



## Research Licence Inspection Report

Project Title	Platform technologies underpinning human embryo stem cell derivation
Centre Name	Department of Gene Expression and Development, Roslin Institute
Centre Number	0202
Research licence Number	R0136/2/b
Centre Address	Department of Gene Expression and Development, Roslin Institute, Roslin Midlothian, EH25 9PS
Proposed treatment centres donating to this research project	0067 St Mary's Manchester 0019 Aberdeen 0004 Ninewells 0044 University College, London 0201 Edinburgh Infirmary Edinburgh Infirmary (not licensed by the HFEA)
Inspection date	18 <sup>th</sup> January 2007
Licence Committee Date	9 <sup>th</sup> May 2007
Inspector(s)	Miss Sarah Hopper Dr Debra Bloor
Fee Paid - date	January 2007
Person Responsible	Dr Paul de Sousa
Nominal Licensee	Professor Ian Wilmut

### **About the Inspection:**

The purpose of the inspection is to ensure that researchers comply 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 patient services and meet regulatory standards. It is primarily written for the Licence Committee who make the decision about the centre's research licence application. The report is also available to patients and the public following the Licence Committee meeting.

### **Brief Description of the Centre and licensed project**

The Roslin institute has held a HFEA licence for this project since July 2003.

Paul de Sousa has been the PR for this project since 2004. He is currently on secondment from the University of Edinburgh and attends the centre for two days a week. It is planned that this will decrease to one day per week in the future.

The project title is: "Platform technologies underpinning Human Embryo Stem cell derivation". Its aim is to develop methods to derive and maintain human embryonic stem cells (hESCs) from a variety of sources as well as derive and maintain hESCs cells in a defined environment free from animal cell products.

The lay summary of the proposed research project, submitted by the centre is as follows:

"The aim of our research is to derive human embryonic stem cells in a manner which would permit those cells to be used in developing treatments for patients with degenerative diseases, such as Parkinson's and diabetes.

Current techniques for deriving stem cells use products which are themselves derived from human or animal cells. Furthermore these techniques are conducted in standard laboratory facilities. This means there is a risk that the cells derived in this way could acquire a disease-producing agent, such as a virus, which could be passed on to subsequent generations of those cells. Consequently, no cells derived in this way, or arising from cells derived in this way, can be transplanted into a patient.

Our research will investigate ways to eliminate the current reliance on human/animal cell based products and we will also develop techniques and protocols to carry out this work in "clean room" facilities. If we are successful, the stem cells we produce could be used as the starting point for producing cells to be used in therapies to address degenerative diseases.

Our research will also investigate the isolation of human embryonic stem cells from eggs and embryos which are clinically unsuitable for infertility treatment. To achieve this we will use methods to mature and/or activate the egg or embryo. If successful, embryonic stem cells will be derived from eggs or embryos which could not have been used in the donor's infertility treatment. Our research will not involve fertilising eggs with sperm. Instead eggs which are either immature or which failed to fertilise will be activated artificially. If we can isolate embryonic stem cells as a result of this process, they will contain only maternal genetic material. Such cells would allow further research into the influence of maternal genes on the development of the embryo."

<b>Research activities of the Centre</b>	Research on human embryos	✓	
	Storage of licensed material		
	Creation of embryos for research	✓	
	Derivation of human embryonic stem cells	✓	
	Cell nuclear replacement		

### Summary for Licence Committee

The centre is applying to renew its research licence to investigate platform technologies underpinning Human Embryo Stem cell derivation.

In the past year oocytes and embryos have been donated from patients undergoing fertility treatment at the IVF unit at the following centres; UCH London ACU (0044) and St. Mary's Hospital, Manchester (0067). In addition oocytes have been donated by patients undergoing voluntary sterilisation at the Edinburgh Infirmary Obstetric/Gynaecology Outpatient Clinic. Between January 2005 and January 2006 the centre received a total of 104 immature oocytes, 5 fresh blastocysts (0044) and 5 clinically unusable oocytes & embryos (0067).

The supply of clinically unusable oocytes and embryos from Centre 0067 is not expected to continue but it is expected that the number of donated oocytes and embryos will remain constant due to a supply from centres 0201, 0019 and 0004. In the next year the PR is proposing to use 100 immature oocytes (sourced from patients attending the Edinburgh Infirmary Ob/Gyn Outpatient Clinic), 400 clinically unusable eggs & embryos (centre 0201), 60 fresh blastocysts (20 from centres 0044, 0019, 0004) and 80 frozen blastocysts (centres 0201, 0044, 0019, 0004).

