Research Licence Interim Inspection Report

Project Title Research Licence Number Person Responsible Nominal Licensee Inspection type Licence expiry date Date Renewal fee paid	**Development of a model to study implantation in the human** R0111 Karen Turner Ian Sargent **Interim** 31 August 2009 N/R
Project Title Research licence Number Person Responsible Nominal Licensee Inspection type Licence expiry date Date Renewal fee paid	**To derive human embryonic stem cells and trophoblast cell lines** R0143 Karen Turner Helen Mardon **Interim** 31 August 2009 N/R
Project Title Research licence Number Person Responsible Nominal Licensee Inspection type Licence expiry date Date Renewal fee paid	**To develop pre-implantation genetic diagnosis (PGD) for mitochondrial DNA disease** R0149 Karen Turner Stephen Kennedy **Interim** 31 August 2009 N/R
Centre Number	0035
Centre Name	Oxford Fertility Unit
Centre Address	Level 4, Women's Centre, John Radcliffe Hospital, Oxford, OX3
Treatment centres donating to these research projects	0035 – Oxford Fertility Unit 0139 – Bath Assisted Conception Clinic 0064 – BMI The Chiltern Hospital Fertility Services Unit
Inspection date	24th June 2008
Licence Committee Date	16th September 2008
Inspector(s)	Andy Leonard, Sarah Hopper, Ellie Suthers (Observer)

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 2007 and 24th June 2008.

Brief Description of the Project

The Centre appeared well organised. All three projects (R0111, R0143, R0149) are housed within the Academic Department of Obstetrics and Gynaecology, Oxford University, in designated licensed premises on the same floor and the floor below the Oxford Fertility Unit (Centre 0035), within the John Radcliffe Hospital, Oxford. The vast majority of embryos used in the research projects are derived from Centre 0035 (968 embryos), the other contributing centres (Centres 0139 and 0064) having contributed 60 embryos between them in the last year. Lay summaries for the projects are:

Project R0111: "**Development of a Model to Study Implantation in the Human**" :
Pre-implantation embryos produce a range of factors which are important in the implantation process. One such factor, called HLA-G, is believed to play a key role in preventing the implanting embryo from being rejected by the mother's immune system. Recent reports in the literature have suggested that measuring HLA-G in the culture medium from IVF embryos may allow embryologists to predict which embryos are most likely to implant and form pregnancies. If true, this could have a major impact on IVF success rates as it would provide a way of selecting the "best" embryos to transfer. However, not all researchers agree with these findings and there are some doubts about the accuracy of the test for HLA-G. We have therefore investigated the expression of HLA-G at different stages of embryo development and, contrary to the published work, have been unable to find it in the early stages (2-8 cell) when IVF embryos are normally replaced in the mother. We are now extending our studies in collaboration with eight other IVF Units as part of a European Network of Excellence set up to investigate the control of embryo implantation. We have also set up novel experimental models to explore further the molecular events that underpin implantation. These have revealed other factors, including soluble growth factors that are produced by both the embryo and the endometrium, and proteins that exist in a matrix surrounding the cells that make up the endometrium that appear to be important in implantation. We will now work out at what stage in the implantation process they are important and what their function is. In addition there are likely to be many other molecules that are produced in the endometrium as the embryo implants that are required for successful implantation. We are identifying such molecules by a technique called DNA microarray profiling, and will go on to validate their production and function in our experimental model systems.

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 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, 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.

Project R0149: "**To Develop Pre-implantation Genetic Diagnosis (PGD) for Mitochondrial DNA Disease derive human embryonic stem cells and trophoblast cell lines**"

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

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

The centre has held licences for these projects since 1998 (R0111), 2003 (R0143) and 2004 (R0149). All three licenses have the same PR and are due to expire on 31st August 2009.

Licensed activities are:

		R0111	R0143	R0149
Research activities	Research on human embryos	✓	✓	✓
	Storage of licensed material	✓	✓	
	Creation of embryos for research			
	Derivation of human embryonic stem cells		✓	
	Cell nuclear replacement			

Summary for Licence Committee

The inspectorate were satisfied that research at centre 0035 is well organised and is carried out in a professional manner which complies in most areas with the Code of Practice, 7th edition. There were a few areas of concern:

- A procedure for reporting serious adverse events to HFEA must be developed to ensure compliance with General Licence Condition A.4.1 and Code of Practice, 7th edition, Standards S.9.4.1 and S.9.4.2.
- In a few cases, one having been revealed during the inspectorate audit of patient consents, consents have been collected close to embryo transfer. The inspectorate expressed concern with this practice as it may be contrary to Code of Practice, 7th edition, Standard, S.8.4.2 (c), as little time is allowed for the patients to consider consenting to the donation. The inspectorate were assured by the PR that all research consenting patients were provided with information well before the start of treatment and were well informed, had considerable time to consider that information, had access to further information via the Research Nurse, were not subjected to coercion or financial incentives, and thus provided appropriate informed consent. The PR also asserted that the practice of consenting close to or at egg collection only occurs if a patient has expressed a desire to complete research consent forms at an early stage, but has failed to do so due to other commitments. The PR considers these patients have had enough time to consider their decision and contact the Research Nurse to discuss their donation prior to signing consent forms, thus informed consent can still be collected.
- The information sheets 'research and training projects using surplus eggs and embryos, Pt Info 2 v.2' and 'Donating frozen embryos to research, v.1' are the only information/consent forms provided to patients consenting to donation of fresh and frozen embryos, respectively, to projects R0111 and R0149. Both sheets were reviewed and were found to not include several pieces of information required by the Code of Practice, (e.g. Standards S.8.2.1 and S.8.3.2, and Guidance G.5.13.1 (a,e,f), as listed in Section 4 in this report. The PR should review these documents and the verbal information provided by the Research Nurse, to ensure patients receive verbally and/or in writing, all information required by the Code of Practice, 7th edition.

