Interim Research Licence Inspection Report

Project Title	**R0170/R0171** – Derivation of human embryonic stem cell lines from embryos, including those created from clinically unused oocytes or abnormally fertilised embryos
Centre Name	St Mary's Hospital, MFS, University of Manchester
Centre Number	0067, 0033, 0175
Research Licence Number	R0170/R0171
Centre Address	Department of Reproductive Medicine St Mary's Hospital, Manchester, M13 0JH Manchester Fertility Services (MFS) Bridgewater Hospital, 120 Princess Road, Manchester M15 5AT University of Manchester Core Technology Facility, 46 Grafton Street, Manchester M13 9NT
Donating treatment centre numbers	0033 – Manchester Fertility Services 0067 – St Mary's Hospital
Inspection date	17 October 2008
Licence Committee Date	15 January 2009
Inspector(s)	Wil Lenton – Lead Inspector Vicki Lamb Allison Cummings
Fee Paid – date (if applicable)	N/A
Person Responsible	Dr Daniel Brison
Nominal Licensee	Dr Sue Kimber/Mr Brian Lieberman/Dr Cheryl Fitzgerald
Licence expiry date	31/12/2009

About the Inspection:
The purpose of the inspection is to ensure that centres are providing a quality service in compliance with the HF&E Act 1990, Code of Practice, licence conditions and directions.

The report is used to summarise the findings of the inspection highlighting areas of firm compliance and good practice, as well as areas where further improvement is required to improve services and meet regulatory standards. It is primarily written for the Licence Committee who make the decision about the centre's licence renewal application. The report is also available to the public following the Licence Committee meeting.

This report is of the initial inspection of new research premises.

Brief Description of the Project

Immature eggs and eggs which have failed to fertilise are not suitable for clinical IVF treatment and are normally discarded. In this project, these eggs have been used to establish and optimise methods for the recovery of clinically unusable oocytes by in vitro maturation and/or parthenogenic activation or fertilisation, in order to generate viable embryos for human embryonic stem cell derivation. We are also using embryos surplus to IVF treatment for this purpose, similar to most other stem cell centres in the UK.

Our results have shown that maturation of immature eggs can be achieved in the laboratory using culture fluid supplemented with various factors which encourage growth. We are studying the expression of various gene patterns in eggs matured in the laboratory and comparing them to normal healthy eggs. At the moment, eggs matured in this way cannot be used for clinical treatment as not enough is known about the normalcy of these eggs but results from this project will increase this knowledge leading to the use of immature eggs for IVF treatment in the future.

Parthenogenetic activation involves an egg being artificially stimulated by chemicals in order to trigger embryo development. Alternatively, eggs which have failed to fertilise may be re-inseminated using donor sperm. Eggs which have failed to fertilise after standard IVF treatment are being treated by both of these methods in the laboratory. Eggs which successfully fertilise after such treatment are cultured in the incubator for up to 7 days and carefully monitored. The normality of such embryos is established by analysing DNA prepared from the embryos for expression of genes which may act as markers of normal development.

The eventual aim of the project is to derive human embryonic stem cell lines from embryos generated using these methods. Any such stem cell lines will be derived in a purpose built facility and will undergo a variety of tests to establish the normality of the cell lines before they will be submitted to the UK Stem Cell Bank.

Licensed Activities

5.1.5	storage of embryos	☒
5.1.6	creation of embryos *in vitro*	☒
5.1.7	use of donated embryos for research	☒
5.1.8	derivation of human embryonic stem cell lines	☒

Summary for Licence Committee

Research has continued to make progress in line with the stated project objectives.

The research team has submitted three papers for publication in peer reviewed, scientific journals based on studies undertaken as part of the project.

Funding is in place for the forthcoming year.

The Executive recommend the continuation of the centres licence.

