Research Licence Interim Inspection Report

Project Title	Biochemistry of early human embryos
Centre Name	University of York
Centre Number	0062
Research licence Number	R0067
Centre Address	Department of Biology, University of York, PO Box 373, York, YO10 5 YW
Treatment centres donating to this research project	Leeds Clarendon Wing 0052 Leeds ACU, St James' University Hospital 0063
Inspection date	12th September 2006
Licence Committee Date	28th November 2007
Inspector(s)	Andy Leonard (Lead)
	Tahir Hussain
Person Responsible	Henry Leese
Nominal Licensee	Judith Hawkhead
Licence expiry date	31/01/2010

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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

Research for this project originally started in 1995. The current licence for R0067 is due to expire on the 31st January 2010.

The Research project is entitled: BIOCHEMISTRY OF EARLY HUMAN EMBRYOS

Licensed for the following purposes:
The project was originally licensed under purposes laid down in Schedule 2 of the Human Fertilisation and Embryology Act 1990

- 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)

Lay Summary for R0067:
Up to 1 in 6 couples find difficulty in conceiving. For many, one solution to their infertility is In Vitro Fertilisation and Embryo Transfer (IVF-ET) the so-called "test tube baby" treatment, which was pioneered in the UK in the late 1970s. This treatment has helped many thousands of couples to have children, but success rates remain disappointingly low with a live birth rate per treatment cycle in the UK of only around 20%. Moreover, since 2, exceptionally 3 embryos may be transferred in any one treatment cycle, there is a risk of multiple births. While the birth of a baby is a cause for joy, multiple births can unfortunately bring problems; the babies are often underweight and peri-natal mortality is above average; the parents may also have problems in coping with the arrival of a large family.

There is now good agreement amongst doctors and embryologists that a solution to these problems would be to transfer single embryos with a high chance of forming a pregnancy. However, we know very little about how human embryos are formed and what makes some embryos more healthy than others. The aim of our work is therefore to carry out a detailed examination of the development of the early human embryo. The focus is on nutrition and metabolism: how the embryo obtains and uses the nutrients it requires; for example, sugars and amino acids. In this way we will learn how to improve culture conditions and devise diagnostic methods that allow the transfer of single, healthy embryos with a high chance of giving rise to pregnancy and limiting multiple births. Eventually, this will help ensure the health of babies born following natural conception as well as IVF.

Lay Summary of Research Undertaken:

Much IVF treatment involves the replacement of embryos which have been cryopreserved, but little is known about the metabolism of such embryos and whether they differ from 'fresh' IVF embryos. We have therefore examined the metabolism, measured non-invasively, of single human embryos post thaw, in terms of their capacity to utilise amino acids - key constituents of embryo culture media. After thawing, the embryos were placed singly in tiny droplets of medium and incubated for up to 24 hours. The droplets were frozen for subsequent analysis, the embryos replaced in culture and their capacity to reach the blastocyst stage – the endpoint of preimplantation development - monitored. It was then possible to relate the 'amino acid profile' of each embryo to its developmental capacity. Reassuringly, the profiles given by the embryos which survived the cryopreservation process were similar to those we have previously measured for fresh embryos. Moreover, we found a difference in the amino acid metabolism of those frozen/thawed embryos which developed successfully and those which failed to reach the blastocyst stage; again, in line with the data for fresh embryos. These results suggest that human IVF embryos which survive cryopreservation are not compromised metabolically and provide further evidence that embryo freezing is a safe technique. In related work, we seek to discover whether the measurement of amino acid profiles can be used to select single embryos with a high chance of giving a pregnancy for replacement in the uterus. The work involves a large clinical trial, which, if it proves successful, would enable acceptable rates of IVF treatment while virtually eliminating the problem of multiple births.

We have also measured, for the first time, a metabolic pathway in early human embryos which involves the energy storage compounds creatine and creatine phosphate. The profile of the enzyme involved has been measured throughout preimplantation development and compared with that given by mouse and pig early embryos. The profile in the human suggests that the pathway acts during the cleavage stages of development to ensure a sufficient supply of energy in the form of ATP.

The project has produced several publications this year

1) Stokes PJ, Hawkhead JA, Fawthrop RK, Picton HM, Sharma V, Leese HJ and Houghton FD (2007). Metabolism of human embryos following cryopreservation: Implications for the safety and selection of embryos for transfer in clinical IVF. *Human Reproduction* **22**: 829-35

2) Forsey KE, Ellis PJ, Sargent CA and Leese HJ (2006) Creatine kinase expression and activity during reimplantation and embryo development in vitro. Biology of Reproduction: Suppl. 1: 125.