The inspectorate also note the following issues regarding which recommendations are made:

- The SOPs related to research practices are not formally document controlled, although SOPs within the clinical (i.e. the Oxford Fertility Service) quality management system are. Document control is not a requirement of a research licence but the inspectorate recommends that some system of document control is implemented in their research SOPs to ensure that all researchers use the same up-to-date procedures.

The inspectorate recommend continuation of research licences R0111, R0143 and R0149

Proposed licence variations

It is proposed to add storage of embryos as a licensed activity to Licence R0149

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 R0111

Principal investigator	Ian Sargent
Scientists	5 research embryologists 2 clinical embryologists
Laboratory technicians	0
Support staff (receptionists, record managers, quality and risk managers etc)	1

Staff R0143

Principal investigator	Helen Mardon
Research technicians	1
Scientists	2
Support staff (receptionists, record managers, quality and risk managers etc)	2

Staff R0149

Principal investigator	Joanna Poulton
Research technicians	0
Scientists	1
Support staff (receptionists, record managers, quality and risk managers etc)	2

Highlighted areas of firm compliance
The Person Responsible (PR) on all the projects is Dr Karen Turner, with each project having a designated lead investigator. The PR has been in post since before the current licenses were issued and has completed the PREP. The PR has extensive knowledge of the regulatory requirements of the HFEA through her long experience as an IVF Laboratory Manager and also as an external advisor to the HFEA. The PR attends the Centre full-time. PR interview and inspection of the premises and activities indicated that the projects are well lead and managed. The PR was obviously familiar with the research projects and hosted a tour of the premises during which it was clear the licensed premises were under her control.

The projects were considered by the inspectorate to be well staffed. A new dedicated Research Nurse is in place to recruit patients to the research projects, working Monday – Thursday, albeit with shorter hours on Tuesday and Thursday. Patients are booked to see her during these times however as a contingency, the Research Nurse has given a presentation to nursing staff to increase their awareness of research so that they can provide advice in her absence if patients require quick answers on an issue. The inspectorate advise that she repeat this presentation annually and to new nursing staff during their induction, to maintain awareness of research amongst clinical staff. The Research Nurse regularly meets with the researchers and is kept up to date with research activities and implications for donors, as evidenced in the minutes of a recent meeting.

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 staff training meets the requirements of Oxford University and involves attendance at conferences, seminars and internal and external training programmes. Continual professional development (CPD) files were available and one was reviewed which detailed 5 items of CPD in the past year.

Funding with finishing dates is in place for each project to the end of the current licence periods, as detailed below;

R0111	R0143	R0149
European Network of Excellence on Embryo Implantation Control (EMBIC) (Sept 2008) MRC (2010) Further applications pending and in preparation	MRC (2010) BBSRC (2009)	IVF Unit Research Funds (on-going)

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 and provided a research staff list on request. The PR stated that all research staff with access to licensed material and patient information are on the research licence and also on the treatment and storage licence. The inspection team were informed by the PR and individual research staff that non-minuted meetings between the PR and lead investigators occur when needed, while 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. A formal research meeting between the PR, all researchers and the Research Nurse is held twice a year, at which research progress and all matters relating to the projects are discussed and minuted.

The Academic Department of Obstetrics and Gynaecology, is a component of Oxford University, which provides the corporate management structure within which the research

licenses operate, supplying 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.

The inspection team were informed by both the PR and individual research staff that minuted meetings between the PR and lead investigators occur when needed (2x per year), while the lead investigators and the researchers meet on a weekly basis. This provides an opportunity for cascading of HFEA Alerts and other essential information. Contacts between the researchers and clinical staff at Centre 0035 are as frequent as required for effective coordination of embryo supply and research activities. The research PR is also Head of Embryology in the clinical laboratory. Regular emails and telephone calls are used to communicate with licensed centres 0064 and 0139. The researchers provide research seminars at the end of the monthly clinical centre all staff meetings, as a means to feedback research progress to clinical staff.

Laboratory standard operating procedures (SOPs) were observed and have been previously reviewed by the inspectorate as fit for purpose. The PR and lead researcher on project R0111 considered the SOPs include all required laboratory methods. These are updated and added to as required.

Issues for consideration

- A procedure for reporting serious adverse events to HFEA should be developed to ensure compliance with General Licence Condition A.4.1 and Code of Practice, 7th edition, Standards S.9.4.1 and S.9.4.2.
- The SOPs related to research practices are not formally document controlled, although SOPs within the clinical (i.e. the Oxford Fertility Service) quality management system are. Document control is not a requirement of a research licence but the inspectorate recommends that some system of document control is implemented to ensure that all researchers use the same up-to-date procedures.

