 – Bath Assisted Conception Clinic
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	Professor Helen Mardon
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 R0143 is housed within a research laboratory in the Oxford Fertility Unit (Centre 0035), on level 4 of the Women's Centre, John Radcliffe Hospital. Research investigation of stem cells, but no licensed activities with viable embryos, is carried out in research laboratories within the Academic Department of Obstetrics and Gynaecology, Oxford University, on the floor below Centre 0035. All embryos used in the research project between 1st March 2008 and 28th February 2009 were derived from Centre 0035 (489 fresh and 13 frozen embryos donated and all used); no embryos were obtained from Centre 0139. The lay summary for the project states:

'**Project R0143: To derive human embryonic stem cells and trophoblast cell lines**
'There is considerable scientific and medical interest in the possibility that stem cells may make new treatments possible for many chronic diseases, including diabetes, heart disease and nervous system diseases such as Parkinson's disease. These new therapies will be possible because stem cells, which are found in the very early embryo, have the potential to form every cell type in the body. It is now possible to isolate these cells from the embryo, maintain them in culture in their stem cell state in the laboratory, and, alternatively, tweak them to develop into different cell types, such as heart, bone and muscle cells.

'This project seeks to understand how to maintain stem cells in culture, and how to promote development into different cell types. Stem cells will be obtained from the early embryo, at a stage known as the blastocyst at about six days after conception, when it is smaller than a pinhead and contains just one hundred cells. At this early stage, there are two types of cells, the stem cells and another type of cell, known as the trophectoderm that will go on to develop into the placenta. Stem cells will be isolated and grown in culture. The factors controlling their maintenance as stem cells as well as the molecular instructions that direct their development into different cell types will be studied. The trophectoderm will also be isolated and cultured so that we can understand what factors are important in development of the placenta.

'The overall aims of our research are to improve our understanding of how stem cells can be maintained and controlled to develop into specific cell types, to study diseases of pregnancy that involve abnormalities in the cells which will become the placenta. It is anticipated that our discoveries will contribute not only to the design of new stem cell based treatments in the future, but to our understanding of how such diseases develop in the first place.'

Summary for Licence Committee

The Centre has held a licence for project R0143 since 2003. The Centre also has research licences for two other projects (R0111 and R0149). The licensed research activities on licence R0143 include research on human embryos, storage of licensed material, and derivation of human embryonic stem cell lines; the licence is due to expire on 31 August 2009. The licence renewal application states that the Centre do not wish to add further activities to licence R0111. The research licence renewal is requested for three years for the same defined purposes as the existing licence: increasing knowledge about the development of embryos; increasing knowledge about serious disease; enabling any such knowledge to be applied in developing treatments for serious disease.

When embryos are accepted into project R0143, they are cultured in the research culture laboratory at Centre 0035 until day 6 post-fertilisation, at which point they are discarded if non-viable or used for stem cell derivation. All embryos used in the research project between 1 March 2008 and 28 February 2009 were derived from Centre 0035 (489 fresh and 13 frozen embryos donated and all used); no embryos were obtained from Centre 0139. The embryo usage differed from that proposed (120 fresh embryos and 0 frozen embryos), because the researchers defined an embryo as having been used in research only if it had been used for stem cell derivation. The estimated usage was based on this definition. Indeed the researchers state that between 1/03/07 and 28/02/2009, only 53 embryos were used for derivation, while 716 embryos were donated to the project. This indicates the relative low quality of embryos donated to research. It should be noted however that the Centre report the derivation of 4 stem cell lines and results which suggest that their derivation protocols have been optimised. The application details an expected usage of 400 fresh and 50 frozen embryos in the coming year.

The inspectorate were satisfied that research at centre 0035 is well led and 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. 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 and 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) Version control on both the general and stem cell specific research information/consent forms indicate that they have not been reviewed in the last year. Updated information sheets are in preparation and a draft version of the general research/consent form provided to the inspectorate was compliant for use at the current premises. It is recommended that the PR ensure both the general and specific information/consenting forms are regularly reviewed such that information provided to patient regarding project R0143 is accurate and compliant with the requirements of the HFEA Code of Practice, 7th edition.

