Research Licence Inspection Report

Project Title	Derivation of human embryonic stem cell lines from embryos created from clinically unused oocytes or abnormally fertilised embryos.
Centre Name	St Mary's Hospital, Manchester (0067), University of Manchester (0175), Manchester Fertility Services (MFS)(0033)
Centre Number	0067; 0175; 0033
Research Licence Number	R0170/1
Centre Address	*0067* – Department of Reproductive Medicine, St Mary's Hospital, Manchester, M13 0JH *0175* – Faculty of Life Sciences, University of Manchester, Floor 2 Core Technology Facility, 46 Grafton Street, Manchester M13 9NT *0033* – Bridgewater Hospital, 120 Princess Road, Manchester M15 5AT
Donating treatment centres	0067; 0033; 0008
Inspection date	30 July 2009
Research Committee Date	18 November 2009
Inspector(s)	Mr Wil Lenton (Lead, HFEA Inspector) Ms Paula Nolan (HFEA Inspector)
Fee Paid – date	R0026 – Yes
Person Responsible	Prof Daniel Brison
Nominal Licensee	Dr Cheryl Fitzgerald; Dr Sue Kimber; Prof Brian Lieberman
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.

Brief Description of the Centre and Description of the Proposed Research

The project, R0170/1 is entitled 'Derivation of human embryonic stem cell lines from embryos created from clinically unused oocytes or abnormally fertilised embryos.

The present licence commenced on 01 January 2006 and is due to expire on 31 December 2009.

This project is based mainly at centres 0067 and 0175, which are within five minutes walk of each other. Active research at centre 0033 has not been undertaken recently as the scientist involved has left.

The project is currently licensed for the following purposes:

- promoting advances in the treatment of infertility
  Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(a)

- increasing knowledge about the development of embryos
  Human Fertilisation and Embryology (Research Purposes) Regulations 2001 s2(a)

- increasing knowledge about serious disease
  Human Fertilisation and Embryology (Research Purposes) Regulations 2001 s2(b)

- enabling any such knowledge to be applied in developing treatments for serious disease
  Human Fertilisation and Embryology(Research Purposes) Regulations 2001 s2(c)

The lay summary is as follows:

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/Proposed Activities

	Activities	**Currently licensed activity**	**Proposed licence activity**
5.1.1	Storage of eggs	☐	☐
5.1.2	storage of eggs within ovarian tissue	☐	☐
5.1.3	storage of sperm	☒	☐
5.1.4	storage of sperm within testicular tissue	☐	☐
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	☒	☐

Changes/Improvements since last inspection

The part-time research nurse coordinators post at 0067 has now been developed into a full time position as of February 2009.

Four new staff have been employed and three have left. All new staff details have been supplied to the Executive via the licence renewal application.

Summary for Licence Committee

This is a renewal inspection report for project R0170/1.

The project has received ethical approval by an appropriately-constituted local research ethics committee (LREC).

Since the last licence renewal in August 2006, research has continued to make progress in line with the stated project objectives and a number of publications, presentations and posters have been realised via data generated from the R0170/1 project work.

The centre has suitably qualified and trained staff with which to pursue the research objectives within secure premises.

The part-time research nurse coordinators post at 0067 has now been developed into a full time position as of February 2009.

Although the present funding is due to expire early in the next renewal period, a new MRC grant (£1.2M, activated June 1st 2009 for 2.5 years) is in place, together with further funding from Arthritis Research Campaign ARC (£200K) for chondrocyte differentiation from Sept 2009 for 3 years (pending written confirmation).

Due to extensive refurbishment work presently underway at St Mary's (0067), between July and October 2009, egg-collections and embryo-transfers are to be performed at centres 0033 (Manchester Fertility Services - MFS) and 0185 (CARE Manchester). Patients have had prior notification of this activity and arrangements made with the other HFEA-licensed centres to provide continuity of care for St Mary's patients during this period.

Patient clinics and consenting for research will continue to take place at St Mary's via a dedicated research nurse, and all aspects of patient and egg/embryo anonymisation will take place exactly as if the patients were being treated at St Mary's. Transport of eggs/embryos from MFS and CARE to the research laboratories at centres 0067 and 0175 will continue to be via dedicated embryo shipping incubator accompanied at all times by a member of research staff.

The Executive recommend the continuation of the centres licence without additional conditions.

