New Research Licence 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	To be assigned. This New Research Licence Application is to allow research associated with project R0143 to be continued on a second site
Centre Address	Centre 0035's proposed new premises at: Institute for Reproductive Sciences, Oxford Business Park North, Oxford, OX4 2HW
Treatment centres donating to this research project	0035 - Oxford Fertility Unit 0064 - BMI The Chiltern Hospital Fertility Services Unit 0139 - Bath Fertility Clinic
Inspection date	25th August 2009
Licence Committee Date	16th September 2009
Inspector(s)	Andrew Leonard Gill Walsh
Fee Paid - date	Fee paid
Person Responsible	Dr Karen Turner
Nominal Licensee	Prof Helen Marden
Licence expiry date	New licence application

About the Inspection:
The purpose of the inspection is to ensure that research will be carried out in compliance with the HF&E Act 1990, Code of Practice, licence conditions and directions. 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 new licence application. The report is also available to patients and the public following the Licence Committee meeting.

Brief Description of the Project

This new research licence application is to allow research associated with project R0143 to be continued on a second site, at the proposed new premises of the Oxford Fertility Unit (OFU; HFEA centre 0035), at the Institute for Reproductive Sciences (IRS), Oxford Business Park North, Oxford, OX4 2HW. A new treatment and storage licence application has been made by the Centre's PR for these new premises from 1st October 2009, to be considered by a HFEA Licence Committee on the 21st September 2009.

Project R0143 is currently housed within a research laboratory at Centre 0035's premises on level 4 of the Women's Centre, John Radcliffe Hospital, and in research laboratories within the Academic Department of Obstetrics and Gynaecology, Oxford University, on the floor below Centre 0035. When the OFU moves to its proposed new premises, project R0143 will continue at the existing premises. This new research licence application is to allow work associated with project R0143 to be carried out at Centre 0035's new premises. Much of the application is therefore common to the recent renewal application for project R0143, approved by research licence committee on the 15th July 2009. The only differences are in the premises and equipment section for obvious reasons, and in the patient information and consenting sections, which have been revised since the renewal inspection.

The new research licence application includes the usage of 400 fresh embryos and 50 frozen embryos in each year of the 3 year licence term. This usage concurs with the proposed usage in project R0143. This is unsurprising because all embryos consented to the new research project will be cultured at the IRS then transported to researchers working on project R0143 at the JRH. In future, stem cell derivation may also be undertaken at the IRS site under this project application.

The lay summary for the project states:
There is considerable scientific and medical interest in the possibility that stem cells may make new treatment approaches 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 them to develop into different cell types. Stem cells will be obtained from the very 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 just

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, and to study diseases of pregnancy that involve abnormalities in the cells which will ultimately 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

This is a new Research Licence Application to allow research associated with project R0143 to continue on a second site, at the proposed new premises of the Oxford Fertility Unit (OFU; HFEA centre 0035) at the Institute for Reproductive Sciences (IRS), Oxford Business Park North, Oxford, OX4 2HW. A new treatment and storage licence application has been made for these new premises from 1st October 2009, to be considered by a HFEA Licence Committee on the 21st September 2009. The new research licence has been requested to also begin on 1st October 2009.

Project R0143 is currently housed within a research laboratory at Centre 0035's current premises on level 4 of the Women's Centre, John Radcliffe Hospital (JRH), and in research laboratories within the Academic Department of Obstetrics and Gynaecology, Oxford University, on the floor below Centre 0035. When the OFU moves to its proposed new premises at the IRS, project R0143 will continue at its current premises in the JRH. This new research licence application is to allow work associated with project R0143 to be carried out at Centre 0035's new IRS premise. The proposed work will principally involve culturing research donated embryos, from Centres 0035, 0064 and 0139, in a designated research incubator and laboratory, before they are transferred to project R0143 at the JRH for stem cell derivation. As a research licence can not cover research on two premises, this new licence application has had to be made.

The researchers consider that research on the two sites will effectively form a common project, i.e. R0143. Thus much of this application is identical to the recent renewal application for project R0143, approved by research licence committee on the 15th July 2009. Indeed the only differences are in the premises and equipment section for obvious reasons, and in the patient information and consenting sections, due to recent document revision.