Executive recommendations for Licence Committee

The Licence Committee is asked to endorse the recommendations made in relation to the areas for improvement cited 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 Centre premises were well organised, clean and tidy on the day of inspection. All three projects (R0111, R0143, R0149) are administered by the Academic Department of Obstetrics and Gynaecology, in designated licensed premises on the same floor and the floor below the Oxford Fertility Unit. The research premises comprise a research-dedicated laboratory accessed via the andrology laboratory in Centre 0035, which contains a class II air flow cabinet, incubator and inverted microscope, and is used for embryo culture in projects R0111, R0143 and R0149. An additional laboratory dedicated to imaging is on the level below within the Academic Department of Obstetrics and Gynaecology, which contains an inverted microscope and a fluorescent microscope for vital time-lapse fluorescent microscopy, along with 2 computers for image process, storage and analysis, an air flow cabinet and an incubator. All licensed material and research records are confined to these two secure licensed laboratories, the latter stored in locked drawers. 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 imaging laboratory is secured with a Yale lock, the key to which is kept in the culture laboratory. Both laboratories are risk assessed annually by the Oxford University Clinical Research Safety Officer, along with other unlicensed premises in the Academic Department of Obstetrics and Gynaecology. The premises were last inspected in April 2007 so this inspection is now due. All research procedures have also 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 during the inspection of the treatment and storage licence, and are secure and equipped with a low oxygen monitor, fan extractor with boost connection to the low oxygen monitor, and low level nitrogen alarms. A procedure for responding to activation of the low oxygen alarm is in place. A documented system for equipment maintenance/servicing is in place; the laboratory head is the designated person responsible for the maintenance of equipment. He related that it was difficult to fund equipment maintenance contracts as such costs were not funded by many grant funding bodies. A microscope had failed last year and a PCR machine this year but the Centre staff thought these failures were not due to lack of maintenance as service contracts are, with difficulty, maintained and both items had been serviced. Inspection of some items of equipment indicated they were all within servicing intervals. 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 by early 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
The treatment and storage and research licences have different PRs and clinical and research practices are separated. The research activities all occur in the licensed research premises on the same floor and the floor below the Oxford Fertility Unit (Centre 0035). One area of close approach is that embryos consented for use in project R0149 are biopsied in the treatment laboratory of Centre 0035 to obtain blastomeres for PGD analysis. Another area 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. Embryos are obtained for research from within Centre 0035 (all projects), and also as frozen stored embryos, from the Bath Assisted Conception Clinic (Centre 0139; Project R0111 only) and the BMI Chiltern Hospital Fertility Services Unit (Centre 0064; Project R0111 and R0149). The Centre has an established research donor recruitment procedure. All couples treated at the centre are provided research information with their treatment information when they first visit the Centre at an open evening. When HFEA consent forms are signed, if patients indicate they wish to consent to research donation their consent is taken on the HFEA form and research information is again provided and a meeting booked with the Research Nurse to discuss donation. After consultation with the Research Nurse, if the patients wish to consent to research and feel all their questions have been answered, they sign a detailed research consent form which allows them to specify the projects to which they consent to donate. If patients need more time to consider their potential donation, this is provided. Research consents are normally collected before the start of treatment and well before egg collection. Cryopreserved donated embryos from Centres 0035, 0139 and 0064 are also used in projects R0111 and R0149. 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 projects R0111 and R0149, and a consent form. Patient information asks them to contact the Research Nurse with any questions they may have. The consent forms are then signed by the patients and embryos are allocated to either research project. 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. Before embryo transfer after 3 days of culture post-fertilisation, embryos are assessed for quality using morphological criteria by the clinical

embryologists, and the best one or two embryos selected for transfer. The centre have a specific procedure which defines the quality of embryos frozen thereafter for subsequent treatment (Grade A and B; 6 cells or more at day 3). Any remaining embryos not suitable for freezing 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 taken by the researchers 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 of the consents provided and on which researchers are available, and logged in the anonymisation book (detailing Centre number; research code; projects consented to; date of arrival in research; project allocated; researcher responsible). The documenting of the Centre number allows back-tracking from research records to patient records if required; patient records remain in Centre 0035 at all times.

The researchers have not carried out a specific audit of stored research material and this is done in conjunction with the treatment and storage dewar audits as embryo storage is in common dewars. The PR also said that cryopreserved embryos were rapidly used after transfer to research, normally within days. To prevent the small likelihood of a breach of a patient consented storage period, the PR outlined that embryos are stored in the dewars used by clinical embryology and documented within their dewar logs. Thus they are subject to the bring-forward system used within the clinical embryology laboratories to prevent storage beyond the consented storage period. If research-consented embryos approach the end of their consented storage interval, the clinical embryologists immediately inform the researchers who arrange for their thaw and use in research.

A research culture sheet labelled with the research code and well number 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.

Issues for consideration

- According to the PR, in a small number of cases consents have been collected close to embryo transfer, as revealed during the inspectorate audit of patient consents, in which one patient was consented the day before embryo transfer. The inspectorate expressed concern with this practice as it may be contrary to Code of Practice, 7th edition, Standard, S.8.4.2 (c), as little time is allowed for the patients to considered consenting to the donation. The inspectorate however were assured by the PR that all research consenting patients were provided with information well before the start of treatment and were well informed, had considerable time to consider that information, had access to further information via the Research Nurse, were not subjected to coercion or financial incentives, and thus provided appropriate informed consent. The practice of consenting

close to or at egg collection only occurs if a patient has expressed a desire to complete research consent forms at an early stage, but has failed to do so due to other commitments. The PR considers that these patients have had considerable time to consider their decision and contact the Research Nurse to discuss their research donation prior to signing consent forms, thus informed consent can still be collected.

- The Research Nurse is funded by the Wellcome Trust Grant which partially funds project R0111, thus her independence from the research projects is not necessarily complete.

Executive recommendations for Licence Committee
The Licence Committee is asked to endorse the recommendations made in relation to the areas for improvement cited above.
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
Eight sets of patient records were reviewed for research consents. The research embryo log book and the ultimate project in which the embryos were used were also reviewed to ensure consents were complied with. While 7 patient notes indicated consents were collected well before oocyte collection as expected given the centres donor recruitment procedures, one patient had signed specific research consent forms the day before embryo transfer. This case is discussed above in Section 3 regarding donation. The embryologists also failed to sign in this patient's notes to confirm that the research consent was checked before transfer to the researchers. It was noted in 2 sets of patient records that only a general research consent was collected. It was apparent that these donations were made more than one year before the date of inspection and reflect an older working practice. Notes related to the 6 recent donations all detailed project specific consent forms which had been appropriately completed. All eight sets of embryos were used in a project to which the patient had consented and there was no evidence that patient consents had been breached
Highlighted areas of firm compliance
The patient information and consent form specifically used for project R0143, 'The generation of human embryonic stem cells, version 1', was reviewed and found to fulfil all the information requirements for stem cell research projects specified by Licence Condition A.19.6 (d). The general patient research information and consent form, which describes projects R0111, R0143 and R0149 was also reviewed (Research and training projects using surplus eggs and embryos, Pt Info 2 v.2), as was the patient information and consent form provided to patients with frozen embryos (Donating frozen embryos to research, v.1), which describes only projects R0111 and R0149. These forms were well presented and broadly compliant with the Code of Practice requirements regarding information provided to research donors, some issues of content are raised below. Variable consents are possible as the consent form allows the provision of consent for each research project individually.
Issues for consideration
The form 'research and training projects using surplus eggs and embryos, Pt Info 2 v.2' is the only information/consent sheet provided to patients consenting in the clinic to projects R0111

and R0149. For project R0143, patients also receive the information sheet 'the generation of human embryonic stem cells, version 1'. This latter form was compliant with the Code of Practice information requirements, but it would appear that patients consenting for R0111 and R0149, who only receive the former, i.e. 'research and training projects using surplus eggs and embryos, Pt Info 2 v.2', are not necessarily informed about:

- The source of funding of the projects, any payments or benefits which accrue to the researchers and/or their departments, and any financial interests in the research projects or their sponsoring organisations, as required by Code of Practice, 7th Edition, Standards S.8.2.1.
- That the decision whether to donate will not affect their treatment in any way, whether the embryos are reversibly or irreversibly anonymised and its implications, and whether any results will be fed back to the research donors, as required by Code of Practice, 7th Edition, Standards S.8.3.2.
- That research is experimental and embryos donated to research can not be taken back and used in treatment, as required by Code of Practice, 7th Edition, G.5.13.1 (a)
- That the patients are under no obligation to donate embryos to the project and that their decision whether to do so will have no repercussion on any treatment they might receive, as required by the Code of Practice, 7th Edition, G.5.13.1 (e)
- That the patient may specify conditions to their consent subject to which the embryos may be used, as required by Code of Practice, 7th Edition, G.5.13.1 (f)

The situation regarding the form 'Donating frozen embryos to research, v.1' is similar as it does not include:

- The source of funding of the projects, any payments or benefits which accrue to the researchers and/or their departments, and any financial interests in the research projects or their sponsoring organisations, as required by Code of Practice, 7th Edition, Standards S.8.2.1.
- That the decision whether to donate will not affect any potential future treatment, whether the embryos are reversibly or irreversibly anonymised and its implications, and whether any results will be fed back to the research donors, as required by Code of Practice, 7th Edition, Standards S.8.3.2.
- That research is experimental and embryos donated to research can not be taken back and used in treatment, as required by Code of Practice, 7th Edition, G.5.13.1 (a)
- That the patients are under no obligation to donate embryos to the project and that their decision whether to do so will have no repercussion on any future treatment they might receive, as required by Code of Practice, 7th Edition, G.5.13.1 (e)
- That the patient may specify conditions to their consent subject to which the embryos may be used, as required by Code of Practice, 7th Edition, Guidance G.5.13.1 (f)

It is accepted that these information requirements may be satisfied verbally by the Research Nurse, albeit this is less likely to happen in the case of patients at home consenting for research donation of cryopreserved embryos. It should also be noted that Standard S.8.3.2 specifically requires the provision of written as well as verbal information regarding the points of information to which this Standard refers.

It is recommended that the PR review the information sheets 'research and training projects using surplus eggs and embryos, Pt Info 2 v.2' and 'donating frozen embryos to research, v.1' and the provision of verbal information by the Research Nurse, to ensure patients are provided with all information required by the Code of Practice, 7th edition. .
Executive recommendations for Licence Committee
The Licence Committee is asked to endorse the recommendations made in relation to the areas for improvement cited above.
Areas not covered in by this inspection
All covered

5. Scientific practice R0104, Maturation and fertilisation of human eggs in vitro

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

Summary of:

- Peer review

Summary
All projects inspected were on interim inspections therefore no peer reviews were collected. When embryos are accepted onto the research projects, they are cultured in the research culture laboratory between day 3 post-fertilisation and day 6, at which point they are discarded if non-viable or used in research. If allocated to project R0111, they are cultured on endometrial cell layers for 2 days then fixed and subjected to further investigation. If allocated to project R0143, embryos at the blastocyst stage are subjected to further culture on feeder cell layers which promote embryo adhesion, impaction and outgrowth and allow stem cell derivation. The embryo as a licensed entity is non-existent after this process. If allocated to project R0149, the embryo is biopsied on day 3 and a blastomere removed, lysed and stored frozen, while the embryo is retained in culture for 24 hours to determine survival and morphology, before being made non-viable and disposed of. Normal working practices mean that no embryos are cultured for more than 8 days post-fertilisation before being rendered non-viable. The centre also has a procedure which states that no embryos should be cultured for 14 days post-fertilisation. *Research project lay summaries:* **Project R0111: "Development of a Model to Study Implantation in the Human"** Pre-implantation embryos produce a range of factors which are important in the implantation process. One such factor, called HLA-G, is believed to play a key role in preventing the implanting embryo from being rejected by the mother's immune system. Recent reports in the literature have suggested that measuring HLA-G in the culture medium from IVF embryos may allow embryologists to predict which embryos are most likely to implant and form pregnancies. If true, this could have a major impact on IVF success rates as it would provide a way of selecting the "best" embryos to transfer. However, not all researchers agree with these findings and there are some doubts about the accuracy of the test for HLA-G. We have therefore investigated the expression of HLA-G at different stages of embryo development and, contrary to the published work, have been unable to find it in the early stages (2-8 cell) when IVF embryos are normally replaced in the mother. We are now extending our studies in collaboration with eight other IVF Units as part of a European Network of Excellence set up to investigate the control of embryo implantation. We have also set up novel experimental models to explore further the molecular events that underpin implantation. These have revealed other factors, including soluble growth factors that are produced by both the embryo and the endometrium, and proteins that exist in a matrix surrounding the cells that make up the endometrium that appear to be important in implantation. We will now work out at what stage in the implantation process they are important and what their function is. In addition there are likely to be many other molecules that are produced in the endometrium as the embryo implants that are required for successful implantation. We are identifying such molecules by a technique called DNA microarray profiling, and will go on to validate their production and function in our experimental model systems.

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.