3) Eckert, JJ, Houghton, FD, Hawkhead JA, Balen AH, Leese HJ, Picton HM, Cameron IT and Fleming TP (2007). Human embryos developing in vitro are susceptible to impaired epithelial junction biogenesis correlating with abnormal metabolic activity.

4) Baumann CG, Morris DM, Sreenan JM and Leese HJ (2007) The quiet embryo hypothesis: Molecular characteristics favouring viability (*Molecular Reproduction and Development*, In Press)

5) A patent protecting the data in paper 1) has also been published: International Patent Application No PCT/GB2007/000277; CRYOPRESERVATION METHOD

Future work

Work will continue to establish the value of non-invasive Amino Acid Profiling (AAP) in diagnosing the health of human IVF embryos prior to transfer; to provide surplus human embryos, following non-invasive amino acid profiling (AAP) to the Institute for Stem Cell Research, University of Cambridge.

A new objective is to be added to the research project: is *to investigate the relationship between Amino acid profiling and DNA damage in early human embryos*. This will investigate the correlation between DNA damage, assessed using the COMET assay, and the amino acid profiles of human embryos in culture. This is a test of the quiet embryo hypothesis in which a low metabolic rate is thought to be indicative of embryo fitness - embryos with the highest metabolism have elevated requirements for nutrients (such as amino acids) due to the need to repair increased molecular and cellular damage. Support has been gained using pig embryos and centre 0062 wish to extend these studies to human embryos.

Activities of the Centre	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

- NONE

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

C	**None**
A	

Summary for Licence Committee

The inspectorate were satisfied that the centre is well organised and running compliantly with the HFE Act (1990). Areas viewed positively by the inspectorate include:

- The responses to issues raised by the licence renewal inspection on 26th September 2006
- Witnessing during laboratory processes was in general considered compliant.
- Laboratory procedures
- Equipment validation and maintenance programme
- New staff induction programme

One breach of the HFE Act (1990) was however noted

- A recently used batch of embryos had been stored past their statutory storage limit for 13 days prior to their use in research, contrary to Section 14 (1) (C) of the HFE Act (1990). Chair's letter (03) 03 implies that centres have a working week from the end of the statutory storage period in which to perish embryos. The inspectorate require the centre to ensure they adhere to the statutory storage limit, with the provisos encompassed in Chair's letter (03) 03

Several breaches of Standards and Guidance in the Code of Practice, 7th edition were also noted which have led to the recommendations detailed:

- Witnessing is robust except that embryo disposal is currently not witnessed. Embryo disposal should be witnessed appropriately, as per Code of Practice 7th edition, G.13.1.1.

- Document control mechanisms were in place but some standard operating procedures dated from 2005, contrary to the maximum annual review requirement of Code of Practice, 7th edition, S.5.2.5. The inspectorate recommend regular reviews of written procedures are performed to comply with S.5.2.5.

- The inspectorate recommend that a risk assessment be performed to cover the possibility of dewar failure in the embryo storage laboratory, given the current lack of a low oxygen monitor and limited ventilation in that room, as required by the Health and Safety Executive, Confined Spaces Regulations (1997). If indicated by risk assessment as an appropriate control measure, a low oxygen alarm should be fitted in the licensed laboratory and a procedure written for responding to it.

- The university employed driver is not currently listed on the licence, yet he has access to patient records (research consent forms) and embryos. The centre should consider the confidentiality issues this arrangement raises. The PR should ensure that they document that all staff with access to embryos and confidential information are assessed by the PR as being of appropriate character and have been advised regarding the HFEA requirements for confidentiality of patient information (Code of Practice, 7th Edition, S.7.2.1)

- The contact point and mechanism for withdrawing consent is not stated in the generic research information leaflet (form Clin 009) nor in the specific project information, though the possibility of withdrawing consent is discussed in form Clin 009, as required by Code of Practice, 7th Edition, G.5.13.1. The inspectorate recommend that form Clin 009 is updated to provide a contact point and mechanism for withdrawing consent.

- Form Clin 009 does not provide details of contacts at the recruiting centres and at centre 0062 from whom further information about the research projects can be obtained, as required by Code of Practice, 7th Edition, G.5.13.1 (h), albeit contact details for the PR of centre 0062 are provided on the specific project information sheets. The inspectorate recommend Form Clin 009 is updated to provide details of contacts at the recruiting centres and at centre 0062, from whom further information about the research projects can be obtained.