This new research licence application includes as licensed activities: Storage of embryos; use of donated embryos for research; derivation of human embryonic stem cell lines. The new research licence application is requested for: increasing knowledge about the development of embryos; enabling any such knowledge to be applied in developing treatments for serious disease. The new research licence application includes the usage in each year of the 3 year licence term, 400 fresh embryos and 50 frozen embryos. These activities and defined purposes, and the usage, are the same as for the project R0143 at its recent renewal. This is unsurprising because all embryos consented to the new research project will be cultured at the IRS then transported to researchers working on project R0143 at the JRH. In future, stem cell derivation may also be undertaken at the IRS site under this project application.

The PR has informed the inspectorate that the organisation of the research covered in this new research licence application will be identical to that used in project R0143. The inspectorate were satisfied that the research will be well organised and carried out in a professional manner which complies with the Code of Practice, 7th edition. The proposed research donation processes, consenting and scientific practices are also the same as those used in project R0143. These were considered compliant at the renewal inspection at the JRH premises in May 2009 and would appear to be so now in this application.

Patient information and consent forms were reviewed by the inspectorate. Those for fresh embryo donation were compliant with all HFEA requirements; those for frozen embryo

donation appeared non compliant as they did not discuss the stem cell derivation projects including the proposed project. The PR has reported however that frozen embryo donors are not actively recruited to the stem cell projects; embryo usage data for project R0143 supports this assertion. Frozen embryos are donated by patients who have contacted the Centre regarding specific donation to a stem cell project. Such patient are then provided with an additional information and consent form, 'The generation of human embryonic stem cells, version 2; dated 01/08/09', which does provide all information required by the HFEA CoP and Licence Conditions. Thus information provision to frozen embryo donors is compliant, albeit minor changes are recommended to 'The generation of human embryonic stem cells, version 2' if the proposed licence application is approved. The inspectorate also recommended that in the event that frozen embryo donors start being actively recruited, the PR audit patient information provided against HFEA requirements, notably S.8.3.1 and S.8.3.2 a-d, G.5.13.1 a-l, and Licence Condition A.19.6 (d).

The PR has informed the inspectorate that research procedures related to this new research licence application will be identical to those used in project R0143, which were considered compliant at its renewal by a Licence Committee in July 2009.

Peer review of this new research licence application was positive and recommended acceptance of the application in its present form.

The inspectorate recommend the granting of a new research licence to this project. Normally new research licences are granted for a one year term, however in this case, the inspectorate recommend the provision of a three year licence since the Centre have held several HFEA research licences, three of which are currently active, and are considered to be in nearly all aspects compliant with HFEA requirements. This new research licence should commence on 1st October 2009.

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	2
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's current premises 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 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. These arrangements will continue at the new premises if this new research licence application is approved. The proposed Person Responsible (PR) is a Consultant Embryologist and the Laboratory Manager at Centre 0035, while the proposed Nominal Licensee is a professor of reproductive science and will lead the research programme. The proposed PR and NL hold the same positions on project R0143. The proposed PR 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 indicate that the proposed project will be well managed, as has been previously observed at the renewal inspection of project R0143. The project appeared to be appropriately staffed, with the PR, the lead scientist and 4 scientists included as licensed personnel in the application. These staff are the same as are licensed for project R0143. Centre 0035 has a research nurse, but she has not been able to work on project R0143 since September 2008, and will not be working on the new project either due to funding limitations. The research nurse is however still employed at the Centre on other projects and can give advice and information if available. The research nurse post may be reactivated if funding becomes available.

The induction procedure for research staff follows the Oxford University requirements and was considered by the inspectorate at the renewal inspection of R0143 in May 2009. Each element of induction requires sign-off and the record sheet is placed in staff records. 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 for the proposed project and project R0143 from The Medical Research Council and the Wellcome Trust for the 3 year term of the proposed licence. Ethical Committee approval has also been confirmed to be still in place.

A formal organisational chart is not present, nor is it a requirement, however the PR described an appropriate organisation structure for the proposed research. A staff list was also provided in the application. All research staff who will have access to licensed material are included in the staff list in the application and will also be placed on the new treatment and storage licence for the new premises.

A formal research meeting between the PR and all researchers on project R0143 is currently scheduled twice a year, at which research progress and matters relating to the project are discussed and minuted. The PR and lead investigator are in regular telephone and email communication, and the lead investigator and the researchers meet on a weekly basis. These communications provide an opportunity to cascade essential information. These arrangements will remain in place to facilitate communication between the research team working under the proposed research licence.