Report of Inspection findings

1. **Organisation**

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

Summary of findings from inspection
Evidence of: *(Delete areas not reporting on)*

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

Full time equivalent staff

Principal investigators	Daniel Brison and Sue Kimber
Laboratory scientists	3
Administrators	
Collaborators	
Support staff (receptionists, record managers, quality and risk managers etc)	Staff at centres 0033 and 0067

Highlighted areas of firm compliance
The PR has extensive knowledge of the regulatory requirements of the HFEA, together with appropriate research and publishing experience. An induction programme for all new staff at centres 0033 and 0067 is currently in place which covers the regulatory requirements of the HFEA. Continuing professional development (CPD) is documented by staff. The centres' hold weekly minuted meetings which include regular updates on the research projects every 4-6 weeks. Additionly there is a minuted meeting which takes place every two weeks for all research staff.
Issues for consideration
None
Executive recommendations for Licence Committee
None

2. Premises and equipment

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

Summary of findings from inspection: *(Delete areas not being reported on)*

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

Highlighted areas of firm compliance
New purpose-built laboratories for Centre 0175 were inspected by the Executive in May 2007 and found to be fit for purpose. The creation and manipulation of viable embryos is carried out on licensed premises using equipment which is regularly serviced and maintained. Embryos are stored in designated secure areas with controlled access under the auspices of the licences of centres 0033, 0067 and 0175.
Issues for consideration
None
Executive recommendations for Licence Committee
None

3. Donation of material

Desired outcome: Ensure donors are recruited in a proper way and their consent is respected.

Summary of findings from inspection: *(Delete areas not being reported on)*

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

Highlighted areas of firm compliance
The information has not changed since the previous inspection. Thus; **Centre 0067 –** All prospective patients have information packs sent out to them in advance of any consultation. Information sheets and consent forms relating to the specific research projects undertaken in the licensed centres in Manchester are included in these packs. **Centre 0033 -** Patients are given the information sheets and consent for both research projects during their initial clinical consultation. A research nurse, who is independent of the clinical service and research work, gives patients information and obtains consent from patients who wish to donate gametes and / or embryos to the embryo development project. Approximately 40% of patients consent to the donation of gametes and embryos for use in the R0171 licensed research project. If patients consent to the donation of gametes and / or embryos to be used in licensed research a coloured sticker (yellow for project R0170/0171) is placed on the treatment cycle embryo tracking form. The transfer of gametes and / or embryos from clinical use to research is witnessed by two appropriate people and this includes checking that appropriate consent is in place. Centrally held records reviewed in the course of the inspection contained necessary information to allow tracking of individual embryos. All the fresh embryos donated to research are those that are unsuitable for use in treatment and do not meet the centre's criteria for freezing. 45% of patients who receive licensed treatment at centre 0067 have embryos frozen and more than 50% of patients receiving licensed treatment at centre 0033 have embryos cryopreserved for potential future use. The decision of whether embryos are unsuitable for use in treatment or cryopreservation is always made by a clinical embryologist not involved in research. Two of the embryologists at centre 0067 are involved, on a part time basis, in licensed research. However, they do not make decisions regarding the suitability of embryos for clinical

use if they are going to be carrying out research on the embryos donated from these patients.
Issues for consideration
The patient consent rate to the project has been lower than anticipated in the last 12 months, due to the loss of the research nurse in September 2007 combined with delays in MRC funding. This has impacted significantly on the number of donated oocytes and embryos. A replacement research nurse has now been in post, on a part-time basis from May 2008 and funding is now in place to make this a full-time position.
Executive recommendations for Licence Committee
None

4. Patient information and consents

Desired outcome: Ensure that patients are informed in order to give informed consent

Summary of findings from inspection: *(Delete areas not being reported on)*

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

Outcome of audit of records
No discrepancies found during the notes audit.
Highlighted areas of firm compliance
Patient information and consents comply with all of the requirements outlined in standard licence conditions and the 7th Code of Practice (CoP7).
Issues for consideration
None
Executive recommendations for Licence Committee
None.
Areas not covered in this inspection
None.