3) The renewal application states that embryo usage in the next year will include 50 frozen embryos. The current and draft updated versions of the general patient information/consent form provided to patients with embryos in storage, do not include information regarding

R0143. It is unclear what information is provided to patients donating frozen embryos regarding project R0143. It may be that the stem cell research specific information/consent form 'The generation of human embryonic stem cells, version 1' is provided. If this is the case, the information it contains would fulfil the regulatory requirements, however the form is suited to fresh, rather than frozen, embryo donation and may be confusing to some patients. The PR should ensure that frozen embryo donors to project R0143 are provided with information which satisfies the HFEA Code of Practice requirements before consent is taken.

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 R0143 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	5
Laboratory technicians	0
Support staff (receptionists, record managers, quality and risk managers etc)	1

Highlighted areas of firm compliance
The treatment and storage licence and research licence for project R0143 at Centre 0035 have different PRs and clinical and research practices are separated. The one area of close approach between clinical and research activities is that 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. There are no research dewars for embryo storage. Removal of embryos from dewars for research use is appropriately witnessed and recorded in patient records. The Person Responsible (PR) is a Consultant Embryologist and the Laboratory Manager at Centre 0035, while the Nominal Licensee leads the research and is a professor of reproductive science. The PR has been in post since before the current licence was issued and has completed the PR Entry Programme. The PR has extensive knowledge of the regulatory requirements of the HFEA and is an external advisor to the HFEA. Discussions with the PR and the research lead, and inspection of the premises, indicated that the project is well lead and managed. The project appeared to be appropriately staffed, albeit two post-doctoral scientists have left and the research nurse at Centre 0035 has 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 on other projects 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 observed by the inspectorate. 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 Medical Research Council for the term of the licence applied for, albeit further funds are always 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 all 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. Regular emails and telephone calls are used to communicate with Centre 0139.

The Academic Department of Obstetrics and Gynaecology, is part 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 were not provided as they are not used to work on viable embryos and tend to be sections of research papers. The principal investigator on the project considered the SOPs used were suitable and are updated as required by technique development in their area of research.

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
Formal research group meetings should be held every six months, according to centre staff. Minutes were provided for meetings in January 2008, May 2008 and 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 and stem cell derivation. All licensed material and research records are confined to this secure laboratory. 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 laboratory is 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. 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 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 R0143 from within Centre 0035 and also as frozen stored embryos, from the Bath Assisted Conception Clinic (Centre 0139). 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 consulation 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 and 0139 are also used in project R0143. 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 their Centre. Frozen embryos at Centre 0139 are sent in batches to Centre 0035, and are used in research within days of arrival. 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 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 was not then used in project R0143 due to the absence of consent, but was used in 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
Initial patient recruitment to project R0143 for fresh embryo donation uses a general patient research information/consent form (Research and training projects using surplus eggs and embryos, Pt Info 2 v.2), which describes project R0143 (as well as describing and collecting consents for projects R0111 and R0149). If patients express an interest in project R0143 after reading this form, they are provided further information and consents are collected using the stem cell research specific information/consent form 'The generation of human embryonic stem cells, version 1'. The two documents were reviewed and they together provide all information required by Licence Condition A.19.6 (d) for stem cell research donors. Variable consents are possible as the general research information/consent form used at Centre 0035 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
Version control on both the general and stem cell specific research information/consent forms indicate that they have not been reviewed in the last year. The general research information/consent document is also non-compliant with regard to the information within it for patients consenting to other research projects, as it is the only document provided to them; this is dealt with in the inspection reports for those projects. Updated information sheets are being prepared for when the Centre move to new premises in August/September 2009 and a draft version of the general research form was provided at the inspection and seen to be

compliant for use at the current premises. It is recommended that the PR ensure both the general and stem cell specific information/consenting forms are regularly reviewed such that information provided to patients is accurate and compliant with the requirements of the HFEA Code of Practice, 7th edition.