Contact between the researchers and clinical staff at Centre 0035, by email and telephone, is currently as frequent as required for effective coordination of embryo supply to project R0143. These arrangements will continue, to coordinate embryo donation to the project covered by this licence application. Communication is facilitated by the PR being the Clinical Laboratory Manager at Centre 0035, though she has no role in consenting patients for research. The researchers provide seminars at all staff meetings at Centre 0035, as a means to feedback research progress to clinical staff. Regular emails and telephone calls are used to communicate with Centres 0064 and 0139, and facilitate the transfer of any donated stored embryos. No embryos were however donated by these centres to project R0143 in the year May 2008 – May 2009. These arrangements will continue as part of the new research project covered by this application.

The Academic Department of Obstetrics and Gynaecology, Oxford University, provides the management structure within which the research staff operate. The University supplies a full range of support services, e.g. health and safety, finance, personnel and facilities management.

Laboratory standard operating procedures (SOPs) related to embryo donation and culture were reviewed by the inspectorate at the renewal inspection of project R0143 in May 2009 and were considered fit for purpose. At this inspection the PR said that these protocols would also be used for the new research project, as the work involved is the same as in R0143, and that they have already been reviewed to determine whether changes were required due to the move to new premises. Few changes were necessary and these have all been made. SOPs related to downstream laboratory analyses on the proposed research project were recently

considered as part of the renewal of project R0143. The principal investigators considered the SOPs were suitable for the analysis they wished to perform and were updated as required if technique development occurred. The Centre has a procedure for reporting adverse incidents to the HFEA. This was reviewed at the renewal inspection for project R0143 and was considered appropriate. This procedure will also be used in the new centre premises research laboratory to ensure effective incident reporting occurs.
Issues for consideration
None
Executive recommendations for Licence Committee
None
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
Centre 0035's new premises comprise a renovated two storey business unit of *ca.* 2400 m^2 floor area. The building, the Institute of Reproductive Science, is a collaboration between the University of Oxford, Oxford Fertility Unit and Reprogenetics (a company providing a genetic analysis service to the fertility sector). Thus 30% of the space is allocated to a reproductive sciences post-graduate teaching facility, 65% is allocated for Centre 0035 to provide licensed treatment services and 5% to the Reprogenetics laboratories. The premises were inspected soon after being handed over by the contractors and were therefore unequipped. This research licence application applies to the dedicated research laboratory (room HG32) and the cryolaboratory (HG26) within the new Centre 0035 premises. The cryolaboratory will be used to store embryos for use in treatment as well as in the new research project. Embryos being stored for treatment, if consented for research use, will remain in the same dewar position until thawed for use in research. This practice is the same as that used by the Centre at its current premises. The cryolaboratory was assessed on inspection of the new treatment and storage licence application for the new premises and was found to be compliant with HFEA requirements. Conditions were attached to this assessment in that the inspectorate required the PR to confirm to the HFEA by 30th September that the low level oxygen monitors, the facilities monitoring system and the liquid nitrogen storage vessel and autofiller manifold had been tested and certificated as being in full working order. The inspectorate have been advised by the IT/business manager of Centre 0035, who is coordinating the move to the IRS, that these conditions will be complied with by 30th September 2009. The dedicated research laboratory is approximately 5 x 5 metres. The research PR said that the room would be equipped by the end of September 2009 with benching, and a research dedicated incubator and air flow cabinet IVF workstation. The research laboratory will allow research donated embryos to be moved from the clinical IVF laboratories into a research environment as soon as possible after donation. Once there, they will be cultured until ready for transfer to the Academic Department of Obstetrics and Gynaecology, Level 3 and 4, JRH, for use in projects R0143, R0143 and R0149. This new research licence application covers the use of the research laboratory for culturing embryos for use in project R0143. It is possible in the future that stem cell derivation will be performed in the research laboratory under the proposed research licence. Both the cryolaboratory and the research laboratory have electronic card key activated locks, by which access will be restricted to research personnel. Both rooms are fitted with fire, movement and temperature detector/monitors, which are integrated into the complex building management system installed at the new premises. This system will summons assistance in

the event of fire or break-in and will also log all persons who access the rooms. Dewars in the cryolaboratory will also be monitored by the facilities management system which will provide warnings to on-call staff in the event of dewar failure.

A documented system for equipment maintenance/servicing will be in place at the new premises; the Clinical Laboratory Manager is the designated person responsible for the maintenance of equipment. On the last 2 inspections of project R0143, all equipment sampled was within servicing intervals and servicing documentation was available. All electrical equipment had been subjected to portable appliance testing. These working practices will continue at the new premises to ensure all equipment used in the proposed new project is safe and well maintained.

