



## Research Licence Renewal Inspection Report

Project Title	Analysis of chromosomes in human preimplantation embryos using FISH and CGH
Centre Name	London Fertility Centre
Centre Number	0088
Research licence Number	R0169
Centre Address	Cozens House, 112a Harley Street, London W1G 7JH
Treatment centres donating to this research project	Leicester Fertility Centre 0068
Inspection date	10 <sup>th</sup> August 2006
Licence Committee Date	
Inspector(s)	Miss Sarah Hopper (Lead)
	Dr Elliot Lawrence
Fee Paid - date	08/06/06
Person Responsible	Dr Alan Thornhill (at time of inspection)
Nominal Licensee	Mr Lawrence Ashford
Licence expiry date	31/12/2006

### **About the Inspection:**

The purpose of the inspection is to ensure that centres are providing a quality service for patients in compliance with the HF&E Act 1990, sixth edition 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 licence renewal application. The report is also available to patients and the public following the Licence Committee meeting.

This report covers the period between January 2006 and August 2006.

### **Brief Description of the Research Project and PR**

#### **Research Project:**

Research for this project originally started in 2003 but work halted and the licence expired in 2004 as the lead researcher left employment of the Centre. This licensed project, R0169, commenced in January 2006 and is due to expire on the 31<sup>st</sup> December 2006.

**The Research project is entitled:** Analysis of chromosomes in human preimplantation embryos using FISH and CGH.

The project was originally licensed under purposes laid down in Schedule 2 of the Human Fertilisation and Embryology Act 1990; 3(2)(e) to develop methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation.

#### **Lay Summary:**

“Certain IVF patient groups have been identified as being at high risk of producing embryos with chromosomal abnormalities. These chromosomal abnormalities usually cause failure of implantation following repeated IVF embryo transfers, or miscarriages. In a minority of cases the embryos can develop to cause a pregnancy affected by a chromosomal abnormality such as trisomy 21 (Down's syndrome). Preimplantation genetic screening (PGS) is a technique, which allows embryos produced during an IVF treatment cycle to be tested for specific chromosomal abnormalities. Following the screening procedure only embryos that are identified as being normal for the chromosomes being analysed are considered for embryo transfer. Due to the increased selective power provided by this procedure, PGS may reduce miscarriage rates and improve both implantation rates and live birth rates in specific patient groups. The screening process involves looking at the chromosomes present in a single cell taken from a 3 day old embryo. PGS relies on the fact that chromosomally, this cell should be an identical copy of the remaining cells in the embryo. By inference if the cell is normal, it likely came from a normal embryo, and if it was abnormal from an abnormal embryo. Previous studies have shown that many human embryos are not made up of chromosomally identical cells. These embryos are called mosaic embryos. Mosaicism can affect the reliability and hence the benefits of PGS.

Our study aims to analyse single biopsied cells using comparative genome hybridization

(CGH) or microarray techniques both of which can detect all the chromosomes in the cell. The results will be compared with the results obtained from the remaining embryo using FISH (a simpler technique able to reliably detect between 5 and 9 chromosomes in a single cell). This strategy will allow us to further investigate the incidence of mosaic embryos and the degree of mosaicism. In this way, we may be able to determine whether mosaicism is linked with a specific patient profile, such as age or IVF techniques such as embryo freezing and what degree of mosaicism an embryo can tolerate. This will ultimately improve the management of patients requesting PGS and help our understanding of early human development”.

Work under this licence involves the biopsy of day three embryos to remove two cells. These cells are then tested, one with Comparative Genomic Hybridisation (CGH) and one with microarray analysis, to determine chromosome constitution. This analysis is carried out at the Human Genetics and Embryology Laboratories, University College, London (Centre 0245). Post biopsy the embryos will be cultured to assess developmental competence.

The PR wishes to extend the research to include a continuation of work reported from a previously licence project R0140. They aim to study additional embryos to:

- Allow a more in depth statistical analysis of the proportion of normal and abnormal embryos.
- Study different age ranges to investigate a link between age and frequency and type of chromosome abnormality.

<b>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	

## Changes/ improvements since last inspection

- The changes to patient information and consent form required by the Licence Committee have been undertaken.
- Implementation of a folder system to allow clear identification of patients which have consented to research, those from which a response is pending and available/used embryos.
- A process of checking consents at each stage prior to thawing embryos has been implemented.
- All documentation is version controlled.
- There have been no changes in staff or equipment since the last inspection.