The renewal application states that embryo usage in the next year will be 400 fresh and 50 frozen embryos. The current and draft updated versions of the general patient information/consent form provided to patients with frozen embryos in storage, do not include information regarding R0143. It is unclear what information is provided to patients donating frozen embryos regarding project R0143. It may be that the stem cell research specific information/consent form 'The generation of human embryonic stem cells, version 1' is provided. If this is the case, the information provided would fulfil the regulatory requirements, however the form is suited to fresh, rather than frozen, embryo donation and may be confusing to some patients. The PR should ensure that frozen embryo donors to project R0143 are provided with information which satisfies the HFEA Code of Practice requirements before consent is taken.

Executive recommendations for Licence Committee

The Licence Committee is asked to endorse the recommendation that the PR ensures both the general and specific information/consenting forms used for project R0143, are regularly reviewed to ensure that information provided to patients regarding project R0143 is accurate and compliant with the requirements of the HFEA Code of Practice, 7th edition.

The Licence Committee is asked to endorse the recommendation that the PR ensures that frozen embryo donors to project R0143 are provided with information which satisfies the HFEA Code of Practice requirements before consent is taken.

Areas not covered in by this inspection

All covered

5. Scientific practice R0143, to derive human embryonic stem cells and trophoblast cell lines

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 on licence R0143 include 'research on human embryos', 'storage of licensed material' and 'derivation of human embryonic stem cell lines'. The Centre do not wish to add further activities. The research licence has been granted for the following defined purposes: increasing knowledge about the development of embryos; increasing knowledge about serious disease; enabling any such knowledge to be applied in developing treatments for serious disease. *Research project lay summary:* 'There is considerable scientific and medical interest in the possibility that stem cells may make new treatments possible for many chronic diseases, including diabetes, heart disease and nervous system diseases such as Parkinson's disease. These new therapies will be possible because stem cells, which are found in the very early embryo, have the potential to form every cell type in the body. It is now possible to isolate these cells from the embryo, maintain them in culture in their stem cell state in the laboratory, and, alternatively, tweak them to develop into different cell types, such as heart, bone and muscle cells. This project seeks to understand how to maintain stem cells in culture, and how to promote development into different cell types. Stem cells will be obtained from the early embryo, at a stage known as the blastocyst at about six days after conception, when it is smaller than a pinhead and contains just one hundred cells. At this early stage, there are two types of cells, the stem cells and another type of cell, known as the trophectoderm that will go on to develop into the placenta. Stem cells will be isolated and grown in culture. The factors controlling their maintenance as stem cells as well as the molecular instructions that direct their development into different cell types will be studied. The trophectoderm will also be isolated and cultured so that we can understand what factors are important in development of the placenta. The overall aims of our research are to improve our understanding of how stem cells can be maintained and controlled to develop into specific cell types, to study diseases of pregnancy that involve abnormalities in the cells which will become the placenta. It is anticipated that our discoveries will contribute not only to the design of new stem cell based treatments in the future, but to our understanding of how such diseases develop in the first place. **Usage and expected usage in next year**: When embryos are accepted into project R0143, they are cultured in the research culture laboratory until day 6 post-fertilisation, at which point they are discarded if non-viable or used for stem cell derivation. All embryos used in the research project between 1 March 2008 and 28 February 2009 were derived from Centre 0035 (489 fresh and 13 frozen embryos donated and all used); no embryos were obtained from Centre 0139. The embryo usage last year differed from that proposed (120 fresh embryos and 0 frozen embryos). This is in part

because the researchers were fortunate to have access to more donated embryos than previously anticipated, but also because the researchers previously defined an embryo as having been used in research only if it had been used for stem cell derivation. Embryos donated to the project were not counted as having been used in research if found to be non-viable after culture to 6 days post-fertilisation.