The research 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 causes of congenital disease  
*Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(b)*
- increasing knowledge about the causes of miscarriages  
*Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(c)*

A Peer Reviewer has assessed the renewal application and concluded that it should be accepted in its current form.

The inspectorate recommends renewal of the centre's licence for a further 3 years.

## 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
- Staffing
- Research governance
- Funding

### Staff

Principal investigator	1
Laboratory scientists	3
Administrators	3
Collaborators	2
Support staff (receptionists, record managers, quality and risk managers etc)	There are research nurses at each of the licensed centre from which donors are recruited.

### Background information

The project is now managed by Roslin Cells Ltd which is a not-for-profit company. Roslin Cells Ltd is currently wholly owned by the Roslin Institute but is in partnership with the University of Edinburgh and the Scottish National Blood Transfusion Service, who contribute to the project through provision of staff with scientific leadership and quality assurance expertise. As a benefit of partnership, all three Institutions are entitled to unencumbered access to Roslin Cells hESC lines for research and commercialisation. It is planned that in the summer months of 2007 the PR will apply for the licence to be varied so that the licensed centre name will become Roslin Cells Ltd.

From July 1<sup>st</sup>, 2006, the project has been funded by Scottish Enterprise Edinburgh and Lothian (SEEL). This funding has allowed for the establishment of Roslin Cells Ltd. The aim of the company is to derive and market research and therapeutic grade hESCs. Revenue from the marketing of hESC lines is intended to make the company self-sustaining. This does not preclude deposition of hESC lines in the UK stem cell bank, in compliance with the terms of an HFEA licence. The PR explained that Roslin Cells will market access to cells in exchange for upfront payment without seeking royalties on the commercialisation of subsequent research. Current funding for the Roslin Cells project runs through to September 30<sup>th</sup> 2008. The PR also plans to try to secure further funding from the MRC and the UK stem cell foundation.

The PR stated that the research team meet twice a month. Executive matters (e.g funding streams) and operational matters (management and coordination of work) are discussed at

alternate meetings.

A quality manager has been appointed and will be in post on the 1<sup>st</sup> February 2007. The PR stated that he is planning to employ two more members of staff in the laboratory and one more administrator.

#### Issues for consideration

Research staff reported that they attend mandatory training sessions. The inspectorate suggested that this information should be recorded within staff training logs.

The PR stated that although he is involved in the recruitment of staff and reviews all references for potential employees, CRB checks are not carried out on staff.

With regard to these issues the PR stated that training and recruitment of staff is currently governed by the Roslin Institute. When Roslin Cells takes over this responsibility, appropriate protocols should be developed to cater for these areas. These should include: a documented policy for the recruitment of staff, which includes checking professional qualifications, experience of prospective staff and the checking of references; a procedure requiring all prospective and existing staff to report to the PR any relevant criminal convictions or breaches of regulations.

The PR should ensure that a written adverse incident handling policy which meets the HFEA requirements is developed.

#### Executive recommendations for Licence Committee

Approve the application for position of NL by Mr Malcolm Bateman.

#### Areas not covered in this inspection

Resource management

## 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

### Background information

Since the last inspection, the original laboratory premises have been renovated in order to satisfy Medicine and Healthcare products Regulatory Agency (MHRA) requirements. The laboratory has been refurbished to good manufacturing practice (GMP), this will enable the PR to achieve his aims of isolating hESCs in high specification facilities working to quality assured, good manufacturing practice defined by UK and EU regulations and directives.

The new facility consists of a series of three rooms with increasing levels of air quality. Researchers will change in the first room, which has a background air quality of grade D. The next room is to be used for preparation of material/media and storage of reagents. The final room, which staff can only enter with full body suits, will be used for the derivation of embryonic stem cells. This room has a grade B background air environment. Entrance to this laboratory is restricted to authorised personnel by use of a key pad lock.

The laboratory is currently undergoing performance validation and the PR anticipates that hESC derivation work will begin within this facility by March 2007.

Currently work is being carried out in temporary laboratory facilities which were inspected by the HFEA in June 2006. This laboratory comprises of three parts, a microscopy room, a preparation room and stem cell derivation clean laboratory. Donated material is brought to the laboratory, removed from the transport incubator and then moved into the stem cell derivation laboratory for further culture and manipulation. This room is secured by key pad lock and the PR confirmed that access is restricted to the research team. The microscope room is shared with other Roslin employees but the PR stated that only fixed and anonymised samples are analysed in this area.