## Additional licence conditions and recommendations and actions taken by centre since last inspection

<b>C</b>	The inspection team advised that the centre should state on consent forms that the form supersedes references to research in the HFEA (00)6 and (00)7 consent forms
<b>A</b>	Complied: Yes

## Summary for Licence Committee

The inspectorate were satisfied that the centre is well organised. No breaches were noted during the inspection but a number of recommendations have been made:

- The research withdrawal consent form should provide clear contact details for the Centre in case it becomes separated from the research information.
- From the summary of the spot check and biopsy audit it is recommended that audits of research embryos in storage and embryo biopsy are performed at least annually.
- The laboratory protocol for research (Lab Pro 15) mentions that embryos can be used in research projects including FISH, PCR projects, ES cell lines. However, the Centre is not licensed for these projects and therefore the protocol does not correspond to the aims and objectives of the licence. Furthermore, the protocol could possibly mislead/confuse any new members of staff. When questioned regarding this, the PR explained that the protocol needed updating and that it would be reviewed appropriately.

The project has been assessed by a Peer reviewer who has confirmed that the application should be accepted for renewal.

The Executive recommend renewal of the licence for 1 year due to the forthcoming change in PR.

## Proposed licence variations

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## 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 investigator	1
Laboratory technicians	2 trained, 2 trainee
Administrators	0
Collaborators	2
Support staff (receptionists, record managers, quality and risk managers etc)	1

### Summary

The PR oversees and coordinates the research project activities. Embryo biopsies are carried out at the London Fertility Centre by two of the embryologists and the genetic analysis occurs at UCL by the principal investigator. Minuted meetings are held on a monthly basis with the co-supervisor and principal investigator at UCL. These were evidenced during the inspection. Regular meetings also take place between the team at Centre 0088. The PR stated that he contacts Centre 0068 to discuss the research project on a quarterly basis; however evidence of this was not seen during the inspection.

Use of the embryos is batched to allow the consistency within and between experiments to be measured. When a batch of embryos is available, the embryo biopsy is coordinated with the principal investigators workload. Before embryos are thawed for use in the research project the PR explained that an informal meeting is held to discuss the number of embryos to be biopsied, recheck patient consents, expiry dates of embryos and the collection of the biopsied cells and fixed embryos.

All submitted documents; the patient information, patient consents and protocols, have appropriate document management system footers.

The project is funded by Life-Force Research Limited. It has received ethical approval from an ethics committee which is properly constituted; the committee has a lay representation of more than five members and members which are independent of the research project.

**Issues for consideration**

The PR stated his intention to leave at the end of August and is in the process of finding a successor. The necessary paperwork required for the PR application should be submitted upon appointment.

Evidence of a training program for the new PR was not provided during the inspection.

**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

<b>Summary</b>
<p>The first stage of research is conducted in the clinical laboratory at Centre 0088. Within this laboratory the embryos are biopsied, cultured and fixed. Following fixation the slides are taken to Human Genetics &amp; Embryology Laboratories, Centre 0245 for analysis. The slides are labelled with the patient number which is non-identifying to the collaborator.</p> <p>During the inspection it was noted the laboratory is manned during working hours and secured out of office hours.</p> <p>Embryos donated to research are stored in locked dewars. Those donated by patients at Centre 0088 are not moved from their original locations and donated embryos transferred from Centre 0068 are allocated a space within the general dewars on arrival. All of the dewars were seen to be fitted with temperature probes connected to alarms. A low oxygen alarm is present in the cryostore and all alarms are connected to an autodialler.</p> <p>A documented emergency procedure for responding to damaged storage vessels was submitted with the laboratory protocols for the interim treatment and storage application. As research embryos are stored in their original dewars the same protocol therefore applies.</p> <p>The equipment within the laboratory had up to date service contracts which were examined during the inspection.</p>
<b>Issues for consideration</b>
None
<b>Executive recommendations for Licence Committee</b>
None
<b>Areas not covered by this inspection</b>
Laboratory premises at 0245 were not assessed as part of this inspection.

### 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

Donors are recruited from two centres; Centre 0088 and Centre 0068. The same recruitment and consenting procedure applies in both centres.

Reference to the research project is first made to patients in their annual invoice for storage. This billing includes a deposition form which outlines their options; continued storage, allowing embryos to perish, donation to another couple or donation to research. Patients who indicate interest in donating embryos for research are sent further information and two copies of the consent form, one for the patient to keep. If required the patients are also sent new 006 and 007 HFEA forms so that their willingness to donate the embryos to research is also indicated on these forms.

The patient information provides contact details should a patient requires further information. This includes contact information for a counsellor should patients wish to discuss the implications of donating embryos to research.

