Research Licence Renewal Inspection Report

Project Title	Development of a model to study implantation in the human
Centre Name	Oxford Fertility Unit
Centre Number	0035
Research licence Number	R0111
Centre Address	Level 3 and level 4, Women's Centre, John Radcliffe Hospital, Oxford OX3 9DU
Treatment centres donating to this research project	0035 – Oxford Fertility Unit 0139 – Bath Assisted Conception Clinic 0064 – BMI The Chiltern Hospital Fertility Services Unit
Inspection date	21st May 2009
Licence Committee Date	TBA
Inspector(s)	Andrew Leonard Paula Nolan
Fee Paid - date	Fee paid
Person Responsible	Dr Karen Turner
Nominal Licensee	Ian Sargent
Licence expiry date	31/08/2009

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

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

Brief Description of the Project

Project R0111 is housed within a research laboratory in the Oxford Fertility Unit (Centre 0035), 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. The vast majority of embryos used in the research project are derived from Centre 0035 (908 fresh and 215 frozen embryos donated and all used between 1 March 2008 and 28 February 2009). In the same period, only 29 frozen embryos were donated from Centre 0139 (all used), and no embryos were obtained from Centre 0064. The lay summary for the project states:

'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. 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 have extended our studies in collaboration with other IVF Units as part of a European Network of Excellence set up to investigate the control of embryo implantation. A multicentre study has failed to show a clear association between HLA-G levels in the culture medium and implantation success. We are continuing to investigate maternal factors which may control embryo HLA-G expression at the time of 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 the embryo and the endometrium, and proteins that exist in a matrix surrounding the cells that make up the endometrium that appear 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.'

Summary for Licence Committee

The Centre has held a licence for project R0111 since 1998. The Centre also has research licences for two other projects (R0143 and R0149). The licensed research activities on licence R0111 include research on human embryos and storage of licensed material; the licence is due to expire on 31 August 2009. The licence renewal application states that the Centre do not wish to add further activities to licence R0111. The research licence renewal is requested for the same defined purposes as the existing licence: Promoting advances in the treatment of infertility; increasing knowledge about the causes of miscarriages; increasing knowledge about the development of embryos.

The vast majority of embryos used in the research project are derived from Centre 0035 (908 fresh and 215 frozen embryos donated and all used between 1 March 2008 and 28 February 2009). In the same period, only 29 frozen embryos were donated from Centre 0139 (all used), and no embryos were obtained from Centre 0064. Embryo usage was above that expected (750 fresh and 200 frozen embryos) but this was explained as being due to a fortunate increase in donor numbers due to increase activity at Centre 0035.

The inspectorate were satisfied that research at centre 0035 is well organised and is carried out in a professional manner which complies in nearly all areas with the Code of Practice, 7th edition. The premises, donation processes, consenting and scientific practices were all compliant. There were only two areas of concern:

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

2) The information sheets 'research and training projects using surplus eggs and embryos, Pt Info 2 v.2' and 'Donating frozen embryos to research, v.1' are the only information/consent forms provided to patients consenting to donation of fresh and frozen embryos, respectively, to project R0111. Both sheets were reviewed and were found to not include several pieces of information required by the Code of Practice, (e.g. Standards S.8.2.1 and S.8.3.2, and Guidance G.5.13.1 (a,e,f), as listed in Section 4 in this report. This issue had been raised at the interim inspection in June 2008 and it was recommended that the PR should review these documents and the verbal information provided by the Research Nurse, to ensure patients receive verbally and/or in writing, all information required by the Code of Practice, 7th edition.

It was noted on this inspection that these non-compliant information and consenting documents were still in use. Centre staff explained that updated information sheets have not been printed because a considerable number of printed copies of the non-compliant versions remain. In addition, the PR was expecting to have to update the information sheets further, since the Centre was due to move to new premises in late 2008, then early, then mid 2009. The delay in the move has delayed the update of the patient information provided. The move is now scheduled for late August 2009 and the NL provided drafts of updated patient information/consent forms. These drafts were reviewed by the inspectorate and were considered to be compliant with the requirements of the

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

Peer review of the project renewal application was positive and they recommended acceptance of the application in its present form.