Project R0149: "To Develop Pre-implantation Genetic Diagnosis (PGD) for Mitochondrial DNA Disease derive human embryonic stem cells and trophoblast cell lines"
Mitochondrial diseases affect about 1 in 10,000 people in the UK. Examples include Maternally inherited Leigh Syndrome (MILS), Myoclonic epilepsy and ragged red fibres (MERRF) syndrome, Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS), and Pearson syndrome. The diseases may involve any parts of the body that have a high energy demand (such as brain, heart muscle and liver), because mitochondria are the "power houses" in cells.

Some women are carriers of defective mitochondrial genes. This means that they carry both normal and damaged mitochondrial genes, and can pass these devastating conditions on to their children. It is difficult to advise these women about the size of their individual risk, because the rules governing transmission of mitochondrial genes are not well understood. The aim of this research is to establish the techniques for identifying defects in mitochondrial DNA in human embryos created by IVF to allow the selection of non-affected embryos for transfer. This technique is referred to as pre-implantation genetic diagnosis (PGD).

We have demonstrated that we can successfully sample embryos and have started to develop our analysis of their mitochondrial genes. So far we have identified one woman in whom we were able to assess the way that the mitochondrial genes are transmitted. Preliminary results from this woman's embryos were generally encouraging. Further work is required to provide basic scientific information and enable us to determine whether PGD will be useful in certain mtDNA diseases.

Licences granted for the purposes of:

	Purpose or purposes of the research project	R0111	R0143	R0149
4.3.1	promoting advances in the treatment of infertility *Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(a)*	☒	☐	☐
4.3.2	increasing knowledge about the causes of congenital disease *Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(b)*	☐	☐	☐
4.3.3	increasing knowledge about the causes of miscarriages *Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(c)*	☒	☐	☐
4.3.4	developing more effective techniques of contraception *Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(d)*	☐	☐	☐
4.3.5	developing methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation *Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(e)*	☐	☐	☒
4.3.6	increasing knowledge about the development of embryos *Human Fertilisation and Embryology (Research Purposes) Regulations 2001 s2(a)*	☒	☒	☐
4.3.7	increasing knowledge about serious disease *Human Fertilisation and Embryology (Research Purposes) Regulations 2001 s2(b)*	☐	☒	☐
4.3.8	enabling any such knowledge to be applied in developing treatments for serious disease *Human Fertilisation and Embryology (Research Purposes) Regulations 2001 s2(c)*	☐	☒	☐

Usage and expected usage in next year:
Project R0111 is supplied with fresh and frozen embryos from Oxford Fertility Unit (0035), Bath Assisted Conception Clinic (0139) and BMI The Chiltern Hospital Fertility Services Unit (0064). Project R0143 is supplied from Centres 0035 and 0064. Project 0149 is supplied from Centre 0035 alone.

(01/03/07 – 29/02/08)	**R0111 (fresh/frozen)**		**R0143 (fresh/frozen)**		**R0149 (fresh/frozen)**	
Usage	*supplied*	*used*	*supplied*	*used*	*supplied*	*used*
0035	719/127	719/116	178/0	36/0	71/0	29/0
0139	0/60	0/47	0/0	0/0	X	X
0064	0/0	0/0	0/0	0/0	X	X
	R0111 (fresh/frozen)		**R0143 (fresh/frozen)**		**R0149 (fresh/frozen)**	
(01/03/08 – 28/02/09)	*fresh*	*frozen*	*fresh*	*frozen*	*fresh*	*frozen*
Proposed total usage	750	200	120	0	100	0

Reasons for discrepant usage were:
R0111: The estimated usage for the last 12 months was 960 fresh and 240 frozen embryos. The actual use was 719 fresh and 179 frozen. The small differences were due to more embryos being suitable for freezing for clinical treatment and others being used in projects R0143 and R0149.

R0143: The estimated use for the last 12 months was 100 fresh and 30 frozen embryos. The

actual usage was 178 fresh embryos supplied and 36 used, while 0 frozen embryos were supplied. The discrepancy in fresh embryos supplied and used (178 versus 36) is due to the centre not counting as used on the project, embryos which have been cultured between day 3 and 6 under the research programme but which have then been considered non-viable and unsuitable for stem cell derivation. This was discussed on inspection and from now on all embryos cultured on the research licence will be counted as used in the project, even if non-viable and discarded at day 6.

R0149: Embryo biopsy to obtain blastomeres for genetic analysis has started and so a number of embryos have been used for this. The numbers of embryos biopsied is not substantially different to those estimated 1 year ago. The number of embryos from which blastomeres were obtained is not the same as the number of embryos used since, particularly in the initial stages, blastomeres were not successfully biopsied. In addition, many of the embryos were of very poor quality and highly fragmented, such that obtaining proper cells was extremely difficult. Blastomeres obtained have been stored as blastomere analysis has not proceeded due to a lack of funding for suitable research staff. This situation will change in the near future with the initiation of the PGD programme aided by the appointment Dr Dagan Wells. Funding for a PhD student to work on the genetics aspect of the project is also in place.

Summary of audit of stored and biopsied material

No licensed materials were in store on the day of inspection.

Project R0111: "Development of a Model to Study Implantation in the Human"

Renewed project objectives
No changes to project aims and objectives

Summary of research undertaken

A) How the work undertaken relates to the objectives.

The purpose of this project is to develop in vitro models to study how the human embryo attaches, invades and interacts with the different cell populations of the endometrium during implantation and the molecules which are involved in these processes. The development of these models has continued at different levels. Firstly, studies are being carried out to look at the expression of molecules thought to be involved in implantation in pre and peri-implantation embryos. Secondly, the dissection of molecular events involved in implantation is being investigated by studying the interactions between embryos and defined molecules in vitro and thirdly, we are studying the interaction between blastocysts and purified populations of endometrial stromal and epithelial cells in the presence or absence of specific receptor agonists/antagonists. The different cellular and molecular events involved in implantation are also being studied in 3-dimensional models. In these models, three-dimensional culture systems that comprise i) human endometrial epithelial and stromal cells and extracellular matrix that imitates the architecture of the normal endometrium, and ii) microbiopsies of endometrial tissue are being used.

B) Research undertaken to date.