5. Scientific practice

Desired outcome: Procedures are robust to ensure material is used appropriately

Summary of findings from inspection: *(Delete areas not being reported on)*

- Standard operating procedures
- Quality assurance systems
- Minimisation of material loss and wastage
- Ability to achieve set aims and objectives

Use of material

Frozen embryos for the research project are donated by patients at St Mary's Manchester (0067) and Manchester Fertility Services (0033).

In the period 01/08/07-31/07/08 the licensed material usage was as follows:

Fresh eggs = 0 (0067) and 0 (0033)

Frozen eggs = 0 (0067) and 0 (0033)

Failed to fertilise eggs = 58 (0067 - recd & used) and 0 (0033)

Fresh embryos = 91 (0067 - recd & used)

Frozen embryos = 19 (0067 - recd & used) and 20 (0033 - recd & used)

'Spare' IVF/ICSI

Created for = 37 (0067 recd & used) and 0 (0033)
Research

33 = parthenogenetic activation
4 = ICSI with donor sperm

The anticipated usage of licensed material over the next 12 months is:

Material	Expected usage
Fresh Eggs*	600 (immature GV/MI)
Frozen Eggs	0
Failed to Fertilise Eggs	1000
Fresh Embryos	500
Frozen Embryos	500

(The figures for usage over the next 12 months represent maximum numbers only)

Project objectives

The overall aim of this project is to derive new hES cell lines from fertilised embryos, that may be suitable for therapeutic use in the future. There are limits to the supply of human embryos and stem cell lines for these purposes. Fresh superior grade embryos produced following in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) represent the preferred source from which to derive new hES lines owing to their likely developmental competence and normalcy however these embryos are usually required for patient treatment. To overcome this, we have been using clinically failed eggs and embryos that require intervention to restore developmental potential, as well as surplus embryos from IVF treatment.

One aim of this project is to evaluate the use of immature oocytes as a source of eggs for stem cell derivation purposes. After in vitro maturation, MII eggs remain fertilisable and in some cases, normal development can be achieved. Research into in vitro maturation may also have clinical benefit as this technique has potential to be routinely used for patient treatment in the future. Although methods exist to successfully recapture the normal developmental potential of these eggs and embryos, safety concerns preclude their clinical use and under current treatment protocols this tissue is routinely discarded. Although these safety concerns are valid when the objective is conception, this is not an issue where the objective is the creation of new embryo stem cell lines, whose normalcy could then be extensively evaluated in the laboratory.

Embryos arising from immature or failed to fertilise eggs were previously shipped to the Roslin Institute, Edinburgh to the derivation laboratory of Dr Paul DeSousa under HFEA R0136-2b (as outlined in the original application and in subsequent reports). This arrangement finished in July 2007 with the expiry of our MRC grant with Edinburgh, and these embryos are now being used in Manchester for derivation attempts in our stem cell centre.

In addition we continue to use surplus embryos donated from IVF treatment to derive embryonic stem cell lines, with a view to establishing lines at Good Manufacturing Practice (GMP) standards for clinical therapeutic use.

Research undertaken to date.

In the past 12 months, work has been continued performing in vitro maturation on immature oocytes.

In vitro maturation of immature (GV or MI stage eggs) has been carried out using 3 different IVM media in an attempt to improve this technique. Eggs which mature to the MII stage then undergo fertilisation using the techniques described below, as used for donated eggs which have failed to fertilise. These oocytes can then be assessed for the integrity of the meiotic spindle, a measure of developmental competence, using birefringent light (Polscope).

Parthenogenetic activation is performed on oocytes donated to research after they have failed to fertilise by conventional IVF/ICSI treatment. Activation is performed by treating the oocytes with calcium ionophore followed by incubation in cyclohexamide and 6-DMAP. As an alternative to parthenogenetic activation, ICSI using donor sperm has also been carried out in order to fertilise the eggs.