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	2
Laboratory scientists	3
Support staff (receptionists, record managers, quality and risk managers etc)	Staff at centres 0033 and 0067

Highlighted areas of firm compliance
The PR has previously successfully completed the PREP, has extensive knowledge of the regulatory requirements of the HFEA, together with appropriate research and publishing experience and has recently been awarded a professorship. 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. Additionally there is a minuted meeting which takes place every two weeks for all research staff. Minutes from the above research meetings were made available to the inspection team. The part-time research nurse coordinators post at 0067 has now been developed into a full time position as of February 2009. The Authority has been notified of all other staff changes. The project has received ethical approval by an appropriately-constituted local research ethics committee (LREC). Current funding (MRC, Northwest Development Agency) is due to expire early in the next renewal period, but a new MRC grant (£1.2M, activated June 1st 2009 for 2.5 years) has been secured, together with further funding from Arthritis Research Campaign ARC (£200K) for chondrocyte differentiation from Sept 2009 for 3 years (pending written confirmation).

Issues for consideration
Due to extensive refurbishment work presently underway at St Mary's (0067), between July and October 2009, egg-collections and embryo-transfers are to be performed at centres 0033 (Manchester Fertility Services) and 0185 (CARE Manchester). Patients have had prior notification of this activity and arrangements made with the other Manchester HFEA-licensed centres to provide continuity of care for St Mary's patients during this period.
Executive recommendations for Licence Committee
A debate was undertaken as to the fees payable by the Manchester centres to the Authority. It was agreed that the issue would be resolved between the Executive and the PR prior to the renewal/issuing of the respective licenses. (R0170/1 – 31/12/2009 and R0026 – 31/01/2010).

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 commissioned in 2007. The inspection team were given a tour of the facilities which were located on both the second and ground floors of the Core Technology building. All access was seen to be secure via personalised/zone-programmed swipe-cards. The second floor laboratories included a stem cell culture room and space where secondary research takes place. Access to licensed material was regulated via a locked incubator. The ground floor included an administration area, microscopy suite, clean rooms and a plant unit which managed the clean room air quality. Again all access was seen to be secure. A cryo-facility for stem cell storage was also viewed. It was seen to be secure with appropriate automated monitoring of both liquid nitrogen within the tank, and oxygen levels within the room. External alarms would alert staff to any problems with either system. An automated monitoring system (monitoring stem cell incubator temperature and % carbon dioxide levels together with the cryofreezer liquid nitrogen temperature) was administered via staff in the office area on the ground floor. An out-of-hours alarm system was in place, which would alert off-site staff should an incident occur outside of normal working hours. No research work was presently being undertaken at centre 0067 laboratories due to major building works to adjacent theatre suite facilities (July- October 2009). This new facility had only been commissioned in February 2008 and was found to be compliant during the inspection of centre 0067 in October 2008.
Issues for consideration
Some critical equipment viewed during the inspection had not been serviced within the prescribed time period.
Executive recommendations for Licence Committee
All critical-use equipment should be serviced/maintained on a regular basis in order to ensure that patient donated licensed material is not lost through equipment failure.

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

Summary
The information has not changed since the previous inspection. Thus; **Centre 0067 –** All prospective patients attend a 'waiting list' meeting where they are introduced to the concept of donating gametes and embryos to research. Patients are also given the information sheets and consent forms relating to the specific research projects undertaken in the licensed centres in Manchester. **Centre 0033 -** Patients are given the information sheets and consent for both research projects during their initial clinical consultation. A medical consultant gives patients information and obtains consent from patients who wish to donate gametes and / or embryos to the embryo development project. Approximately 70% of patients consent to the donation of gametes and embryos for use in the R0026 licensed research project. If patients consent to the donation of gametes and / or embryos to be used in licensed research a coloured sticker (green for project R0026) 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. 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
During the planned refurbishment work between July and October 2009, patient clinics and consenting for research will continue to take place at St Mary's via a dedicated research nurse, and all aspects of patient and egg/embryo anonymisation will take place exactly as if the patients were being treated at St Mary's. Transport of eggs/embryos from MFS and CARE to the research laboratories at centres 0067 and 0175 will continue to be via dedicated embryo shipping incubator accompanied at all times by a member of research staff.
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