The research laboratory and cryolaboratory will be risk assessed before the start of licensed activities and then annually by Centre 0035 staff. All research procedures to be used on the proposed project have been risk assessed.

The research laboratory and cryolaboratory will be cleaned by the same contractor who will be cleaning the clinical IVF laboratories. The contractor is committed to providing named cleaners to the premises, thus the cleaning team of 5 persons will be stable and known to Centre staff and educated regarding the materials to use for cleaning and hazards in their workplace.

After inspection of the research premises and discussions with the PR, the inspectorate consider that the proposed research premises and equipment seemed appropriate and should provide a safe, clean and secure environment in which to pursue this research project.

Issues for consideration
None
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
The application details that embryo donors will be recruited to the proposed project from Centre 0035, BMI The Chiltern Hospital Fertility Services Unit (Centre 0064) and the Bath Assisted Conception Clinic (Centre 0139). Currently, fresh embryo donors are recruited at Centre 0035 to three licensed research projects (R0143, R0143 and R0149) by a common research donor recruitment procedure which will also be used for the proposed project. All couples are provided research information with their treatment information at an initial orientation open evening. HFEA consent forms are signed at a consenting consultation some time afterwards, and general consent to research is taken on the HFEA treatment consent form if patients are interested. This practice will change slightly at the new premises as the new HFEA treatment consent form does not contain a general consent to research, rather it allows a patient to provide consent to allow the Centre to inform them about research donation opportunities. Thus if such consent is provided, research information will be discussed, if required, and research consents taken by nursing staff. If further information or time to consider is required, a further consultation is arranged at which research will be discussed and consent taken if patients so wish. The Centre's 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, 0064 and 0139 will also be used, but only if patients ask specifically to donate to a stem cell derivation project. At present, patients with frozen embryos are annually asked to confirm storage arrangements for the forthcoming year and are sent a research information sheet and a consent form for research, continued storage or disposal. The consent forms are signed by the patients and returned to their Centre, who then organise the research donation. If the patients write that they wish to donate specifically to a stem cell derivation project, further information must be provided as the stem cell projects are not referred to on the research information initially provided. These recruitment procedures will remain in place after the move to new premises and be used for recruitment to the proposed new research project. Defined processes are in place to prevent a breach of patients' research consent and, according to the PR, will remain so after the move to new premises. 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. These procedures for verifying consents will remain in place after the move to new premises and for recruitment to the proposed new research project.

At the new IRS premises, research consented embryos will be transferred to anonymised dishes in the clinical IVF laboratory, then passed to the research laboratory with an affidavit from the clinical embryologist detailing the consents applying and placed in culture. The research donation will be logged by the clinical embryologist in a book, kept in the clinical laboratory, detailing: anonymised research code; patient name; centre number; projects consented to; date of donation to research; developmental stage with length in culture post-fertilisation). After delivery to the research laboratory, the embryos will be allocated to a research project, depending on the consents provided and on which researchers are available. Embryos consented to the proposed project will then be cultured to blastcyst stage before transfer to the Academic Department of Obstetrics and Gynaecology, JRH, for stem cell derivation under licence R0143. In the future, stem cell derivation may be undertaken at the IRS research laboratory under the proposed research licence.

Cryopreserved embryos consented for the proposed research project, will be rapidly used after consent is provided, normally within days. Thus research storage audits are not planned however all storage dewars are audited two-yearly to comply with the treatment and storage licence conditions. Currently, research consented embryos in storage 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 and arrange for their thaw and use in research. These practices will continue if this new research project licence is approved.

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

The donation procedures currently 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.

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
A consent audit was not performed on this new research licence application inspection. At the recent renewal inspection for project R0143 in May 2009, consenting procedures and documentation in patient records was reviewed. This indicated that, in the opinion of the inspectorate, consenting at Centre 0035 was robust and compliant with HFEA Code of Practice requirements.
Highlighted areas of firm compliance
Patients attending for treatment at Centre 0035 and consenting for the use of fresh embryos to the proposed research project, will be provided with general (Research and training projects using surplus eggs and embryos; PIS v.4; dated 01/08/09, 5 pages) and stem cell specific information/consent documents (The generation of human embryonic stem cells, version 2; dated 01/08/09, 4 pages). The two documents were reviewed by the inspectorate and were considered well presented; they together provide all information required for general research and stem cell research donors by the HFEA Code of Practice and Licence Condition A.19.6 (d), and allowed effective consent to be provided. 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. Fresh embryos will not be sourced from patients at Centres 0064 and 0139.
Issues for consideration
The application states that embryo usage will be 400 fresh and 50 frozen embryos per year. The research information/consent forms provided to patients donating frozen embryos at Centre 0035 (Frozen embryos to research.docv v.3, dated 02/03/09, 1 page) and at Centres 0064 and 0139 (Frozen embryos to research - External v.1, dated 02/03/09), do not however include information regarding stem cell research, i.e. project R0143 or this proposed project. The consent form associated with these documents also does not mention the stem cell derivation projects. This issue was noted at the renewal of project R0143 in July 2009. The PR responded in appendix C of the inspection report: '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.'