Information regarding patients' consent to research is tracked via a database detailing from whom consent is pending and received. The database also details the embryos available for research, embryos used and embryos discarded. Each spreadsheet has columns to indicate if the consent and patient information has been verified. The consents for patients donating embryos to research are kept in 3 research folders. The folders separate information on patients considering research but pending consents, embryos available/used for research and embryos discarded due to storage expiry date.

Embryos received from centre 0068 are accompanied by the patients' consent forms. These are checked upon arrival then filed and details added to the database.

Patients are able to withdraw consent to research at anytime up to when they are used, this is explained in the patient information. To withdraw consent patients have to send in a signed withdrawal form. The pending consent spreadsheet and folder shows that one patient withdrew their consent to research and the process works in practice.

#### Issues for consideration

The research withdrawal consent form should provide clear contact details for the Centre in case it becomes separated from the research information.

Executive recommendations for Licence Committee
None
Areas not covered on this inspection
Donor and patient records at Centre 0068

#### 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

<b>Summary</b>
<p>The patient information and consents for the research project are considered satisfactory by the Executive.</p> <p>Patients are sent information about the project and are provided with contact details should they require further information. The consent forms are also included with this literature and patients can therefore keep one copy of their consent form for reference.</p> <p>The PR explained that the patients consent for use is witnessed before embryos are used in research; two members of staff have to match signatures with the completed research form to the original HFEA 00(6) and 00 (7) forms. This procedure was also explained in the research protocol which was submitted with the application.</p>
<b>Summary of records audit</b>
<p>10 sets research notes were examined for appropriate consents. No discrepancies were noted.</p>
<b>Issues for consideration</b>
<p>The research consent form informs patients that they can withdraw consent from the project but there is no explanation provided of how they can do this. However, a withdrawal form is contained in the research information pack sent to patients. This form should be amended to provide clear contact details for the Centre in case it becomes separated from the research information.</p> <p>Patient information does not explain to patients from Centre 0068 that identifying information, which is present on consent forms and the labelling of embryo storage vessels, will be disclosed to members of the laboratory team at Centre 0088. This information should be added to both the patient information and consent forms provided to patients at Centre 0068.</p>
<b>Executive recommendations for Licence Committee</b>
<p>Note the need for amendments to the patient information and consent forms, particularly for patients donating embryos from Centre 0068.</p>
<b>Areas not covered on this inspection</b>
<p>The original patient files at Centre 0068 were not inspected.</p>

## 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

<b>Use of material</b>
The centre expects to use approximately 100 frozen embryos per year. Frozen embryos for the research project are donated by patients at the London Fertility Centre (0088) and the Leicester Fertility Centre (0068). In the period October 2004-May 2006, 37 embryos were received from Centre 0088 and 88 embryos from Centre 0068. None were used in this period. However, in the period following the submission of the renewal application, June 2006 to August 2006, 54 embryos were used in the research project.
<b>Summary of audit of stored and biopsied material</b>
Material for research is stored in general dewars and laboratory staff carried out an audit on these between Feb-May 2005.  <b>Inspectorate audit of stored material:</b> Four embryos donated to research (two from 0068 and two from 0088) were tracked from the research database to the dewar to the laboratory database and paper records. One discrepancy was found. For one patient the freeze sheet indicated one straw in storage, however two straws were observed in the dewar and this correlated with the research and laboratory databases.  Four embryo biopsies procedures were tracked from the practitioners' biopsy sheets to the research database and their laboratory records. One discrepancy was found. The date of biopsy on biopsy record was before the date of embryo thaw on the database. The thaw date was confirmed as correct and no date of biopsy was found in the laboratory records.
<b>Renewed project objectives</b>
The PR stated that:  "To complete the first stage of this study, we wish to analyse at least 50 human embryos. Ideally, 2 cells need to be analysed from each embryo with a CGH and microarray single cell result from 90% of the cells. From previous single cell CGH work, we can reasonably aim to achieve 90% efficiency. However, we have little experience with single cell microarray analysis. Thus 90% efficiency is probably optimistic. We have recently modified our culture policy for treatment to allow more blastocyst culture. As a result more spare frozen cleavage or PN stage embryos donated for research may develop into blastocysts, thus providing sufficient working material to draw valid, scientific conclusions from our results with a view to publication".
<b>Summary of research undertaken</b>
No research occurred from the issue of the licence in January 2006 to June 2006. The PR

stated that this was due to the modification required to the treatment and storage licence to include Preimplantation Genetic Screening to allow biopsy of embryos. In addition, the centre had been liaising with their collaborators at UCL to determine the optimal time to begin work on single biopsied blastomeres.