During the period of this report, work has continued in two main areas of research:

(i) Detection of molecules involved in the implantation process in pre- and peri implantation embryos

Pre-implantation embryos express a range of molecules that may be involved in the implantation process. Documentation of both their pattern and levels of expression throughout embryo development to the blastocyst stage are essential to our understanding of their potential roles. To develop the necessary techniques for these studies we have focussed on one particular molecule, HLA-G, with which we have extensive experience.

Human leukocyte antigen G (HLA-G) is a virtually non-polymorphic HLA class I gene expressed by the implanting embryo, which is believed to play a key role in maternal immune tolerance of the fetus. In addition to its effects on T cells, it is now known that both decidual macrophages and natural killer (NK) cells express specific receptors for HLA-G and that binding of these receptors may trigger the release of a range of cytokines involved in implantation and angiogenesis. HLA-G mRNA can be alternatively spliced into six principal transcripts, which encode four membrane bound isoforms (G1, G2, G3, G4) and two soluble isoforms (G5 and G6).

There has been a growing interest in HLA-G expression by human embryos as there have been published reports suggesting that the levels of soluble HLA-G secreted into the culture medium by an IVF embryo could be used as a diagnostic marker of its potential to implant. However, not all studies support this finding. Our previous work on this project has shown that the percentage of embryos expressing each HLA-G isoform mRNA increased with developmental stage but, contrary to expectation, soluble HLA-G5 mRNA was not detected in

single 2-8 cell embryos and was only expressed by 20% of morulae and blastocysts. This is at a lower frequency than the reports of the detection of soluble HLA-G protein. This disparity between mRNA and protein may be due to the HLA-G protein in the embryo being produced from maternal oocyte mRNA stores prior to embryonic genome activation and brings into question the measurement of soluble HLA-G for clinical evaluation of embryo quality.

(ii) Experiments involving models to study the molecular basis of implantation
The implantation models described above are currently being used to investigate further the function of extracellular matrix molecules, and of members of the Rho family of GTPases in implantation. This is being achieved by the use of targeted inhibition of specific molecules in the endometrial cell-blastocyst co-cultures by specific inhibitors that we have previously used successfully for inhibition of endometrial cell function. In particular we aim to target the Rho family of GTPases, by the use of small RNA inhibitors and/or dominant-negative mutant cDNAs transfected into the trophectoderm of peri-implantation blastocysts. All the techniques are worked up in mouse embryos prior to experiments with human embryos.

In a second approach, the modulation of molecules expressed by the endometrium in response to the implanting blastocyst is being investigated. Several studies have previously identified molecules that are either up- or down- regulated in the endometrium during the window of implantation. However it is likely that the implanting embryo induces expression or inhibition of specific molecules in the endometrium as it attaches and invades the tissue, as has been shown in the rodent. The models we have developed thus provide an excellent system to investigate this, by means of DNA profiling, in the human.

C) Results
Measurement of soluble HLA-G in IVF embryo culture medium
The Oxford Fertility Unit is part of a European Network of Excellence on Embryo Implantation Control (EMBIC), which, amongst many other projects, is investigating whether there is a correlation between soluble HLA-G expression and embryo quality in vitro. This study has involved collaboration between two IVF Units in France (Paris and Toulouse), one in Belgium (Liege) and Oxford. Unfortunately, the embryo culture conditions used in Oxford (500µl wells) means that any soluble HLA-G present is too dilute to be measured by the current HLA-G ELISA. However, 1405 embryo culture supernatants from the other IVF Units who all culture embryos in 50µl (i.e. 10x more concentrated) have been studied. In only one centre was a significant correlation between the presence of sHLA-G and successful implantation found. Furthermore, it was apparent that the numbers of sHLA-G positive embryos and the levels of sHLA-G they produced were dependent of the culture media and conditions use in each IVF Unit. Overall these results do not suggest that sHLA-G is a reliable marker for selecting the best embryos to transfer in IVF. As a follow up to this, EMBIC has organised an International Workshop on the sHLA-G and implantation, which will be hosted by members of the Oxford Fertility Unit research team at Keble College Oxford in June 2008.

Maternal factors which may upregulate HLA-G expression during implantation
Although measurement of sHLA-G in embryo culture supernatants may not yet be a reliable method of selecting the best embryos for transfer, HLA-G expression is still very likely to play an important role in blastocyst implantation. An important question is how factors (growth factors and cytokines) produced by the endometrium might upregulate embryo HLA-G expression and thereby improve implantation. In particular LIF, IFNγ, progesterone and IL-10

have been reported to upregulate HLA-G on trophoblast. Our aim is therefore to investigate the effect of these factors on HLA-G expression by human blastocysts. The ultimate aim would be to use these factors clinically to improve blastocyst implantation. To study this we have initially been using two models; human trophoblast (choriocarcinoma) cell lines and bovine embryos:

Human trophoblast cell lines

These studies have utilised two human HLA-G expressing choriocarcinoma (trophoblast) cell lines called JEG3 and AC1M59, together with an HLA-G negative choriocarcinoma (JAR) as a control. LIF and IFNγ have been found to upregulate HLA-G protein expression using flow cytometry in JEG3 cells but progesterone and IL-10 had no effect. Recently, we have also looked at the effect of Galectin 1, an immunoregulatory molecule which plays a pivotal role in materno-fetal tolerance in the mouse, and shown that it significantly upregulates HLA-G protein expression on JEG3 cells, as measured by flow cytometry.

It is also proposed to study the changes in expression of the 6 different HLA-G mRNA isoforms using real-time PCR. However this has proved to be technically challenging as the JEG3 cells have low copy numbers and sizes of the amplicons required to discriminate between the different isoforms are at the limits of the of optimal conditions for the SYBR green system used. When these problems have been resolved, the primers will be used to measure HLA-G isoforms in human embryos with and without LIF and IFNγ stimulation. Two human blastocysts have been examined to date and successful amplification of the 18s and RPL19 housekeeping genes has been achieved. A further 23 blastocysts have been snap frozen prior to lysis for RNA extraction.