It is accepted that recruitment of frozen embryo donors from Centres 0035, 0064 and 0139 to the stem cell derivation projects is unlikely if the projects are not discussed in patient information provided to patients with embryos in storage, or the associated consent form. The inspectorate also note that the licence application form indicates some frozen embryo donors will be recruited. It is accepted that these donors are not actively recruited but are those who have enquired about donation to stem cell derivation specifically, as per the PR's response above. It is important that such patients are provided with all required information before they consent to donate. The provision of 'The generation of human embryonic stem cells, version 2; dated 01/08/09' to these interested patients, as the PR's response indicates currently happens, will accomplish this. The document will need modifying to include the project number, assuming this application is approved by Licence Committee. Given the scope of the proposed research, the assigned project number could be included together with that of R0143 in a single consent. It is also recommended that the consent form is revised to not just state that consent can be withdrawn, but to include the mechanism for withdrawal, as outlined in the patient information.

In the event that the review discussed in the PR's response above occurs and frozen embryo donors become actively recruited to the proposed project (and project R0143), it is recommended that the PR audit patient research information provided against HFEA requirements, notably S.8.3.1 and S.8.3.2 a-d, G.5.13.1 a-l, and Licence Condition A.19.6 (d).

Executive recommendations for Licence Committee

The Licence Committee is asked to endorse the recommendation that the PR makes the minor revisions detailed in this report to 'The generation of human embryonic stem cells, version 2; dated 01/08/09'.

The Licence Committee is asked to endorse the recommendation that in the event that frozen embryo donors are actively recruited to this proposed project, the PR audit patient information provided to those donors against HFEA requirements, notably S.8.3.1 and S.8.3.2 a-d, G.5.13.1 a-l, and Licence Condition A.19.6 (d).

Areas not covered in by this inspection

All covered

5. Scientific practice

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

Summary of:

- Peer review

Summary
The proposed licensed research activities include research on human embryos; storage of licensed material; derivation of human embryonic stem cells. Downstream analytical methods to be used include: analysis of gene expression by RT-PCR analysis; high resolution immunohistochemical analysis after fixation. The research licence is requested for: increasing knowledge about the development of embryos; enabling any such knowledge to be applied in developing treatments for serious disease. The PR states in the application: 'The proposed research aims are to increase the efficiency of derivation of human embryonic and trophoblast stem cells in the absence of animal- based reagents, and to determine conditions that drive self- renewal and differentiation. In addition, the in vivo niche and the fate of pluripotent embryonic stem cells will be investigated. In particular, the composition of the extracellular matrix, and expression of markers of pluripotency and differentiation in human embryos at peri- and post- implantation embryos will be performed using an in vitro model of implantation. The research will increase knowledge of early human development past the implantation stage, and improvement of enabling technologies will have an impact on the realisation of translation of embryonic stem cell science to the clinic.' The proposed licence is almost identical to the application made for renewal of research project R0143, inspected at Centre 0035 in May 2009. This is because the proposed licence is to allow culture of research donated embryos at the new IRS premises for Centre 0035, before they are transported to the old JRH premises for experimental use in project R0143. In the future, some experimentation relevant to project R0143 may be performed on embryos under the proposed research licence at Centre 0035's new premises. **Usage and expected usage in next year**: The proposed project estimates it will use 400 fresh and 50 frozen embryos per year for the three year term of the licence. These usage estimates were also provided in the renewal application for project R0143 in May 2009. It is foreseen that embryos used in the proposed project will then be used in project R0143.
Summary of audit of stored and biopsied material
No licensed materials were in store on the day of inspection so no audit was performed.
Project objectives
The PR states: '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, and to study diseases of pregnancy that involve abnormalities in the cells which will ultimately 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.

The PR also states: 'The main objectives of the research are identical to those in R0143 namely:

i) establish new methods to efficiently derive new hES cell lines in the absence of animal reagents
ii) determine how hES cells are driven along one tissue pathway versus another, and
iii) isolate and grow trophoblast stem cells for use in models of implantation.'