This issue was addressed at the interim inspection in June 2008. The research lead was advised that all embryos consented to the research project should be considered to have been used in the project if they were cultured within the research laboratory, even if they were non-viable after culture to 6 days post-fertilisation. This definition was not though used in defining the proposed embryo use for the past year. Indeed the researchers state that between 1/03/07 and 28/02/092009, only 53 embryos reached early blastocyst stage and were used in the project. Data submitted to the HFEA indicates the number of embryos donated to the project and cultured to produce the 53 blastocysts, was 214 in the first year and 502 in the second. This indicates the relative low quality of embryos donated to research in that only 53 of 716 embryos cultured was capable of developing to the blastocyst stage. The application details an expected usage of 400 fresh and 50 frozen embryos in the coming year.

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.

The objectives of the project are to i) derive new human embryonic stem cell (hESC) lines from blastocysts, and ii) determine the expression and function of specific extracellular matrix molecules in hESC renewal and differentiation. These will enable the development of stem cell technologies that increase efficiency of derivation and avoid the use of animal products. In order to achieve this, blastocysts were grown in culture from day three embryos that are surplus to clinical requirements and donated by couples attending the Oxford IVF Unit. They were then processed for stem cell derivation by mechanical isolation of the inner cell mass (ICM) which was plated out onto either mouse embryonic fibroblast (MEF) or human foreskin fibroblast (CCD 1112Sk) irradiated feeder cells. Four hES cell lines were successfully derived and characterised. The capacity of extracellular matrix to support hES derivation and early stage hES cell renewal will now be addressed.

Scientific justification.

Human embryonic stem (hES) cell technology offers remarkable scope for the development of new therapies for a diverse range of diseases, including degenerative diseases such as cardiovascular, musculoskeletal and neurodegenerative diseases, as well as diabetes and tissue damage caused by injury. In addition hES cell research provides novel opportunities for drug discovery and testing, and to provide new inroads into human developmental biology.

There are several major challenges facing successful delivery of hES cell-based therapeutics to the clinic. These include i) derivation of a large number of well-characterised hES cell lines;

and ii) overcoming the requirement for animal reagents for sustained ES cell maintenance. This project addresses these problems using a combination of the principles and methods of biochemistry and cell biology.

It is clear that different hES cell lines have different phenotypes and properties, and are therefore likely to have different propensities for differentiation along certain pathways. Derivation of a large number of hES cell lines will be necessary to gain a better understanding of the underlying mechanisms and conditions that regulate differentiation along specific pathways and the properties of hES cell lines that determine specific differentiation potential. In addition, methods that improve streamlining the basic stem cell technology are under-researched.

Some, but not all, hES cell lines maintain their pluripotent status when cultured on the extracellular matrix (ECM) Matrigel or laminin, in the presence of medium conditioned by mouse embryonic fibroblasts (Richards, 2000; Xu et al, 2001; Rosler et al, 2004, Mallon et al, 2006). The maintenance of hES cells cultured on the ECM component fibronectin (Amit et al, 2004), and vitronectin (Braam et al, 2008) in a defined culture media has been reported. Fibronectin, and a combination of fibronectin and laminin, has also been shown to support higher hES cell growth rates compared to collagen type IV and laminin alone, or cells grown in the absence of ECM components (Draper et al, 2004). Ultimately, for many therapeutic applications it will be efficacious to grow and differentiate stem cells within a three-dimensional scaffold containing ECM instead of on two-dimensional substrate, not only to mimic the in vivo environment but also for ease of transplantation to a localised site.