Two sets of embryos were used for further analysis. Firstly those activated / ICSI oocytes showing signs of fertilisation (the presence of pronuclei). Secondly, normally fertilised embryos donated to the project which were not suitable for embryo transfer or cryopreservation due to poor embryo quality. Both groups were cultured in the research laboratory for up to 7 days. Blastocysts generated from recovered oocytes and from surplus, poor quality embryos were used for stem cell derivation in Manchester. A subset of embryos from both groups were fixed for analysis by immunofluorescence or lysed for gene expression analysis by PolyA PCR and or microarray techniques. This work is undertaken in Manchester using the standard protocols of our existing embryo research licence R0026.

In addition we continue to use surplus embryos donated from IVF treatment to derive embryonic stem cell lines, with a view to establishing lines at Good Manufacturing Practice (GMP) standards for clinical therapeutic use.

We also continue with our stem cell research programme aimed at developing progenitor cells for target tissues cartilage and insulin producing beta cells. We have not reported data from this research programme as it lies outside the remit of the HFEA licence. However we include a brief summary of activities:

The NWESCC GMP (Good Manufacturing Practice) Clean Laboratories are the first in the North West to engage in the production of GMP embryos and the derivation of human embryonic stem cell lines and will allow us to develop stem cell products to standards of GMP for potential therapeutic use. To date the work in this area has established standardised methods for maintenance of pluripotency using existing hES cell lines (HES-3, HUES-3 and HUES-7) and these have been applied to the derivation of new hES cell lines. Using embryos donated by patients at centres 0067 and 0033 three hES cell lines

have been derived, RCM-1, MAN-1 and MAN-2, which have been characterised and banked with the UK stem cell bank. In order to eliminate the use of non-human contaminants, such as mouse embryonic fibroblasts commonly used as feeder cells in ES culture, protocols are being developed using human placental stromal fibroblasts (PSF) feeder cells. A GMP protocol has been initiated for the screening, passaging and stockpiling of PSF cells for the derivation and culturing of future MAN hES cell lines. The goal is to use such a protocol to generate a GMP immortalised PSF line using integrating viral vectors, in an attempt to attain reproducibility between ongoing derivations and during culture under full GMP conditions.

Lay summary of research undertaken

Immature eggs and eggs which have failed to fertilise are not suitable for clinical IVF treatment and are normally discarded. In this project, these eggs have been used to establish and optimise methods for the recovery of clinically unusable oocytes by in vitro maturation and/or parthenogenic activation or fertilisation, in order to generate viable embryos for human embryonic stem cell derivation. We are also using embryos surplus to IVF treatment for this purpose, similar to most other stem cell centres in the UK.

Our results have shown that maturation of immature eggs can be achieved in the laboratory using culture fluid supplemented with various factors which encourage growth. We are studying the expression of various gene patterns in eggs matured in the laboratory and comparing them to normal healthy eggs. At the moment, eggs matured in this way cannot be used for clinical treatment as not enough is known about the normalcy of these eggs but results from this project will increase this knowledge leading to the use of immature eggs for IVF treatment in the future.

Parthenogenetic activation involves an egg being artificially stimulated by chemicals in order to trigger embryo development. Alternatively, eggs which have failed to fertilise may be re-inseminated using donor sperm. Eggs which have failed to fertilise after standard IVF treatment are being treated by both of these methods in the laboratory. Eggs which successfully fertilise after such treatment are cultured in the incubator for up to 7 days and carefully monitored. The normality of such embryos is established by analysing DNA prepared from the embryos for expression of genes which may act as markers of normal development.

The eventual aim of the project is to derive human embryonic stem cell lines from embryos generated using these methods. Any such stem cell lines will be derived in a purpose built facility and will undergo a variety of tests to establish the normality of the cell lines before they will be submitted to the UK Stem Cell Bank.

Issues for consideration

The research team has submitted three papers for publication in peer reviewed, scientific journals, based on studies undertaken as part of the project.

De Sousa et al. manuscript describing RCM-1, due to be submitted to Cell Stem cells in September 2008

Sneddon et al. manuscript describing characterisation of parthenogenetically activated oocytes, due to be submitted September 2008

Camarasa et al. manuscript describing Man-1 and Man-2 stem cell lines, due to be submitted to RBMOnline October 2008.