Summary
Five sets of patients notes, who had donated licensed material to research, were reviewed on the day of inspection and found to be compliant. In two of the cases the material donated to research was traced, via an audit trail from procurement, use and storage, through to donation to research and subsequent end-use in the research project. All licensed material donated to research was traceable via the audit trail. Patient information and consents comply with all of the requirements outlined in standard licence conditions and the 7th Code of Practice (CoP7).
Areas for improvement
None
Executive recommendations for Licence Committee
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

Projected use of material for project R0170/1 in previous inspection report (17 October 2008)

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

***These figures represent maximum numbers only**

Actual use of material (from licence renewal application)

Material	0067		0033	
	Received	Used	Received	Used
Fresh Eggs	36 (immature)	36	0	0
Frozen Eggs	0	0	0	0
Failed to Fertilise Eggs	56	56	0	0
Fresh Embryos	47+ 48 = 95	47+ 48 = 95	0	0
Frozen Embryos	0	0	29	14**
Embryo's created	26*	26	0	0

* **parthenogenetic activation**

** **used from previous years**

Projected use of material for project R0170/1 in forthcoming twelve months (2009/10)

Material	Expected usage
Fresh Eggs	600(immature)
Frozen Eggs	0
Failed to Fertilise Eggs	800
Fresh Embryos	500
Frozen Embryos	300

As noted by the peer reviewer, the amount of licensed material used over the last year, has been lower than previously anticipated, mainly due to the absence of a research nurse coordinator (from September 2007), which lead to a lower rate of patient consent to the project. Due to delays in MRC funding the nurse coordinator was only replaced in May 2008, on a part time basis (2 days per week) to January 2009. This impacted significantly on the number of donated oocytes and embryos. The centre have now appointed a new full-time research nurse, as of February 2009, and do not anticipate further problems with egg and/or embryo supply.

Project objectives

Renewed objectives

Since our current licence was granted (January 2007), our core objectives have remained essentially unchanged, in that we continue work towards derivation of embryonic stem cell lines from surplus IVF embryos, and activated oocytes, under GMP conditions.

During the summer of 2009 (July-October) we will continue to collect oocytes and embryos from Manchester Fertility Services as previously, and we will also add CARE Manchester as one of our donation sites. This is because the NHS laboratories at St Mary's Hospital are closed for a refurbishment of the hospital building, and St Mary's NHS patients will have egg recoveries and embryo transfers performed at Manchester Fertility Services and CARE Manchester. These two centres have signed contracts with St Mary's for this work and agreed to facilitate donation of oocytes and embryos in line with patient wishes. Patient clinics and consenting for research will continue to take place at St Mary's via our dedicated research nurse, and all aspects of patient and egg/embryo anonymisation will take place exactly as if the patients were being treated at St Mary's. Transport of eggs/embryos from MFS and CARE to the research laboratories at centres 0067 and 0175 will continue to be via dedicated embryo shipping incubator accompanied at all times by a member of research staff.

Summary of research undertaken

In the past 8 months, work has been continued performing in vitro maturation on immature oocytes and activation of failed to fertilise and IVM oocytes at metaphase II. Previous work is summarised in the appended manuscript Sneddon et al, submitted to Human Reproduction.

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.

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.

Two sets of embryos were used for further analysis: firstly those activated 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.

Since September 2008, we have received 12 immature oocytes and 7 of these reached metaphase II after in vitro maturation. Six IVM oocytes were activated and 100% cleavage rate was observed. Of the 56 MII failed to fertilise oocytes donated to research, 18 were activated, 16 cleaved and 1 blastocyst was generated for stem cell derivation. Of the fresh metaphase II oocytes, 2 cleaved after parthenogenetic activation but no blastocysts were generated.

Derivation of embryonic stem cell lines

The 3 research grade lines we have derived have now been submitted to and accepted by the UK Stem Cell Bank, are fully characterised, and described in two published papers (DeSousa et al., 2009; Camarasa et al., in press). We have also submitted details to the European Registry (http://www.hescreg.eu/index_reg.php?nav=55&id=450#AI ; http://www.hescreg.eu/index_reg.php?nav=55&id=451 and have enrolled these lines in the International Stem Cell Initiative (ISCI) (http://www.stemcellforum.org/isci_project/isci_2.cfm) for karyotypic and SNP analysis at early and late passages.
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We have not derived another research grade line, but are continuing with our efforts to develop GMP compatible protocols for derivation, culture and targeted differentiation of hES cell lines. To this end we have developed human feeder cells from placental fibroblasts (McKay et al. appended) and a feeder-free culture medium (Baxter et al 2009). We have also developed a detailed protocol for differentiation of human ES cells to chondrogenic cells for cartilage repair (Oldershaw et al., Nature Biotech, under review).