Bovine embryos

Given the small numbers of human embryos available for research, we have also been exploring the use of bovine embryos as a model. One of our EMBIC collaborators, Dr Trudee Fair at University College, Dublin has shown that bovine embryos express a class I MHC molecule very similar to HLA-G. Dr Anna Swales from our Unit has therefore spent two months in Dublin investigating the effect of IFNγ and progesterone on bovine embryo development and class 1 MHC expression. No significant effects of these molecules on bovine embryo development were found. Class I MHC expression will be evaluated using real time PCR as for the human work and appropriate primers are being developed.

In vitro co-culture models for implantation

We will determine signalling pathways that are triggered in response to ECM-integrin interactions and that have a function in embryo implantation. These include focal adhesion kinase phosphorylation, which we will determine by high-resolution immunohistochemistry, and Cdc42/Rho/Rac pathways, which we will analyse with the use of specific inhibitors transferred into the trophectoderm of human blastocysts.

The endometrial cell-blastocyst co-culture models are being used to identify genes that are modulated in response to the implanting human blastocyst in vitro. This has been achieved by co-culturing peri-implantation human blastocysts and endometrial epithelial or stromal cells for 24-48 hours. The RNA from both the blastocysts dissected from the cultures at the end of the experiment, and the endometrial cell layer, cultured either alone or together, has been amplified and subjected to hybridisation with three types of either cDNA or oligo arrays. We have identified a number of potential candidates that are induced in response to the

blastocyst. We are now validating some of these molecules by real time RT-PCR and have begun to extend these validation studies to protein analyses. These experiments are particularly important since it has not been possible previously to study in the human genes that are modulated by the blastocyst, but such cross talk is likely to be very important in implantation, as shown in the mouse.

The cell-embryo co-culture model is being used to investigate the effect of gonadotrophin (GnRH) analogues used in IVF cycles on the stromal invasion stage of implantation. The experiments are performed in the presence or absence of GnRH agonist or antagonist and embryos are assessed according to the degree of invasion through the stromal layer. There is no difference in either attachment or invasion of the embryo indicating that this stage of implantation may not be affected by GnRH analogues.

The 3- D models are beginning to yield data showing expression of members of the EGF family and integrins, complementing our work on these molecules in solid phase end cell-embryo co-culture systems.

D) If progress was slower than anticipated, the reasons for this.
1) Problems with the real time PCR machine and primer design
The development of real time PCR for the measurement of HLA-G mRNA isoforms in human embryos has been delayed as the real time PCR machine broke down early this year and has yet to be replaced. Some progress has been made recently using a demonstration model, but primer design has proved difficult. It is hoped the Department will be in a position to purchase a new real time PCR in the near future. These problems are also comprimising our progress with validation of candidate genes identified in the array experiments
2) 3-D culture models
Robust 3-D cultures are difficult to establish, partly because the engineered endometrial cultures need to coincide with the availability of good quality human blastocysts. However more information can be gleaned from these cultures than the cell- embryo systems and we will therefore continue to perform these experiments.

E) Publications which have arisen from work under the licence.
There have been no new publications this year.

Previous Publications:
Sargent IL, Swales AK, Ledee N, Kozma N, Tabiasco J and LeBouteiller P (2007) sHLA-G production by human IVF embryos: can it be measured reliably? J.Reprod Immunol 75, 128-132.
Yao YQ, Barlow DH and Sargent IL. (2005) Differential expression of Alternatively Spliced Transcripts of HLA-G in Human Blastocysts and Inner Cell Masses. J Immunol, 175, 8379-8385.
Carver J, Martin K, Spyropoulou I, Barlow D, Sargent IL and Mardon, HJ (2003) An in vitro model for stromal invasion during implantation of the human blastocyst. Human Reproduction. 18, 2 283-290
Chobotova K, Spyropoulou I, Carver J, Manek S, Heath JK, Gullick WJ, Barlow DH, Sargent IL and Mardon, HJ (2002) Heparin binding epidermal growth factor and its receptor ErbB4 mediate implantation of the human blastocyst. Mechanisms of Development. 119, 137-144.

F. Future work

Detection of molecules involved in the implantation process in pre-, peri- and post-implantation embryos.

There is no future work proposed in this section which is not already included in any other licence submission

Experiments involving models to study the molecular basis of implantation

The work described above using culture systems will continue, and will be extended to study the expression of key molecules involved in very early development as the embryos progress through the implantation process.

The proposed future work builds on the depth of experience we have gained working with human embryos. We have already met many of our primary objectives and through the study of particular molecules of interest, have put in place a number of the fundamental tools necessary to study the events leading up to and surrounding implantation. Thus, the techniques developed so far provide a foundation for the study of the role of a whole range of molecules in this process and we now wish to build on these findings using new technology that has become available since the original licence application. The clinical importance of these studies is that by identifying the key molecules secreted by human embryos and the endometrium, it may be possible to either stimulate their production or supplement them to improve implantation rates. The data generated by these studies will contribute to the development of better treatments for infertility and IVF protocols.

Peer review comments (if applicable)
Not required at this interim inspection
Issues for consideration
NONE
Executive recommendations for Licence Committee
NONE
Areas not covered on this inspection
All covered

Project R0143: "To derive human embryonic stem cells and trophoblast cell lines"