Frozen embryos are not stored at the centre as all donated frozen embryos are thawed prior to transport to the Roslin Institute.

Derived stem cell lines are stored in freezers which are held in a securely locked cryostore. The cryostore is a shared facility with other staff at the Roslin Institute. A low oxygen monitor is fixed in this room. A dedicated cryostorage consisting of a secure room containing -154, -80, and -20 C freezers, and low oxygen sensor all with a centralised alarms is currently undergoing performance validation and will form part of the new laboratory facilities.

Research records were seen to be securely locked in cabinets within the PR's office.

### Issues for consideration

No areas for improvement were identified during the inspection.

Executive recommendations for Licence Committee
None
Areas not covered in this inspection
Storage facilities Safety of equipment Servicing and maintenance of equipment

### 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
- Ensuring patient consent is not breached
- Donor and patient records

#### Background information

Research nurses are in post at all supplying centres. Funding for these research nurses is both private (Centres 0201 and 0044) and public from the MRC (Centres 0067, 004, 0019). All supplying centres have written protocols for the provision of information to people wishing to donate oocytes/embryos to research. These protocols were submitted with the renewal application.

Donors are recruited using a similar process, regardless of the supplying centre. Patients wishing to donate fresh embryos or oocytes are approached regarding research on day 6-9 of their cycle and consent is confirmed on the day of HCG administration or on the day of egg collection.

A verbal checklist is completed by the research nurse to confirm that consent for donation of embryos has been obtained. This checklist is used to confirm that participation is voluntary and that the implications of research have been discussed. An anonymised version of this checklist is provided to the research team and stored within the research files. Examples of completed checklists were provided during the inspection.

Patients with embryos in storage are provided with information about the research project by post. They are then given the opportunity to discuss the project with the research nurse coordinator either by phone or in person. If the patients are interested in donation to research they are then sent the specific consent forms.

Donors are made aware that they can withdraw consent. This information is provided in the patient research information sheets.

Each donor's consent for use in research is checked, witnessed and documented before transfer to the research project. A detailed embryo/oocytes acquisition form is completed by the research nurses (or in certain cases by an embryologist independent of the treatment process). This checklist includes:

- Supplying centre confirmation that the HFEA and research patient consent (PI&C) forms have been signed and witnessed.
- Confirmation that copies of signed research and HFEA forms have been placed in patient notes.

- Confirmation that the donor eligibility form has been checked for donor specified variations in terms. If the donors have placed restrictions on the use of their oocytes/embryos this information is also detailed.
- Arrangements for transport of oocytes and embryos (if applicable this includes courier details)

Once samples are received at the Roslin Institute an oocyte/embryo receipt and record checklist is completed. The form also contains an area to indicate any donor restrictions on consent. This ensures that the researchers are made aware of any limitations or restrictions to the usage of the donated material. During the course of the inspection, the issue of restricted consent was discussed and the PR recognised that restrictions cannot be placed on the use of embryonic stem cells. However, the PR stated that in some circumstances he will accept and use embryos donated with restricted consent as these could be incorporated into parts of the project which will not lead to the generation of stem cells lines.

Copies of the acquisition and receipt forms were provided to the inspection team and also seen during the audit of research records.

All identifying information is removed before transfer to researchers. A tracking number that is independent of the patient ID is assigned to each donation and a key correlating patient ID information and egg/embryo tracking number is kept at the supplying centres. All records audited during the inspection were seen to be anonymised.

Issues for consideration
None noted during inspection
Executive recommendations for Licence Committee
None
Areas not covered in this inspection
Prevention of coercion of prospective donors

#### 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

##### Background information

Patient information provided to potential donors, at all supplying centres, has the same format. There are slight differences depending on the type of donation concerned, i.e. donation of failed to fertilise oocytes, surplus embryos or frozen embryos. These patient information sheets contain relevant information required by the HFEA. The inspectorate were satisfied with the clarity and agreed that the information appears lay intelligible.

Information regarding research is provided to prospective donors during their treatment cycle (with the exception of patients donating frozen embryos to research) but the inspectorate reasoned that as the information is provided in stages the risk that patients would become overwhelmed by information has been considered and minimised.

Independent counselling is promoted in the patient information.

Patient consent forms cover all HFEA requirements. It is possible for prospective donors to give conditional or restricted consent.

All patient information and consent forms make the specific implications of donation to embryonic stem cell derivation projects clear. The information includes guidance that any stem cell lines created may continue indefinitely and that stem cell lines may be used for commercial purposes but that the donors will not benefit financially from this. The consent form confirms that the donor understands that samples of any stem cell line will be deposited in the UK stem cell bank and also confirms permission for a copy of the consent forms to be kept by the Secretary to the UK Stem Cell Bank Steering Committee.