A completed application form has been submitted to the HFEA by the PR and the licence renewal fee has been paid

The inspectorate recommend renewal of research licence R0111 for a 3 year period.

Proposed licence variations

None

Report of Inspection findings

1. **Organisation**

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

Summary of findings from inspection
Evidence of:

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

Staff

Principal investigators	2
Scientists	3
Laboratory technicians	0
Support staff (receptionists, record managers, quality and risk managers etc)	1

Highlighted areas of firm compliance
The treatment and storage and research licences at Centre 0035 have different PRs and clinical and research practices are separated. The one area of close approach between clinical and research activities is that embryos in storage for treatment for which consent for research use is then obtained, remain stored in the same dewar position until used in research. There are no research dewars for embryo storage. Removal of embryos from dewars for research use is appropriately witnessed and recorded in patient records. The Person Responsible (PR) is a Consultant Embryologist and the Laboratory Manager at Centre 0035, while the Nominal Licensee and another professor of reproductive medicine lead the research. The PR has been in post since before the current licence was issued and has completed the PREP. The PR has extensive knowledge of the regulatory requirements of the HFEA and is an external advisor to the HFEA. Discussions with the PR and the research leads, and inspection of the premises, indicated that the project is well lead and managed. The project appeared to be appropriately staffed, albeit the a post-doctoral scientist has left and the research nurse at Centre 0035 has not been able to work on the project since September 2008, both due to funding limitations. The research nurse briefed nursing staff regarding the project prior to leaving it, so that they are informed and able to provide information to patients about it. Furthermore, the research nurse is still employed at the Centre on other projects and can give advice and information if she is available. The research nurse post on this project may be reactivated if funding becomes available. The induction procedure for research staff follows the Oxford University requirements and was observed by the inspectorate. Each element of induction requires sign-off such that a record of induction is prepared and placed in the staff record. The PR said that research staff

training meets the requirements of Oxford University and involves attendance at conferences and internal and external training programmes. Weekly departmental seminars are also held and researchers are encouraged to attend.

Funding is in place from The Medical Research Council and the Wellcome Trust. It does not cover all aspects of the research and further funds are being sought.

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

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

Contact between the researchers and clinical staff at Centre 0035 is as frequent as required for effective coordination of embryo supply and research activities. Communication is facilitated by the PR being the Head of Embryology at the Centre and the NL being the Scientific Director, though neither has a role in consenting patients for research. The researchers provide seminars at monthly all staff meetings, as a means to feedback research progress to clinical staff. Regular emails and telephone calls are used to communicate with Centres 0064 and 0139.

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

Laboratory standard operating procedures (SOPs) related to embryo donation and culture were provided to the inspectorate and were considered fit for purpose. SOPs related to downstream laboratory analyses were not provided as they are not used to work on viable embryos and tend to be sections of research papers. The principal investigators on the project considered the SOPs used were suitable for the analysis they wished to perform; they are updated as required by technique development in their area of research.

At the last inspection in June 2008, it was recommended that a procedure for reporting serious adverse events to HFEA should be developed to ensure compliance with General Licence Condition A.4.1. At this inspection, the inspectorate was advised that the research team now have this procedure, provided by the clinical staff at Centre 0035, and it was displayed on the culture laboratory wall. This subject was seen to be minuted in the last research group meeting minutes from April 2009.

Issues for consideration
• Formal research group meetings should be held every six months, according to centre staff. Minutes were provided for meetings in January 2008, May 2008 and April 2009. It would appear that a meeting was missed in November 2008. It is recommended that the Centre maintain the 6 month periodicity of these meetings, to ensure continuing management and coordination between the research and clinical activities.
Executive recommendations for Licence Committee
The Licence Committee is asked to endorse the recommendation made in relation to maintaining the six monthly periodicity of the formal research group meetings, discussed above.
Areas not covered in by this inspection
All covered

2. Premises and equipment

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

Summary of findings from inspection:

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

Highlighted areas of firm compliance
The Centre premises were well organised, clean and tidy on the day of inspection. The research premises comprise a research-dedicated laboratory within Centre 0035, accessed via the andrology laboratory, on Level 4 of the Women's Unit, John Radcliffe Hospital. This laboratory contains a class II air flow cabinet, incubator and inverted microscope, and is used for embryo culture. An imaging laboratory within the Academic Department of Obstetrics and Gynaecology on the floor below is also used for licensed work. It contains an inverted microscope and a fluorescent microscope with a controlled environment stage, for vital time-lapse fluorescent microscopy. The laboratory also contains 2 computers for image processing, storage and analysis, and an air flow cabinet and incubator for embryo culture and manipulation. All licensed material and research records are confined to these two secure licensed laboratories. 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 Oxford University health and safety staff. Detailed health and safety documentation for research activities was provided to the inspectorate and all research procedures have been risk assessed. There are no dedicated research embryo storage facilities, and all such embryos are stored in dewars used for clinical storage, until required on the research project. These storage facilities were considered fit for purpose during the inspection of the treatment and storage licence, and are secure and equipped with a low oxygen monitor, fan extractor with boost connection to the low oxygen monitor, and low level nitrogen alarms. A procedure for responding to activation of the low oxygen alarm is in place. Research staff must receive training before using liquid nitrogen and the storage facilities at Centre 0035. A documented system for equipment maintenance/servicing is in place; the laboratory head is the designated person responsible for the maintenance of equipment. Inspection of some items of equipment indicated they were all within servicing intervals and servicing documentation was provided. All electrical equipment inspected also evidence of portable electrical testing certification and the PR said that such testing was up to date. The centre ensures that appropriate training is provided to all staff using specialist equipment to enhance safety and prevent equipment damage.
Issues for consideration
There is an anticipated change to new premises in October 2009, these being in a renovated

and refurbished building on the edge of Oxford. The Oxford Fertility Unit will move to the same premises.
Executive recommendations for Licence Committee
None
Areas not covered in by this inspection
All covered

3. Donation of material

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

Summary of findings from inspection:

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

Highlighted areas of firm compliance
Embryo donors are recruited to the Centre's three licensed research projects (R0111, R0143 and R0149) by a common research donor recruitment procedure. Embryos are obtained for research on project R0111 from within Centre 0035 and also as frozen stored embryos, from the Bath Assisted Conception Clinic (Centre 0139) and the BMI Chiltern Hospital Fertility Services Unit (Centre 0064). All couples treated at Centre 0035 are provided research information with their treatment information when they first visit the Centre at an initial orientation open evening. When HFEA consent forms are signed at a consenting consulation some time afterwards, if patients indicate they wish to consent to research donation their consent is taken on the HFEA form. Research information is then discussed, if required, and research consents are taken by nursing staff. If further information or time to consider is required, a further consultation is arranged at which research is discussed and consent taken if patients so wish. The research consent form is detailed and allows them to specify the projects to which they consent to donate. Research consents are normally collected before the start of treatment and well before egg collection. Cryopreserved donated embryos from Centres 0035, 0139 and 0064 are also used. Patients with frozen embryos are annually asked to confirm storage arrangements for the forthcoming year. If they express an interest in donating to research they are sent a 'donating frozen embryos to research' information sheet, which describes the project, and a consent form. The consent forms are signed by the patients and returned to their Centre. Frozen embryos at Centres 0139 and 0064 are sent in batches to Centre 0035, and are used in research within days of arrival. Defined processes are in place to prevent a breach of patients' research consent. Prior to egg collection, patient notes are reviewed for all consents including those for research and a note taken of research consented patients. After transfer of the best one or two embryos, the Centre has a specific procedure which defines the quality of embryos frozen for treatment; any remaining embryos are available for research. At this point research consents are verified in the patient notes, then checked again and witnessed by another clinical embryologist. Research consented embryos are then passed to the researchers with an affidavit from the clinical embryologists detailing the consents applying. The research donation is logged by the clinical embryologists in a book (detailing patient name; centre number; projects consented to; date of donation to research; developmental stage with length in culture post-fertilisation). Embryos are then taken to the research culture laboratory and placed in the incubator to

equilibrate. Soon thereafter embryos are anonymised in that they are transferred to a dish labelled only with a unique research code. They are also allocated to a research project, depending on the consents provided and on which researchers are available according to the donation rota, and logged in the anonymisation book (detailing Centre identification number; research number; projects consented to; date of arrival; project allocated; researcher responsible). The documenting of the Centre identification number allows back-tracking from research records to patient records; patient records remain in Centre 0035 at all times.