B) Research undertaken under the term of the current licence.
Blastocysts were cultured to day 6 from donated embryos. Either the embryos were explanted directly onto irradiated mouse embryonic or human foreskin fibroblast feeder cells, or the inner cell mass was isolated mechanically with fine glass needles made using a pipette puller and placed onto feeders. Explanted whole embryos were cultured on feeders for a maximum of 6 days during which time there were some limited outgrowth. None of the whole blastocysts explanted onto feeders gave rise to hES cell lines. Isolated inner cell masses were plated onto feeders and their outgrowth monitored for up to 4 weeks. When an ES-like colony appeared, it was cut into pieces and transferred onto fresh feeders. Thereafter the cells were propagated by cutting colonies and passaging the pieces onto fresh feeders until the line was established.

C) Results during the term of the current licence
Summary of the numbers and fate of embryos used: The total number of embryos consented for this project between the 1 March 2008 and 28 February 2009 was 489. However the majority were of very poor quality and either arrested prior to morula stage or degenerated, and thus were not suitable for derivation of hES cell lines. A total of 53 embryos reached early blastocyst stage and were used in the project, between 1/03/07 and 28/02/09, from which 2 hES lines were derived. A further 2 lines were derived during March 2009 from 15 embryos. Many of the first 53 blastocysts were of poor quality, grade C or lower and many were cultured on a line of human foreskin fibroblasts (Hs27), which subsequently was shown to be unable to support the growth of established hES cells. The results in the table below are the data from September 2008 to April 2009, after conditions for derivation had been optimised. Our work has demonstrated that the quality of the embryos is critical for successful derivation, as is the quality of the feeders cells.

	MEF feeders		CCD 1112S. feeders	
	Grade A embryos	Grade B embryos	Grade A embryos	Grade B embryos
No. embryos	9	9	10	10
No. hES cell lines	4	0	0	0

Table 1: Summary of the numbers and fate of embryos used in hES cell derivation experiments

Four human embryonic stem cell lines, OxF1, OxF2, OxF3 and OxF4 were successfully derived, all from grade A embryos, and on MEF feeders. Attempts to derive on human feeder CCD fibroblasts were unsuccessful, despite the ability of CCDs to support established hES cell lines in our laboratory. The first hES cell line, OxF1, has been best characterised.

OxF1 was derived in September 2008 from a day 6 blastocyst graded as 4AA. The inner cell mass (ICM) was isolated mechanically after Ström et al, (2007), but using fine glass needles manufactured with a pipette puller (Narishige Int. Ltd). The ICM was plated onto a feeder layer of irradiated MEFs of the MF1 strain. The culture medium was DMEM/F12 (Invitrogen) containing 20% Knockout Serum Replacer (KSR, Invitrogen), 1 mM glutamine, 1% non-essential amino acids and FGF 2 (Peprotech) at 4 ng/ml.

Cells with the morphology of human embryonic stem (hES) cells first appeared eight days after plating the ICM, and the single colony was cut into several pieces and passaged into a new feeder well four days later. Expansion of the line and subsequent routine culture involved mechanically cutting undifferentiated colonies and transferring the pieces onto fresh feeders every five days. The feeder layer and culture media were the same as for derivation. OxF1 has currently been cultured for twenty-seven passages, the last eight of which have been achieved by enzymatic dissociation using TrypLE Select (Invitrogen) to produce a single-cell suspension. The interval between enzymatic passages was extended to 7 days.

Expression of pluripotency markers was demonstrated by immunocytochemistry, as shown in Figure 1. Cells at passage 9 were fixed in 4% paraformaldehyde, permeabilised in phosphate-buffered saline containing 0.2% Triton X-100 and non-specific binding was blocked with 10% goat or donkey serum. The primary antibodies used were anti-Oct 4 (1:200, Santa Cruz), anti-Nanog (1:10, R&D) and anti- TRA-1-60 1:100, Chemicon). The secondary antibodies were AlexaFluor anti-mouse IgG 555, anti-goat IgG 488 and anti-mouse IgG 488 respectively, all at a dilution of 1:400. Nuclei were counter-stained with DAPI. Control samples received either the appropriate IgG or no primary antibody.