According to the protocols for obtaining donor consent, a copy of the consent forms is given to the donors once completed.

The previous inspection team recommended that patient information should be amended to note that an independent person is available should a potential donor wish to discuss the project. Patient information has been updated accordingly.

##### Outcome of record audit

Four research records were audited by the inspectorate. The records were detailed and contained supplying centre evidence that the patient consent forms had been checked to ensure valid consent to research. No discrepancies were noted.

Issues for consideration
Some slight amendments to the patient information were recommended by the inspectorate. The PR stated that the patient information will be revised accordingly.
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

Summary
<p>Standard Operating Procedures (SOPs) supplied to the inspectorate has evidence of version control.</p> <p>Quality assurance systems are being developed by the PR. It is planned that aliquots of stem cells lines will be tested within a separate quality assurance laboratory at the Roslin Institute. A Quality Assurance Manager has been appointed and is due to commence employment in February 2007.</p> <p>Within this study the embryos are not subjected to immunosurgical techniques to isolate the inner cell mass, instead whole embryos are outgrown in culture. This means that embryos are cultured beyond 14 days. This was discussed during the inspection and the PR commented that by day 12, embryos that have attached and outgrown on the surface of the dish no longer have the organisation structure of a viable embryo and are not representative of a 3D suspended embryo undergoing gastrulation.</p>
Embryo usage
<p>Embryo donation and usage figures provided by the PR relate to the embryos received by the Roslin Institute not the total number of embryos donated to the project. The PR confirmed that he considers it more effective for the donating/supplying centres to thaw research embryos and to continue culturing the embryos until they reach an appropriate stage for the research project. Embryos which do not survive the thawing procedures or which do not continue successfully in culture are not sent to the Roslin Institute and are therefore not included in the PR's calculated figures for embryo donation and usage.</p> <p>In the last 12 months, January 2006 to January 2007, 104 immature oocytes have been donated from Edinburgh Ob/Gyn Outpatient Clinic, 5 clinically unusable oocytes and embryos (at the blastocyst stage) have been donated by St Mary's Hospital Manchester (0067) and 5 fresh embryos have been donated from UCH London (0044). These numbers are substantially lower than those estimated in the original proposal. The PR stated that this was because of the following reasons:</p> <ul style="list-style-type: none"><li>• Immature oocytes (Edinburgh Infirmary): Supply was lower than predicted because the consent rate was lower than anticipated (on average of 22 donors per year)</li><li>• Fresh surplus blastocysts (0044): Embryo donation from this centre was halted during a research funding gap of 16 months.</li></ul>

- Clinically unstable eggs and embryos (0067): Supply was as anticipated until the funding gap.

In the next year the PR expects to use 100 immature oocytes, 400 failed to fertilise oocytes, 60 fresh blastocysts and 80 frozen blastocysts.

#### Summary of research undertaken

The PR stated that to date their achievements specifically include;

- a. Derivation of 7 new hESC lines, 6 of which have now been deposited in the UK Stem Cell Bank.
- b. Derivation of one hESC line in a defined media and a defined substrate (purified human extracellular matrix laminin), with transitional reliance on human fibroblast feeder cells.
- c. Derivation of hESCs from both fresh blastocysts that were surplus to infertility treatment, and a clinically unusable failed to fertilise egg whose developmental potential was recovered by artificial parthenogenetic stimulation.
- d. Evaluation of a role for Brain Derived Neurotrophic Factor in human oocyte in vitro maturation, and the discovery that it is downstream of and can substitute for hormone signalling responsible for cumulus expansion during oocyte maturation.
- e. Establishment that in vitro matured human oocytes from small antral follicles retain a competence for early cleavage development following parthenogenetic activation, although development to the blastocyst stage is poor.

The lay summary of work conducted so far (provided by the centre):

“So far, we have produced seven new embryonic stem cell lines. The techniques used to derive these cell lines have used increasingly fewer products derived from human or animal cells. One of these cell lines was derived without any reliance on nutritional products that originated from animals. However, it still remained necessary for this cell line to come into contact with other human cells as part of the derivation process.

Six of the seven cell lines were derived from donated embryos which were not required for infertility treatment. The other line was derived from an egg which failed to fertilise and which was activated as part of our research. DNA analysis of this cell line showed that it contained genetic material from a sperm as well as the egg. Although sperm penetration had occurred it was insufficient to elicit the development of an embryo. These results demonstrate the potential to use clinically unusable eggs for stem cell derivation.