Since submission of the renewal application (until 31 July 2006), 55 embryos have been biopsied, with 80 biopsied cells processed for CGH or Microarray analysis (results pending) while the biopsied embryos were further cultured and spread for FISH analysis.

PR summary of work carried out so far:

“Successful biopsy was achieved in 87% cases. The Blastocyst rate post-biopsy was 29% which is very reasonable considering the fact that these were surplus frozen-thawed embryos, frozen at different developmental stages and biopsied for one or two blastomeres. Of particular interest is the 63% blastocyst rate after biopsy of embryos frozen at the 2PN stage. This is not unexpected since the 2PN stage is a robust stage at which to effectively freeze human embryos.

The finding that FISH was only successful on 63% biopsied embryos is disappointing but not unexpected considering the relatively low quality of the embryos available for biopsy. Indeed, only 16/55 (29%) embryos were at least 7 cells at the time of biopsy and many embryos failed to develop extensively beyond 24 hours after biopsy - an indication that they might not yield scoreable FISH signals. The single blastomeres have not yet been subjected to CGH or microarray analysis. Since they have been lysed and stored at -80C (or in some cases subjected to whole genome amplification before being stored at -80C) there is little danger that the samples will degrade prior to analysis.

We potentially have 13 results (FISH plus microarray/CGH) from 104 thawed embryos. To achieve a further 37 results (FISH plus microarray/CGH) we may need to thaw another 300 embryos. We are probably recruiting at a rate of 100-150 embryos per year (we have 80 unused embryos already consented and available for use at present) and a further 100-150 embryos per year from centre 068. Based on these numbers, we estimate that the project would only need to run for a further year (rather than a further 3 years). ”.

#### Peer review comments (if applicable)

The Peer Reviewer initially found it impossible to review the project proposed by the applicant as results from the previous year were not included and no work appeared to have been conducted.

In response to this, the PR resubmitted the application with further information and this was then accepted by the Peer Reviewer.

#### Issues for consideration

From the summary of the spot check and biopsy audit it is recommended that audits of research embryos in storage and embryo biopsy are performed at least annually.

The laboratory protocol for research (Lab Pro 15) mentions that embryos can be used in research projects including FISH, PCR projects, ES cell lines. However, the Centre is not licensed for these projects and therefore the protocol does not correspond to the aims and

objectives of the licence. Furthermore, the protocol could possibly mislead/confuse any new members of staff. When questioned regarding this, the PR explained that the protocol needed updating and that it would be reviewed appropriately.

Executive recommendations for Licence Committee

None

Areas not covered on this inspection

None

Report compiled by:

Name: Sarah Hopper and Elliot Lawrence

Designation: Inspectors

Date: 15<sup>th</sup> August 2006

## Appendix A: Centre Staff interviewed

The PR, Dr Alan Thornhill and 1 other member of the team.

## Appendix B: Licence history for previous 3 years

Licence	Status	Type	Start date	Expiry date
<a href="#">R0169/1/a</a>	Active	Research Project	01/01/2006	31/12/2006
<a href="#">R0140/1/a</a>	Expired	Research Project	01/10/2003	30/09/2004

- The R0140 was allowed to expire due to the research PRs departure from the Centre.
- In 2006 the project R0140 was renewed as R0169 by the research PR Dr Alan Thornhill.

**Appendix C:**  
RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number...0088.....

Name of PR Dr Alan Thornhill.....

Date of Inspection 10/08/06.....

Date of Response.....31/08/06.....

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

- The research withdrawal consent form has been amended to provide clear contact details for the Centre in case it becomes separated from the research information (version 2).
- Annual audits of stored research embryos will be performed (see LabAdm10, V2).
- The laboratory protocol for research (Lab Pro 14, V3) has been amended and updated to reflect the fact that no work on ES cells is currently licensed at LFC.
- LFC consent form and patient information sheets amended to reflect the fact that identifying information, which is present on consent forms and the labelling of embryo storage vessels, will be disclosed to members of the laboratory team at London Fertility Centre but not to the diagnostic testing centre. We have decided to continue to use the same consent forms and information sheets for both LFC and centre 068 to avoid potential confusion and maintain document control (see amended consent form).
- Result summary for embryos used up until July 31<sup>st</sup>, 2006 is attached (with embryo usage flow chart and raw data sheet from UCL)
- Professor Craft (PR) and Andrew Berkley will appoint a new research license PR.
- All amended documents are attached

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).

Lab Pro 15 should read Lab Pro14 (amended)  
Mr Alan Thornhill should read Dr Alan Thornhill (amended)

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