Executive recommendations for Licence Committee

Good progress made in achieving the stated aims of the project.

The research team has submitted three papers for publication in peer reviewed, scientific journals based on studies undertaken as part of the project.

Areas not covered in this inspection

None.

Report compiled by:

Name…… ……Wil Lenton…….

Designation…… …Regulatory Inspector…….

Date……17 October 2008……

Appendix A: Centre Staff interviewed

PR plus seven other staff

Appendix B: Licence history for previous 3 years

9th January 2008 – Research Committee re: Inspection report 26/09/2007

26th September 2007 - Inspection

9th May 2007 – Research Committee re: Inspection of new laboratories (0175)

Status	**Licence**	**Type**	**Active From**	**Expires**
Active	R0170/1/b R0171/1/b	Research Project	01/06/2006	31/12/2009
Replaced by new version	R0170/1/a R0171/1/a	Research Project	01/01/2006	31/12/2009
Expired	R0156/2/a	Research Project	27/10/2004	31/10/2005

R0156 was replaced by R0170/0171 for administrative purposes.

R0170/0171 were issued without any additional conditions.

R0156/2/a was issued with one additional condition:

Once 200 embryos have been created as part of the project, the Person Responsible must contact the Authority to communicate that fact, to update the Authority about the results of the research and to demonstrate whether the creation of more embryos is necessary. In addition the Authority would like to be told how many blastocysts have been derived from the embryos used.

Appendix C:

RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number…….0067, 0033, 0175…….

Name of PR…… Daniel Brison ……..

Date of Inspection…… 1710 08……..

Date of Response…… 26 11 08……

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

None

I have read the inspection report and agree to meet the requirements of the report.

Signed……  …….

Name…… Daniel R Brison ……..

Date…… 26 11 08……

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).

3. Donation of material – highlighted areas of firm compliance Centre 0067 no longer holds a waiting list meeting for patients, due to the requirement to treat within 18 weeks (DH 18 week pathway). As of mid-2008, all information including for research projects is now posted out to patients in advance, similar to most other units. Consent for licence R0171 is taken by a dedicated research nurse who is independent of clinical treatment or research, not by a medical consultant as is the case for licence R0026.

We also welcome comments about the inspection on the inspection feedback form, a copy of which should have been handed out at the inspection. If you require a copy of the feedback form, please let us know.

Please return this section of the report to:
Dr Chris O'Toole
Head of Research Regulation, HFEA
21 Bloomsbury Street

London
WC1B 3HF

Research Licence Committee Meeting

15 January 2009
21 Bloomsbury Street London WC1B 3HF

MINUTES Item 3

Research Projects R0170 and R0171
based at St Mary's Hospital, Manchester Fertility Services and University of Manchester (0067, 0033, 0175)
Interim Inspection

Members of the Committee:	In Attendance:
Emily Jackson, Lay Member – Chair	Chris O'Toole, Head of Research Regulation
Richard Harries, Lay Member	Claudia Lally, Committee Secretary
David Archard, Lay Member	
Neva Haites, Professor of Medical Genetics, University of Aberdeen	Providing Legal Advice to the Committee:
Hossam Abdalla, Director, Lister Fertility Clinic	Mary Timms, 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 (61 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 this research project is concerned with generating clinical grade embryo stem cell lines. The actual derivation takes place at centre 0175, with the two fertility centres, 0067 and 0033, providing and growing the embryos to be used. Dr O'Toole reported that in the period covered by the inspection, the project has made good progress, with researchers having submitted three papers to peer reviewed journals based on studies undertaken as part of the project.

The Committee's Decision

2. The Committee noted the good progress being made with this project of research, and the submission of three papers based on the research findings.

The Committee also took into account the fact that no issues of concern had been raised at the interim inspection and that a satisfactorily completed Person Responsible Entry Programme (PREP) assessment has been submitted by the Person Responsible.

3. The Committee decided that the research licence should continue with no additional conditions.

Signed..... Date 26-1-09.
Emily Jackson (Chair)