We have also developed cell culture environments which support the maturation of eggs in the laboratory. This research has used immature eggs donated by women undergoing elective sterilisation. The means of supporting the maturation of eggs which we have developed has used a natural growth factor produced in the brain called neurotrophin. Our results support earlier work in this area and confirm that further study may lead to a better understanding of process by which human eggs develop.

Our future work will continue the development of methods to derive new embryonic stem cells without the reliance on products derived from human or animal cells and the development of techniques for deriving stem cells in “clean room” facilities.”

<b>Peer Reviewer comments</b>
<p>The Peer Reviewer assessed the application and concluded that:</p> <p>“The presented results and publications demonstrate that the group have done very important sequential steps in the creation of chemically defined environments to support the derivation of therapeutic grade hESC lines. The group have now completely refurbished laboratory to Good Manufacturing Practice (GMP) clean room standards which is of huge benefit to fulfil planned aim to derive new lines of clinical grade.”</p> <p>A minor point made by the reviewer was that the list of publications needs to be updated.</p> <p>The Peer Reviewer recommended that the application is accepted in its current form.</p>
<b>Issues for consideration</b>
None noted during inspection
<b>Executive recommendations for Licence Committee</b>
None
<b>Areas not covered in this inspection</b>
None

Report compiled by:

Name.....Sarah Hopper .....

Designation...Inspector... .....

Date.....18/01/07.....

## Appendix A: Centre Staff interviewed

PR and two members of the research team.

No conflicts of interest were declared

## Appendix B: Licence History

Licence	Status	Type	Active From	Expiry Date
<a href="#">R0136/2/b</a>	Active	Research Project	10/01/2005	30/06/2007
<a href="#">R0136/2/a</a>	Replaced by New Version	Research Project	01/07/2004	30/06/2007
<a href="#">R0136/1/b</a>	Expired	Research Project	12/03/2004	30/06/2004
<a href="#">R0136/1/a</a>	Replaced by New Version	Research Project	01/07/2003	30/06/2004

There are no conditions or recommendations on the current licence.

### Research Licence Committee 26 July 2006

The Committee noted that licensed work will be taking place within a temporary laboratory inside the existing premises whilst the main laboratory is refurbished.

### Research Licence Committee 12<sup>th</sup> January 2005

The papers were presented by Chris O'Toole, Head of Research Regulation. Dr O'Toole explained that a request had been made by the licence holder of R036 to vary that licence in order to allow the use of embryonic material from the research projects licensed under R0156 and R0158. The Committee agreed to vary the licence as requested.

### Research Licence Committee 28<sup>th</sup> October 2004

The papers were presented by Ross Thacker, Research Officer, who explained that the Licence Committee on 16 June 2004 imposed the licence condition that the Person Responsible makes certain changes to the patient information and consent forms related to this project. These changes have now been carried out to the satisfaction of the Executive, and the Person Responsible, Dr De Sousa, has requested that the condition is therefore removed from the licence. The Committee agreed that the patient information and consent forms have been amended in accordance with the condition and agreed therefore that this condition be removed from the project licence.

## Appendix C:

### RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number...0202

Name of PR...Paul A. De Sousa

Date of Inspection..18<sup>th</sup> January, 2007

Date of Response...6<sup>th</sup> February, 2007

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

First institutional ethics board introductory meeting occurred on 1<sup>st</sup> February, 2007  
Ethics board meeting to discuss review of HFEA licence renewal application scheduled for 8<sup>th</sup> March, 2007. The Executive will be informed of the outcome of this meeting.

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

Signed.....

Name.....

Date.....

#### 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).

Please note the following corrections under the relevant headings and associated page numbers:

#### Brief Description (pg 2)

Statement 1- Presently the PR attends Roslin 2 days per week and is shifting to 1 day/week.  
Statement 2 – In a “defined environment” not a “culture free environment”.

#### Staff (pg 4)

Admin. : 3

Collaborators: 2

#### 1. Organisation

Background info (pg 5): We are planning to employ to more laboratory staff and 1 more administrator.

2. Premise and equipment (pg 6)

The current cryostorage area is a shared facility however dedicated cryostorage consisting of a secure room containing -154, -80, and -20 C freezers, and low oxygen sensor all with a centralised alarms is undergoing performance validation.

3. Donation of material (pg 8)

Funding for research nurses is both private (0201 and 0044) and public from the MRC (0067, 004, 0019).

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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