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

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

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

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

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

4. Patient information and consents

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

Summary of findings from inspection:

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

Results of consent audit
Six sets of patient records were reviewed for research consents. All but one contained appropriately completed specific research consent forms, as well as HFEA research consents. In one case, the female partner but not the male partner had signed to consent for project R0143, whereas both had consented to projects R0111 and R0149. This discrepancy had been detected by the embryologists reviewing consents before egg collection, and noted in the patient record. The embryos had subsequently been used in research on project R0111, which was consented to by both patients. This indicates that the Centre's procedure for reviewing patient consents, and using embryos in research according to those consents, is robust. No consents were seen to have been taken on the day of embryo transfer. Indeed all the consents appeared to have been taken before the start of stimulation in the cycle of embryo donation. There was no evidence that patient consents had been breached in any way.
Highlighted areas of firm compliance
The patient research information/consent form, which describes and collects patient consent for project R0111, as well as the other research projects, was reviewed (Research and training projects using surplus eggs and embryos, Pt Info 2 v.2), as was the information/consent form provided to patients with frozen embryos (Donating frozen embryos to research, v.1). These forms were well presented and broadly compliant with Code of Practice requirements regarding information provided to research donors, however some issues of content are raised below. Variable consents are possible as the consent form allows the provision of consent for each research project individually. Procedures within Centre 0035 prevent the use of embryos in research without patient consent.
Issues for consideration
The document 'research and training projects using surplus eggs and embryos, Pt Info 2 v.2' is the only information/consent sheet provided to patients consenting in the Centre 0035 to the use of fresh embryos in project R0111. Likewise, the form 'Donating frozen embryos to research, v.1' is the only information/consent sheet provided to patients consenting in the Centres 0035, 0139 and 0064 to the use of frozen embryos in project R0111. At the last inspection it was noted that these information sheets were non-compliant with the HFEA Code of Practice (e.g. Standards S.8.2.1 and S.8.3.2, and Guidance G.5.13.1 (a,e,f). It was accepted in the last inspection report that these information requirements may be satisfied verbally by the Research Nurse, albeit this was unlikely to happen for patients donating frozen

embryo and the Research Nurse is now not employed on the project.

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

The inspectorate recognise that the updated information/consent forms will need further updating in the near future when the Centre moves to new premises. It would thus be wasteful to commercially print them in large numbers at the present time. It is also noted that these revised versions are compliant with the requirements of the HFEA Code of Practice, 7th edition, whereas the versions currently in use at the Centre are not. To ensure patient information and consent forms are compliant, it is recommended that the PR immediately brings the revised versions of the patient information/consent forms into use at the Centre. It is up to the PR to decide, but the inspectorate suggest they be printed from copiers within the Centre until the move to the new premises, then commercially printed versions suitable for the new premises can be prepared after the Centre's move in August/September 2009.

.

Executive recommendations for Licence Committee

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

Areas not covered in by this inspection

All covered

5. Scientific practice R0111, Development of a model to study implantation in the human

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

Summary of:

- Peer review

Summary

The current licensed research activities include research on human embryos and storage of licensed material. The Centre do not wish to add further activities but state that downstream analytical methods used include: PCR analysis of gene expression in embryos from early cleavage stage to blastocyst; High resolution immunohistochemical analysis after fixation; Transfection of siRNA and/or dominant-negative mutant cDNAs into the trophectoderm of peri-implantation blastocysts for gene knockdown. The research licence has been granted for the following defined purposes: Promoting advances in the treatment of infertility; increasing knowledge about the causes of miscarriages; increasing knowledge about the development of embryos.