Renewed project objectives
No changes have been made to the 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; enable stem cell technologies that increase efficiency of derivation and avoid the use of animal products. In order to achieve this blastocysts are grown in culture from day two or day three embryos that are surplus to clincial requirements and donated by couples attending the Oxford IVF Unit. They are then either processed for stem cell derivation. The function of extracellular matrix molecules on renewal of established embryonic stem cells is being investigated in parallel studies with lines brought in from Sheffield (Shef 1-6) and Wisconsin (H1). *B) Research undertaken to date.* Blastocysts or arrested morulae are cultured to day 6 from donated embryos and manipulated in one of two ways to derive stem cells. Either the embryos are explanted directly onto irradiated Hs27 feeder cells, or the inner cell mass is isolated mechanically with pulled glass pipettes and placed onto feeders. Explanted whole embryos are cultured on feeders for a maximum of six days during which time there is some limited outgrowth of a minority of embryos. The outgrowth is then cut and pasted onto fresh feeders and maintained using this method. *C) Results* Of the 178 embryos that were consented for this project, 36 embryos were considered to be viable for processing for hESC derivation. 34 were explanted directly onto feeder cells and 2 were subjected to mechanical biopsy of the inner cell mass. Most of the embryos attached but showed little outgrowth. Two outgrowths were obtained in February 2008 and are still being maintained by cutting and pasting. The cells are growing extremely slowly and so far there are too few cells to analyse for marker expression. *D) If progress was slower than anticipated, the reasons for this.* The number of good quality embryos donated is very low, and the majority of embryos we received were arrested morulae. In future we will only use good quality blastocysts and hope to improve culture efficiency at early stages of derivation. *E) Publications which have arisen from work under the licence.* None as yet *F) Future work* All future work was included in the original licence application. The objectives of the project are considered by the centre to be the same as in the licence application.
Peer review comments (if applicable)
Not required at this interim inspection

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

Project R0149: "**To Develop Pre-implantation Genetic Diagnosis (PGD) for Mitochondrial DNA Disease derive human embryonic stem cells and trophoblast cell lines**"

Renewed project objectives
No changes have been made to the project aims and objectives
Summary of research undertaken
A) How the work undertaken relates to the objectives. Embryo biopsy to obtain blastomeres for genetic analysis has started and so a number of embryos have been used for this: 71 embryos supplied to the project and 29 have been biopsied. The numbers of embryos biopsied is not substantially different to those estimated 1 year ago. The number of embryos from which blastomeres were obtained is not the same as the number of embryos used, since blastomeres were not successfully biopsied from many embryos. In addition, many of the embryos were of very poor quality and highly fragmented, such that obtaining proper cells was extremely difficult. Blastomeres obtained have been stored as blastomere analysis has not proceeded due to a lack of funding for suitable research staff. This situation will change in the near future with the initiation of the PGD programme aided by the appointment Dr Dagan Wells. Funding for a PhD student to work on the genetics aspect of the project is also in place. *B) Research undertaken to date:* See A) above *C) Results:* See A) above *D) If progress was slower than anticipated, the reasons for this:* See A) above *E) Publications which have arisen from work under the licence.* Currently being redrafted for submission to Human Molecular Genetics: A major component of the mitochondrial bottleneck has occurred by the time oocytes are mature: a significant step towards genetic management of mtDNA disease. Malik S, DR Marchington, K Turner, V Macaulay, SJ Dutton, P Oakeshott, DH Barlow, SH Kennedy, J Poulton. *F) FUTURE WORK:* As originally described in the project application
Peer review comments (if applicable)
Not required at this interim inspection
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	31st JULY 2008

Appendix A: Centre Staff interviewed

PR only and research leads on each project

Appendix B: Licence history

Licence	Active From	Expiry Date	Changes
R0111/1/a	Expired	09/03/1998	09/04/2000
R0111/1/b	Expired	10/03/2000	09/04/2000
R0111/2/a	Expired	10/04/2000	30/04/2003
R0111/2/a	Expired	10/04/2000	30/04/2003
R0111/4/a	Active	01/09/2006	31/08/2009

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

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

Appendix C:

RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number 0035

Name of PR Dr Karen Turner

Date of Inspection 24th June 2008

Date of Response 12th August 2008

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

A protocol for adverse incident reporting has been done. In order to get around the issue of the University not being subject to document control in the same way as the IVF Unit, this protocol will become part of the Laboratory protocols within the IVF Unit and thus will be subject to document control.

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).

All meetings between myself and the Lead Investigators are Minuted. The report says these are not Minuted and is therefore incorrect. A copy of a recent set of Minutes was presented in the Inspection 'pack'. These meetings are had as and when required and on average are held once or twice a year.
[Corrected in text – Lead Inspector]

We do have embryos donated to research in storage. These are monitored in the same way as our clinical embryos so form part of any audit and are checked for forthcoming expiry dates.
[Corrected in text – Lead Inspector]

Biopsy for project RO149 is usually done on day 3, not day 4 as stated.
[Corrected in text – Lead Inspector]

Research Licence Committee Meeting

16 September 2008
21 Bloomsbury Street London WC1B 3HF

MINUTES Item 6

Research Projects: R0111, R0143 and R0149
based at Oxford Fertility Unit (0035)

Members of the Committee:	In Attendance:
Emily Jackson, Lay Member – Chair	Chris O'Toole, Head of Research Regulation
Richard Harries, Lay Member	
Clare Brown, Lay Member	Claudia Lally, Committee Secretary
Neva Haites, Professor of Medical Genetics, University of Aberdeen	Providing Legal Advice to the Committee: Sarah Ellson, FFW Solicitors

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 (93 pages)
- no papers were tabled.

1. The papers for this item were presented by Chris O'Toole, Head of Research Regulation. Dr O'Toole informed the Committee that an interim inspection of all three research projects took place on 24 June 2008. The inspection found that progress has been made in relation to all three projects and the inspection team had been satisfied with the centre is well organised and carries out research in a profession manner largely compliant with the Code of Practice.

2. Dr O'Toole informed the Committee that a number of areas for improvement were identified at the inspection visit:

    - a procedure was required for reporting serious adverse incidents to the HFEA
    - in a few cases patients had been approached about donating material for research too close to the point of embryo transfer
    - information sheets were found not to include information required by the Code of Practice

- the inspectorate had recommended that the SOPs relating to research practices be document controlled.

3. Dr O'Toole informed the Committee that a detailed response had been received from the Person Responsible which had addressed the issue of a protocol for adverse incident reporting and the issue of document control. This response was included at appendix C of the report.

The Committee's Decision

4. The Committee noted the response to the report from the Person Responsible and agreed that the three licences should continue with no additional conditions.

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