Peer reviewers comments

The peer reviewer noted that, ‘progress had been as might have been expected’ and that, ‘the group were well qualified to carry out the research’ and accepted the licence renewal application in its current form.

Issues for consideration

Progress has been very good.

The group have not continued to derive research grade hESC lines, but as of June 2009 will be focussing on the derivation of GMP grade lines.

Since the last licence renewal in August 2006 a number of publications, presentations and awards have been realised via data generated from the R0170/1 project work:

Publications (*see Appendix H for manuscripts and abstracts)*:

Baxter M A, Camarasa M V, Bates N, Small F, Murray P, Edgar D H, Kimber S J - Analysis of the distinct functions of growth factors and tissue culture substrates necessary for the long-term self-renewal of human embryonic stem cell lines; *Stem Cell Research* 2009

Camarasa M V, Kerr R W, Sneddon S F, Bates N, Baxter M A, Oldershaw R A, McKay T R, Small F, Brison D R, Kimber S J. Derivation of Man-1 and Man-2 research grade human embryonic stem cell lines.

McKay T R, Camarasa M V, Bates N, Sharp T V, Foxler D, Mee M, Aplin J, Brison D R & Kimber SJ - Human Feeder Cell Line for the Derivation, Culture and Maintenance of Human Embryonic Stem Cell Lines. For submission to *Nature Methods* 2009

Oldershaw RA, Baxter M A, Lowe E T, Bates N, Grady L M, Brison D R, Hardingham T E, Kimber S J; The targeted differentiation of human embryonic stem cells towards chondrocytes Submitted to Nature Biotech (Manuscript under review)

Sneddon S F, DeSousa P A, Arnesen R E, Lieberman B A, Kimber S J, Brison D R.
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A new source of human eggs and embryos for infertility and stem cell research (Human Reproduction, under review)

Oral Presentations:

Oldershaw RA. (February 2009) Stem Cells. Invited presentation at Stockport Grammar School as part of the Junior Cafesci programme.

Oldershaw RA. (April 2009) Differentiation of human embryonic stem cells towards chondrocytes using a step-wise protocol. UK National Stem Cell Network, Second Annual Scientific Conference, University of Oxford.

Oldershaw RA. (Pending June 2009) Invited guest speaker at the Institute of Physics (IoP) Manchester teacher's conference "Stem Cells – Hype and Hope". This presentation is aimed to present an overview of the stem cell

research being carried out at the University of Manchester in order that teachers can pass on to their students information about entering careers in science.

Oldershaw RA. (Pending June 2009) Invited guest panellist for the annual Pathways Careers Conference hosted by the University of Manchester. This is event is a question and answer session in which prospective and current PhD students have the opportunity to question past PhD alumni about their chosen career paths.

Posters: see Appendix I

(i) The differentiation of human embryonic stem cells towards chondroprogenitors using a chemically defined step-wise protocol (Oldershaw)

(ii) Novel human embryonic stem cell lines derivation and adaptation to xeno-free culture conditions (Camarasa)

(iii) Generation of a Novel Human Feeder Cell Line for Derivation and Culture of Human Embryonic Stem Cells (McKay)

Executive recommendations for Licence Committee
The project work has made good progress and generated peer-reviewed scientific publications.

Report compiled by:

Name…….....Wil Lenton…….

Designation…… Inspector…….

Date……..30 July 2009……

Appendix A: Centre Staff interviewed

PR, NL and two staff members.

Appendix B: Licence history for previous 3 years

30th July 2009 – Renewal inspection

15th January 2009 – Research Committee – no issues of concern

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.

Appendix C:

RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number……0067. 0033, 0175……

Name of PR……Daniel Brison…….

Date of Inspection…….July 30th 2009……..

Date of Response……October 9th 2009 ……

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

We have reviewed our equipment maintenance schedules as suggested during the inspection. This has already been, done, and maintenance will be routinely performed on all equipment within the next 12 months as required.

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

Signed…….

Name……Daniel Brison…….

Date……9th October 2009……

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).

None, other than the number for the 3rd donating centre, 0008, is incorrect. You may mean CARE Manchester, which is 0185 I believe.

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
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WC1B 3HF