When embryos are accepted into project R0111, they are cultured in the research culture laboratory until day 6 post-fertilisation, at which point they are discarded if non-viable or used in research. If allocated to project R0111, they are cultured on endometrial cell layers for 2 days while being observed using vital fluorescent microscopy, then fixed and subjected to further investigation. Embryos may also be disrupted at 3 – 6 days post-fertilisation and used for molecular analysis of embryonic HLA-G expression and factors which impinge on this process. Normal working practices mean that no embryos are cultured for more than 8 days post-fertilisation before being rendered non-viable. The centre also has a procedure which states that no embryos should be cultured for 14 days post-fertilisation. This is also discussed in patient information

Research project lay summary:

'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. 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 have extended our studies in collaboration with other IVF Units as part of a European Network of Excellence set up to investigate the control of embryo implantation. A multicentre study has failed to show a clear association between HLA-G levels in the culture medium and implantation success. We are continuing to investigate maternal factors which may control embryo HLA-G expression at the time of 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 the embryo and the endometrium, and proteins that exist in a matrix surrounding the cells that make up the endometrium that appear 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.

Usage and expected usage in next year:
The vast majority of embryos used in the research project are derived from Centre 0035 (908 fresh and 215 frozen embryos donated and all used between 1 March 2008 and 28 February 2009). In the same period, only 29 frozen embryos were donated from Centre 0139 (all used), and no embryos were obtained from Centre 0064. The embryo usage last year differed from that proposed (750 fresh and 200 frozen embryos) because more embryos were available for research due to an increase in the number of treatment cycles carried out at Centre 0035. Usage next year is estimated at 750 fresh embryos and 250 frozen embryos.

Summary of audit of stored and biopsied material

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

Renewed project objectives

No changes to project aims and objectives.

Summary of research undertaken

A) How the work undertaken relates to the objectives.
The 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 under the term of the current licence.

During the period of the current licence, work has focussed on:

1) 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.

2) HLA-G

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 several 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 much 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.

3) Experiments involving models to study the molecular basis of implantation

Expression and function of known molecules:

The 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.

Investigation of novel molecules:

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 during the term of the current licence

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, has investigated whether there is a correlation between soluble HLA-G expression and embryo quality in vitro. This study 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) meant that any soluble HLA-G present was 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) were 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 organised an International Workshop on the sHLA-G and implantation, which was hosted by members of the Oxford Fertility Unit research team at Keble College Oxford in June 2008.

The workshop was attended by 20 key workers in the field from Austria, Belgium, France, Germany, Ireland, Italy, Japan, Spain and the UK. Other workers from the USA, Canada and France were invited, but were unable to attend. The programme covered the role of MHC genes in embryo development, the problems of measuring sHLA-G and the types of assays used, clinical findings and the effect of embryo culture conditions on sHLA-G production. The main conclusions were that while there is clearly an association between sHLA-G (and its homologues in other species) and embryo development, problems with current assay systems and variability in embryo culture conditions currently preclude its use as a reliable marker for the selection of IVF embryos. The participants agreed to establish a system of sample and assay exchange to try to identify where the problems lie.

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 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 are determining the 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 have determined by high-resolution immunohistochemistry, and Cdc42/Rho/Rac pathways, which we have analysed 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.
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.
Tabiasco, J., Perrier d'Hauterive, S., Thonon, F., Parinaud, J., Léandri, R., Foidart, J-M., Chaouat, G., Munaut, M., Lombroso, R., Selva, J., Bergère, M., Hammoud, I., Kozma, N., Aguerre-Girr, M., Swales, A.K., Sargent, I.L., Le Bouteiller, P. and Lédée, N. (2009) Soluble HLA-G in IVF-/ICSI-embryo culture supernatants does not always predict implantation success: a multicentre study. RBM Online 18, 374-381.
Grewal S, Carver JG, Ridley AJ, Mardon HJ (2008) Implantation of the human embryo requires Rac1-dependent endometrial stromal cell migration. PNAS 105(42):16189-16194.
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 the previous licence submission or discussed above.