Research proposal

Lay Summary

There is considerable scientific and medical interest in the possibility that stem cells may make new treatment approaches 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 them to develop into different cell types. Stem cells will be obtained from the very 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 just 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, and to study diseases of pregnancy that involve abnormalities in the cells which will ultimately 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.

Abstract

The early human embryo, at day six post-conception when it is called the blastocyst, contains two types of cells. The inner mass of cells contains embryonic stem (hES) cells, which have the capacity to form all the tissue types of the embryo and adult organism. The outer layer, the trophectoderm, forms the placenta and also contains stem cells that have the capacity to form the placenta and membranes.

hES cell technology potentially offers remarkable scope for the development of new therapies for a diverse range of degenerative diseases, including heart disease, neurodegenerative

diseases such as Parkinson's and Alzheimer's 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 how the human organism develops and the mechanisms that underlie diseases.

However there are several major challenges facing successful delivery of hES cell-based therapeutics to the clinic. These include i) how to obtain a large number of hES cell lines; and ii) overcoming the requirement for animal reagents for sustained ES cell maintenance to avoid possible viral contamination of the hES cells. 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 properties, each having different propensities for developing into different tissues types, and thus potential use in therapies for different diseases. Derivation of a large number of hES cell lines will be necessary to gain a better understanding of the underlying mechanisms and conditions that control differentiation along specific tissue type pathways. We also need to improve streamlining the basic stem cell technology to make it more efficient.

Impaired placental development causes conditions such as intrauterine growth retardation and pre-eclampsia, and affect up to 15% babies. Apart from the high mortality rate, these small for dates babies are at risk of developing significant complications in later life. Isolation and growth of trophectoderm, or trophoblast, stem cells provides an experimental system with which we will be able to understand better how the placenta develops and factors that cause impaired placental development.

The objectives of the project are to i) establish new methods to efficiently derive new hES cell lines in the absence of animal reagents, ii) determine how hES cells are driven along one tissue pathway versus another, and iii) isolate and grow trophoblast stem cells for use in models of implantation. In order to achieve this, we will obtain embryos that are surplus to clinical requirements and donated by couples attending the Oxford IVF Unit with informed consent. These will be processed for stem cell derivation by mechanical isolation of the pluripotent stem cells from the inner cell mass of the embryo and of the trophectoderm, and maintaining them in culture. So far we have derived and characterised four hES cell lines, OxF1-4, and we will now go to test their differentiation capacities.

Proposed work

The main objectives of the research are essentially identical to those in RO143. 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 extracellular matrices that mimic that of feeder cells that support hES cell derivation and of the in vivo niche of pluripotent embryonic stem cells, and to test the capacity of this synthetic extracellular matrix to support derivation and early stage self-renewal.

Fresh embryos donated for research will be obtained from the Oxford Fertility Unit in the new IRS building. For some experiments embryos will be cultured to the blastocyst stage in the new embryo research laboratory in the IRS facility under the new research licence, dissociated and processed for hES cell derivation. However, for other experiments embryos will be transported at the cleavage or blastocyst stages to the existing facility in the Nuffield

Department of Obstetrics and Gynaecology (NDOG) and cultured under the existing R0143 licence. The reason for this is that the feeder cells, and other growth substrates are prepared in NDOG and the image analysis system used for these studies is also located there. Given the space limitations and the cost it is not possible to duplicate these facilities in the IRS.

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. Once the embryo has been dissociated it is no longer viable.

The proposed studies involve essentially a continuation of the current research in RO143. 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, selfrenewal 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.

So far we have been unable to derive trophoblast stem cells. However the method of mechanical isolation of the inner cell mass, which we adopted latterly in preference to immunosurgery, leaves the trophectoderm more or less intact, and we will plate these onto feeders with the aim of deriving trophoblast stem cells.

In addition we will determine the fate of pluripotent embryonic stem cells in vivo in peri- and post- implantation human embryos by immunohistochemical detection of markers of pluripotency and differentiation in an in vitro model for implantation, as approved in RO111. This will greatly enhance our understanding of very early human development, which so far has been intractable.

Background

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.

Research undertaken to date under licence R0143 relevant to the proposed project
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.

Results
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.