OxF1 demonstrates positive expression of the transcription factors Oct 4 and Nanog (Figure 1) and the cell surface marker TRA-1-60, as shown in Figure 1. Control samples showed no staining and nor did the MEF feeder cells.

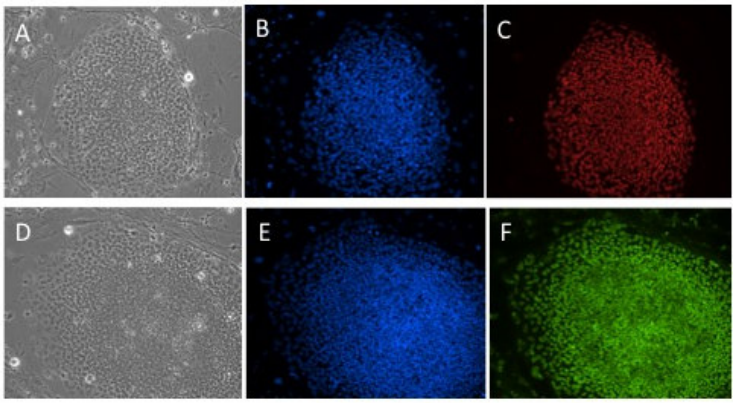

Figure 1: Staining of two colonies of OxF1 (A-C and D-F) for markers of pluriotency. A, D phase contrast; B, E DAPI; C, Oct 4; F Nanog.

Further evidence that OxF1 is pluripotent was provided by in vitro differentiation studies. Embryoid bodies were formed by culturing aggregates of OxF1 cells of passage 22 in suspension for 4 days, and were then plated onto gelatinised coverslips and cultured for 11 - 18 days in DMEM/F12 containing 20% FCS. Samples were fixed and prepared for immunocytochemistry as above to determine expression by differentiated cells of markers of all three germ layers. The primary antibodies used were anti-βIII tubulin (1:100, Chemicon, a marker of ectoderm), anti-desmin (1:100, Lab Vision, a mesoderm marker) and anti-Gata 6 (1:200, an endoderm marker). The secondary antibodies were AlexaFluor anti-mouse IgG 488 and anti-rabbit IgG 546 at 1:400.

Positive staining was observed for βIII-tubulin, desmin and Gata6, indicating that OxF1 can differentiate into cells which represent all three germ layers. Control samples showed no staining. OxF1 and OxF2 have been karyotyped, OxF1 at passages 13 and 26, and both are normal 46XX.

These early stage hES cell lines will be used in experiments to assess the capacity of recombinant extracellular matrix components, in particular focussing on integrin binding fragments of fibronectin, to support their growth and maintenance of pluripotency. Preliminary studies with established cell lines indicate that the fibronectin fragments we have generated support self-renewal and potentially better restrict differentiation compared to hES cells grown on mouse embryonic fibroblasts. If we can reproduce these results on our very early stage hES cells and in the future successfully derive new lines on such defined substrates, this will represent an important step towards overcoming the challenge of avoidance of animal reagents in generating and maintaining hES cell lines.

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D) If progress was slower than anticipated, the reasons for this.
N/A

E) Publications which have arisen from work under the licence.
Brook F, Evans E, Turner K and Mardon H, Derivation and characterisation of the human embryonic stem cell line, OxF1 (submitted).

F. Future work
Having achieved relatively efficient derivation of new hES cell lines, our next aim is learn how to derive efficiently, further hES cell lines on defined, non-animal extracellular matrix substrates. Our objectives are to synthesise an extracellular matrix that mimics that of feeder cells that support hES cell derivation and test the capacity of this synthetic extracellular matrix to support derivation and early stage self- renewal.

Methods
Blastocysts will be cultured to day 6 from donated embryos. The inner cell mass will be isolated mechanically with fine glass needles made using a pipette puller, as described above, and will be placed onto feeders or extracellular matrix substrates, including fibronectin fragments. Early stage hES cell outgrowths will be passaged initially by cutting and pasting. The efficacy of enzymatic dissociation of cells will also be tested in conjunction with growth on fibronectin.