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 solid phase, 2-D and 3- D models for human embryo implantation will be used to investigate:-

i) the function of adhesion- related and EGF family molecules in implantation.

ii) the effect of procedures and drugs used in ART on the capacity of embryos to invade the endometrial stroma.

iii) the fate of early human stem cells in the peri- and post implantation embryo in vitro.

Methods
We have now established robust and highly reproducible methods for performing experimental models of human embryo implantation, and these are now routinely used in our laboratory. We will continue to use all three models: i) solid phase testing single or mixtures of molecules; ii) 2- D testing interaction of embryos with endometrial epithelial and stromal cell; and iii) 3- D allowing us to dissect the cellular and molecular events that mediate progression of the embryo through the implantation process. The co-cultures will be monitored for between 8- 12 days pc and then fixed in either acetone or paraformaldehyde and assessed.

The expression of candidate molecules or differentiation markers will be achieved by high resolution fluorescence and image analyses techniques. The function of specific molecules will be tested by specific inhibitors, transfection of the trophoblast with fluorescently- labelled cDNAs or RNAi in the co- cultures.

The proposed studies are designed to determine molecules that are critical for early human development during the implantation process. These are both embryonic and maternal, and further our knowledge of these will increase our understanding of the maternal-embryo dialogue that is likely to direct these developmental processes. The resource we have built up during the course of the last fifteen years that allows us to construct and perform human embryo-endometrial co-cultures puts us in a unique position in being able to address questions of early human development events. Not only will the results of the proposed studies increase our knowledge of early human development, which would otherwise be impossible to achieve, but also contribute to the development of better treatments for infertility and improved IVF protocols.

Peer review comments (if applicable)

The peer reviewer considered that they had enough information available in the application to recommend acceptance of the application in its current form.

Regarding progress on the project, the reviewer stated:

'The group has contributed extensively to an international collaboration to investigate the claims that levels of HLA-G secreted by early human embryos may be a good indicator of their suitability for selection for transplantation. They have found that in fact levels of soluble HLA-G may arise from stores of maternal RNA, and may not therefore be used to identify the best embryos. Additionally, the variation in culture conditions between IVF units results in different readouts that do not always correlate with embryo quality.

'The group has established a culture system to identify factors important for implantation. One component of this system is to apply inhibitors of specific pathways to the trophectoderm, another is to perform DNA profiling of the endometrial response to an implanting blastocyst. The validity of these systems has been tested using mouse embryos and is now set up for the human. They have successfully established RT-PCR on human embryos and are ready to explore expression of candidate genes in response to the implantation interaction, both in the embryo and the endometrium. They are also preparing to investigate proteins using this system.

‘Reflecting the success of this project, there have been several publications over the last few years in high profile journals resulting from this work.’

Regarding embryo usage, the peer reviewer stated:

‘The activities have been pursued in accordance with their original application, but they have been fortunate to have access to more donated embryos than previously anticipated, so the number of embryos used has been greater than predicted.’

Regarding the justification for future work, the peer reviewer stated:

‘The plans for the future are sure to achieve decisive results since all the systems are now in place for the molecular studies proposed. At least the outcome of this project will be an increase in knowledge about human embryo implantation, with the potential to identify a suitable factor to monitor embryo quality. At best, the knowledge will lead to improvement for the selection of culture conditions to improve the efficiency of embryo implantation.’

The peer reviewer finally noted:

‘This group has an excellent track record and extensive experience in the field of embryo implantation. The IVF community is likely to benefit greatly from this project.’

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	9th June 2009

Appendix A: Centre Staff interviewed

PR and research leads

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

Appendix C:

RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number 0035

Name of PR Dr Karen Turner

Research project RO111

Date of Inspection 20th May 2009

Date of Response 6th July 2009

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

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

i. We currently plan six monthly research meetings, but one meeting was missed in 2008. We will continue to plan these meetings every six months and ensure that we do indeed meet on this regular basis.

ii. We are in the process of finalizing revised patient information sheets and consent forms in accordance with the inspectorate's recommendations. The Inspectorate suggests in the Report that the documents are prepared in-house rather than commercially since they will need revising when the OFU moves to new premises. This will avoid delays and updated versions will be used as soon as possible.