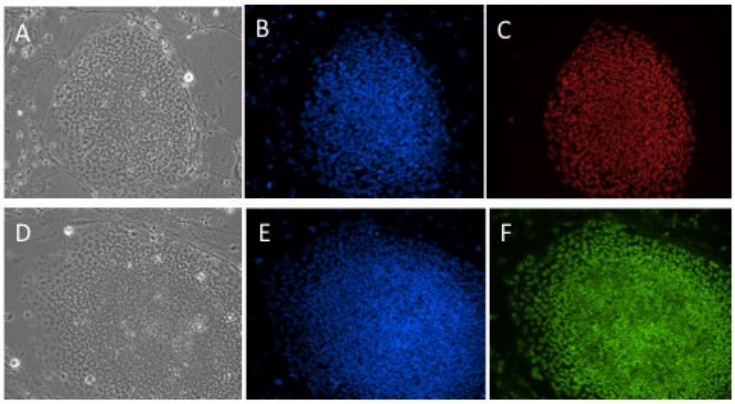


Figure 1: Staining of two colonies of OxF1 (A-C and D-F) for markers of pluriotency. A, D phase contrast; B, E DAPI; C, Oct 4; F Nanog.

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.

Publications arising from R0143 to date and relevant to the proposed research project
Brook F, Cowley S, Evans E, Turner K, James WS and Mardon H, Characterisation of the OxF1 human embryonic stem cell line. In vitro Cell Dev Biol (revised manuscript submitted).

References
Amit M, Shariki C, Margulets V, Itskovitz-Eldor J (2004) Feeder layer- and serum-free culture of human embryonic stem cells Biol Reprod 70(3), 837-45
Braam SR, Zeinstra L, Litjens S, Ward-van Oostwaard D, van den Brink S, van Laake L, Lebrin F, Kats P, Hochstenbach R, Passier R, Sonnenberg A, Mummery CL Recombinant vitronectin is a functionally defined substrate that supports human embryonic stem cell self-renewal via alphavbeta5 integrin, Stem Cells, 2008, 26(9):2257-65
Draper JS, Moore HD, Ruban LN, Gokhale PJ, Andrews PW (2004) Culture and characterization of human embryonic stem cells Stem Cells Dev 13(4), 325-36
Mallon BS, Park K-Y, Chen KG, Hamilton RS, McKay RDG (2006) Towards xeno-free culture of human embryonic stem cells. Int. J. Bioch.Cell Biol. 38, 1063-1075
Richards M, Fong C-Y, Chan W-K, Wong P-C and Bongso A (2002), Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells, Nature Biotech, 20 933-936
Rosler ES, Fisk GJ, Ares X, Irving J, Miura T, Rao MS Carpenter MK (2004) Long-term culture of human embryonic stem cells in feeder-free conditions. Dev Dyn 229, 259-274
Strom S, Inzunza J, Grinnemo K-H, Holmberg K, Matilainen E, Stromberg A-M, Blennow E, Hovatta O (2007) Mechanical isolation of the inner cell mass is effective in derivation of new human embryonic stem cell lines. Human Reprod 22, 3051-3058
Xu C, Inokuma M, Denham J, Golds K, Kundu P, Gold J, Carpenter M (2001), Feeder-free growth of undifferentiated human embryonic stem cells, Nat Biotech, 19, 971-974

Peer review comments (if applicable)

The peer reviewer used a renewal application peer review form, submitted on 24/08/2009. It was clear that they considered that they had enough information available in the application

to recommend acceptance in its current form.

The peer reviewer concurred with the applicants in that the research proposed complied with the following defined purposes: increasing knowledge about the development of embryos; enabling any such knowledge to be applied in developing treatments for serious disease.

The peer reviewer noted the previous reasonable progress on project R0143 – 4 stem cell lines derived.

The peer reviewer considered the research proposed was justified but did not add a reason for this. They later stated regarding their opinion that the derivation of stem cells was justified:
'Derivation of a large number of hESC 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 hESC lines that determine specific differentiation potential.

Issues for consideration
None
Executive recommendations for Licence Committee
None
Areas not covered on this inspection
All areas covered

Report compiled by:

Name	Andrew Leonard
Designation	HFEA inspector
Date	6th September 2009

Appendix A: Centre Staff interviewed

PR

Appendix B: Licence history

None. New licence application

Appendix C:

RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number 0035

Name of PR Dr Karen Turner

Date of Inspection 25th August 2009

Date of Response

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

No appendix C response was received

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).