Discussion
The proposed studies involve essentially a continuation of the current research. New human embryonic stem cell lines will be derived from day 6 blastocysts. Each cell line has different properties and by characterising and studying different cell lines we will aim to discover mechanisms of, and conditions that regulate, self-renewal and differentiation of hESCs, which will in turn inform our understanding of early human development. For example we will investigate the ability of different lines to proliferate or differentiate on ECM components and the interactions with integrins. The development of techniques for efficient derivation of hES cell lines in the absence of animal reagents on defined extracellular matrix substrates will represent a significant step in overcoming some of the challenges faced in taking hES cells into the clinic.

Peer review (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 R0143 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	9th June 2009

Appendix A: Centre Staff interviewed

PR and research leads

Appendix B: Licence history

Licence	Active From	Expiry Date	Changes
R0143/1/a	Expired	14/08/2003	31/08/2006
R0143/2/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 Licence: RO143

Date of Inspection 20th May 2009

Date of Response 6th 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 and NL recognized 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 revised versions of the patient research information compliant with the requirements of the HFEA Code of Practice, 7th edition, and iii) frozen embryo donors are provided with information specifically relevant to frozen embryos.

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 suggest 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.

iii. It is uncommon for frozen embryos to be donated to RO143. At present, due to the more involved nature of this project, we do not routinely give this information to patients with frozen embryos who may wish to donate their material to a research project (advice from previous inspector). However if we receive a specific request for information on stem cell research we will follow this up by providing the patient information sheets and consent forms for the stem cell project, and patients are given the opportunity to discuss the project further with a nurse. We will review this policy during the course of this year.

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).

HFEA Research Licence Committee Meeting

15 July 2009

21 Bloomsbury Street London WC1B 3HF

Minutes – Item 5

Oxford Fertility Unit (0035; R0143) – 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 (86 pages)
- tabled papers (2 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 1 September 2006, 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 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 6 July 2009.
4. It was noted by the Committee that the report observed that at the time of the inspection the current draft updated versions of the general patient information and consent forms do not include information specific to this research project and that it was unclear if specific information was being provided for patients providing frozen embryos and specific information regarding stem-cells. Further, the Committee noted that in the PR's response it was explained that the project rarely receives donated embryos and that they will address this if required and revise the policy over the course of the next year.
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.

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

6. The Committee identified the activities to be authorised by a licence as the use of donated embryos for research. The Committee agreed that they were satisfied that these activities were not prohibited under the HFE Act 1990 (as amended).
7. The Committee decided that these activities were necessary and desirable for the following purposes:
    - increasing knowledge about the development of embryos
      HFE Regulations 2001 2(a)
    - increasing knowledge about serious disease
      HFE Regulations 2001 2(b)
    - enabling any such knowledge to be applied in developing treatment for serious disease
      HFE Regulations 2001 2(c)

8. 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 improve understanding of how stem cells can be maintained and controlled to develop into specific cell types, to study diseases of pregnancy that involve abnormalities in the cells which will become the placenta, with the anticipation of not only contributing to the design of new stem cell based treatments in the future, but increasing the understanding of how diseases develop in the first place. The Committee agreed that in order to understand how to maintain and control the development of human embryonic stem cells, and to understand diseases which occur because of abnormalities in the human placenta, there was no suitable alternative to human embryonic stem cells.
9. 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. Subject to this approval, the Committee agreed that it was satisfied of the suitability of the patient information and consent forms.
10. The Legal Adviser confirmed that the legislation required an application from the Centre but did not specify that it had to be signed. Although the provision of signatures was helpful in clarifying the position the Committee could proceed if it was satisfied that it had an application "from the Centre" which might be evidenced by an email from the PR.
11. 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.
12. The Committee agreed that it continued to be satisfied as to the character, qualifications and experience of the Nominal Licensee. Also, 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).
13. 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.
14. 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)