2. Correction of factual inaccuracies

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

HFEA Research Licence Committee Meeting

15 July 2009

21 Bloomsbury Street London WC1B 3HF

Minutes – Item 4

Oxford Fertility Unit (0035; R0111) – Renewal

Members of the Committee:	Committee Secretary:
Emily Jackson (lay) – Chair Richard Harries (lay) David Archard (lay) Lesley Regan (clinician) Hossam Abdalla (clinician)	Kristen Veblen Legal Adviser: Sarah Ellson, Field Fisher Waterhouse

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

The following papers were considered by the Committee:

- papers for licence committee (77 pages)
- tabled papers (2 pages)s.

The Committee also had before it:

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

1. The Committee noted that the research project had commenced on 9 March 1998, that the current licence would expire on 31 August 2009, and that the renewal application had been made for 3 years.
2. The Committee considered the papers, which included the renewal inspection report, peer review and Licence Committee minutes from 16 September 2008, 25 July 2007 and 26 July 2006. Additionally, the Committee noted the tabled papers, an email from the Person Responsible (PR) covering the application form and a letter concerning Ethics Committee approval.
3. The Committee noted that the renewal inspection had taken place on 21 May 2009 and that the PR had responded to the report on 6 July 2009.
4. It was noted by the Committee that at the time of the inspection, non-compliant patient information and consent forms were in use. Further to this finding, the Committee noted the response of the Person Responsible which indicated that revised patient information sheets and consent forms were nearly complete.
5. The report also observed that that one of the scheduled meetings, to be held every six months had been missed. Further to this, the Committee noted the response of the PR, indicating that these meetings would continue to take place regularly every six months and be recorded appropriately.

The Committee's Decision

6. The Committee identified the activities to be authorised by a licence as storage of embryos and use of donated embryos for research. The Committee agreed that they were satisfied that these activities were not prohibited under the HFE Act 1990 (as amended).
7. The Committee decided that these activities were necessary and desirable for the following purposes:
    - promoting advances in the treatment of infertility
      HFE Act 1990 (as amended) 2 3(2)(e)
    - increasing knowledge about the causes of miscarriages
      HFE Act 1990 (as amended) 2 3(2)(c)
    - increasing knowledge about the development of embryos
      HFE Regulations 2001 2(a)
8. The Committee decided that it was satisfied that the proposed use of human embryos was necessary for the purpose of research. In making this decision the Committee took into account that the purpose of the project was to understand the factors that affect implantation. The Committee agreed that the use of human embryos was necessary and desirable, as it would not be

possible to understand factors which affect the implantation of human embryos without using human embryos in the research.

9. The Committee noted that the patient information and consent forms were due to be updated, as explained in the response of the PR, and requested that the Centre submit these forms for Executive approval of their suitability by 28 August 2009. This approval would be sufficient for the Committee to be satisfied of the suitability of the patient information and consent forms.
10. The Legal Adviser confirmed that the legislation required an application from the Centre but did not specify that it had to be signed. Although the provision of signatures was helpful in clarifying the position, the Committee could proceed if it was satisfied that it had an application "from the Centre" which might be evidenced by an email from the PR..
11. The Committee considered itself satisfied that it was appropriate to grant a licence, noting that it was in possession of an application from the Centre, indication of ethics committee approval and that the appropriate fee had been paid.
12. The Committee agreed that it continued to be satisfied as to the character, qualifications and experience of the Nominal Licensee. Also, the Committee noted that the PR had completed the PR Entry Programme and agreed that, it also continued to be satisfied as to the character, qualifications and experience of the PR as required for the supervision of the activities to be discharged under Section 17 of the HFE Act 1990 (as amended).
13. Further, the Committee agreed that it continued to be satisfied that the premises for which the Licence was to be renewed, as described by the report, were suitable for the activities.
14. The Committee, in accordance with the recommendation of the Executive, decided to grant a licence for a period of 3 years, with no additional conditions.

Signed..... Date 30.07.09.
Emily Jackson (Chair)