No appendix C response was received

HFEA Research Licence Committee Meeting

16 September 2009

21 Bloomsbury Street London WC1B 3HF

Minutes – Item 2

Oxford Fertility Unit (0035; R0143) – New licence application to allow project R0143 at Centre (0035) to be carried on in new premises

Members of the Committee:	Committee Secretary:
Emily Jackson (lay) – Chair Richard Harries (lay) Hossam Abdalla (clinician)	Kristen Veblen Legal Adviser: Stephen Hocking, Beachcroft
Apologies: David Archard (lay) Lesley Regan (clinician)	

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 (104 pages)
- no tabled papers

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 considered the papers, which included the new licence inspection report, the application form with ethical approval, staff

curriculum vitae, peer review, patient information and consent forms and relevant publications. Additionally, the Committee noted that a response to the report from the Person Responsible (PR) tabled for the previous item also applied to this item.

2. The Committee noted that the inspection had taken place on 25 August 2009 and that, in the PR's response, she had undertaken to make all of the necessary amendments to the patient information that had been outlined in the report. Further the Committee noted in her response that the PR had committed to recruit no further patients until the patient information was fully compliant.

3. Additionally, the Committee noted that on the form titled, "Patient Consent to Research: Research and training projects using surplus eggs and embyros" point 5 should qualify the statement that "consent may be withdrawn at any time" with the statement that this is only possible until the embryos had been used in research, as was the case in the third paragraph of the patient consent to research form titled 'Derivation of human embryonic stem cell lines'.

4. The Committee additionally endorsed the inspectorate's recommendation that, in the event that frozen embryo donors were actively recruited to this proposed project, the PR should audit patient information provided to those donors against HFEA requirements S8.3.1 and S.8.3.2 a-d, G.5.13.1 a-l, and Licence Condition A.19.6 (d).

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

5. The Committee identified the activities to be authorised by a licence as storage of embryos, use of donated embryos for research and derivation of human embryonic stem cell lines. The Committee agreed that they were satisfied that these activities were not prohibited under the HFE Act 1990 (as amended).

6. The Committee decided that these activities were necessary and desirable for the following purposes:
   - increasing knowledge about the development of embryos
     HFE (Research Purposes) Regulations 2001 2(2)(a)
   - enabling any such knowledge to be applied in developing treatments for serious disease
     HFE (Research Purposes) Regulations 2001 2(2)(c)

7. The Committee decided that it was satisfied that the proposed use of embryos was necessary and desirable for the purposes of this research.

In making this decision, the committee took into account that the derivation of a significant number of hESC lines would be necessary to gain a better understanding of the underlying mechanisms and conditions that regulate differentiation along specific pathways and the properties of hESC lines that determine specific differentiation potential.

8. The Committee noted that, with the changes the PR had undertaken to make, as well as the two amendments outlined above, it considered itself satisfied that the patient forms were fit for purpose. Additionally, the Committee again noted and commended the PR's commitment not to recruit any further patients until the forms were fully compliant. The Committee also again endorsed the recommendation outlined above that the PR should audit the patient information forms if frozen embryo donors were to be actively recruited.

9. The Committee considered itself satisfied that it was appropriate to grant a licence, noting that the appropriate fee had been paid.

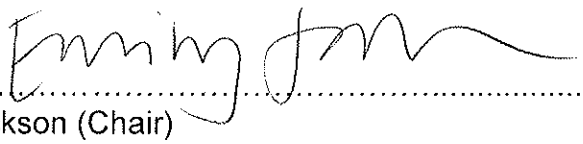
10. The Committee agreed that it was satisfied as to the character, qualifications and experience of the Nominal Licensee. Also, the Committee agreed that it was satisfied as to the character, qualifications and experience of the Persons Responsible as required for the supervision of the activities to be discharged under Section 17 of the HFE Act 1990 (as amended).

11. Further, based on the evaluation of the inspectorate, the Committee agreed that it was satisfied that the premises for which the Licence was to be granted were suitable for the activities outlined.

12. The Committee considered that usually a one year licence was given for a new research project. However, the Committee considered that in this case, and with the recommendation of the inspectorate, a three year licence would be more appropriate, considering that this project was, in fact, a continuation of an existing project at different premises, and that none of the reasons usually given for granting a one year licence applied in this case.

13. In the interest of efficiency and to reduce the regulatory burden on the clinic, the Committee decided to grant this licence for a period of 2 years and 11 months, with no additional conditions, so that the course of the related licences would now run concurrently, and be subject to renewal inspections and licence committee consideration at the same time.

14. The Committee requested that in future matters related to these related licences be brought to the same meeting of the Committee.

Signed.....  Date.....25.9.09.
Emily Jackson (Chair)