- The IVF Research Consent Form does not provide the ability to limit consent to specific individual projects or vary the consent, as required by Code of Practice, 7th Edition, G.5.13.1 (f,g). This ability is discussed in form Clin 009 but only research in general is consented to on the IVF Research Consent Form. The inspectorate recommend the IVF Research Consent Form is updated to provide patients the ability to limit or vary consent to individual research projects

Proposed licence variations

NONE

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:

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

Full time equivalent staff

Principal investigator	1
Laboratory scientists	3
Administrators	0
Collaborators	4
Support staff	0

Summary

The centre functions within the Department of Biology, University of York, which provides an appropriate level of health and safety, facilities and human resources support to the centre. On inspection, the centre seemed well organised in terms of its premises, staffing, operations and interactions within the Department of Biology.

The PR oversees the research project activities which are performed by an experienced laboratory technician (also the laboratory manager), a post doctoral research fellow and two research students. Staff experience and numbers were considered appropriate for the project and facilities/funding available. Because of the team's small size, one to ones and review meetings are held when required, but all centre staff meet approximately once a month to discuss laboratory and university related issues, resource management and HFEA Alerts. These meetings are now minuted, as required by Licence Committee after the licence renewal inspection on 26th September 2006. Interviews with the staff and PR and general research progress indicate appropriate leadership and management of the project

The PR stated communication with centres 0052 (Clarendon Wing, Leeds General Infirmary) and 0063 (ACU, St James University Hospital, Leeds) occurs regularly via email and telephone to determine when donated embryos can be collected. Fresh embryos are usually collected weekly by a regular university-employed driver, considered trustworthy by the PR, or sometimes by the PR himself. Frozen embryos are collected in batches, and are transported to centre 0062 using an appropriate transport container, observed during the inspection, again by the regular university employed driver or the PR. Visits by the PR to centre 0052 and 0063 allow discussion of the research project with the collaborators at these centres to facilitate a coordinated approach. The PR provides seminars at centre 0052 and 0063 annually to inform on research progress. The PR stated he has liaised closely with the

research nurses at centre 0052 and 0063 to ensure research information and consenting procedures are compliant.

The centre's research activities are currently funded by the Medical Research Council (grant soon to expire) and departmental funds. Grant applications are in preparation for submission. They have received ethical approval from an appropriate ethics committee at York University. Research progress is evidenced in that the centre is embarking on a multi-centre clinical trial to test the applicability in IVF clinical practice of its 'quiet embryo' hypothesis. This project involves centre 0062 producing amino acid profiles for media sent in from multiple IVF treatment centres, after it has been used to culture embryos which are then implanted. Patient outcomes will be correlated with the amino acid profiles obtained.

Embryo entry into storage and usage in experiments is recorded and witnessed on a spreadsheet log, evidenced as being appropriate during the inspection, and also experimental notes and data are documented in laboratory notebooks and held on computer. The centre has incorporated a GMP (Good Manufacturing Practice) approach in its research activities due to the clinical trial it is coordinating. It has developed a quality manual containing 58 protocols, covering a wide variety of centre activities; they were considered well written and fit for purpose. Mechanisms of research governance were considered appropriate

Issues for consideration
The university employed driver is not currently listed as staff on the licence yet he has access to patient records (research consent forms) and embryos. The centre should consider the confidentiality issues this arrangement raises. The PR should ensure that they document that all staff with access to embryos and confidential information are assessed by the PR as being of appropriate character and have been advised regarding the HFEA requirements for confidentiality of patient information (Code of Practice, 7th Edition, S.7.2.1)
Executive recommendations for Licence Committee
None
Areas not covered on this inspection
NONE

2. Premises and equipment

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

Summary of findings from inspection:

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

Summary
Licensed activities are undertaken in a single laboratory, and analysis of embryo extracts and media is undertaken in a second laboratory. The centre also contains three offices and an open lobby area. The premises were considered fit for purpose by the inspectorate. The storage and culturing of embryos occurs in a locked laboratory, access to which is limited to staff on the HFEA research licence. The laboratory contains a biohazard cabinet, laminar flow hood, fridge/freezer, incubators and a dewar for embryo storage. Biochemical analysis of media and embryo extracts occurs in a laboratory opposite which is well equipped and maintained to GMP standards. Frozen embryos donated to research are stored in a locked dewar. The dewar is checked weekly when embryos are in storage and topped up with liquid nitrogen if required, evidenced by the filling log sheet. The centre has a small spare dewar in case of emergency. The laboratory does not have a low oxygen alarm or direct air extraction system. The laboratory manager stated that a Health & Safety inspection considered air circulation to be adequate in the event of dewar failure however no hard copy report was available. The inspectorate were not satisfied with this and requested that a risk assessment of dewar failure be performed taking into account the lack of a low oxygen monitor and the limited, in the opinion of the inspectorate, air circulation in the laboratory. If the risk assessment indicated it, a low oxygen alarm should be fitted in the laboratory and a procedure written for responding to its activation. . The sealed research consent forms are initially kept in a locked draw in the licensed laboratory. After being checked they are stored in a locked filing cabinet in the PR's office. Document security was considered appropriate. Carbon dioxide cylinders and the temperatures of incubators and fridge/freezers are checked each working day, evidenced by log sheets. The equipment within the laboratory had up to date service contracts which were evidenced during the inspection. Written procedures were in place for operating all equipment. Equipment safety, servicing and maintenance were considered appropriate
Issues for consideration
A risk assessment for dewar failure should be performed and, if indicated by risk assessment as an appropriate control measure, a low oxygen alarm should be fitted in the licensed laboratory and a procedure written for responding to it.

Executive recommendations for Licence Committee
None
Areas not covered by this inspection
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:

- 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; Leeds General Infirmary (0052) and St. James' University Hospital, Leeds (0063). Patients at centre 0052 and 0063 donate frozen embryos to research. Fresh embryos are also donated from 0052. Provision of information and the consenting process is managed by the recruiting centres and is undertaken by their research nurses. The PR has liaised with the centres to ensure he understands the recruitment process and that it is compliant. Patient information makes it clear that donation to research will not affect present or future treatment. Further guidance for patients regarding the research projects is available from the research nurses, from the nominated personnel at the recruitment centres, and from the PR of centre 0062. The inspectorate considered that this availability of guidance was compliant with the Code of Practice, 7th edition. A time-plan for the recruitment process was provided which showed that patients had appropriate time in which to consider research donation. It should be noted that the PR stated during inspection that the centre had recently dealt with a situation of withdrawn consent in an appropriate manner, and this was evidenced in the records by the inspectorate. Embryos from centres 0052 and 0063 are accompanied by the patient's consent form and a copy of the centre's embryo laboratory sheet in a sealed envelope. These are checked upon arrival by a person not involved in the research, but who is on the licence, then filed securely in the PRs office. If a discrepancy is found the recruiting centre is contacted. The embryos are given a unique York identification number and this is detailed on the embryo stock/experimentation spreadsheet log, along with the research number, estimated grade and statutory storage expiry from the donating Centre. Records are indexed according to the expiry date of the consent, so embryos closer to their expiry are used first. When records were inspected it was noted that a recently used batch of embryos had been stored past their statutory storage limit for 13 days prior to use. When asked, the Laboratory Manager stated that she had been informed by another centre that it was legal to use embryos in consented research up to the end of the month in which the statutory storage period had lapsed. The inspectorate informed the staff at centre 0062 that the consented statutory storage period could not be extended. Review of centre records indicated this incident had not been repeated in the previous year. Review post inspection found that Chair's letter (03) 03 implies that centres have a working week from the end of the statutory storage period in which to

perish the embryos. The centre have been informed of this.

Frozen embryos arrive with the patient's name on the storage straw but this is seen only by licensed personnel. This cannot be avoided and the patient's name is used as a secondary identity check. This situation is described in the patient information used by centres 0052 and 0063. Fresh embryos donated to research are transferred to a Petri-dish in the air flow cabinet then placed in the incubator, as are frozen embryos when thawed for research. Embryos are regraded and their culture location and subsequent gradings and media changes, with dates, are logged on the spreadsheet. Used media samples are stored for later amino acid profiling.

The inspectorate considered that patient/donor records are securely stored by the centre. The inspectorate consider that patient confidentiality is, and consents will be, protected compliant with the Code of Practice, 7th edition, now the centre have been informed regarding the inviolability, without consent, of the statutory storage periods.

Issues for consideration

A recently used batch of embryos had been stored past their statutory storage limit for 13 days prior to their use in research, contrary to Section 14 (1) (C) of the HFE Act (1990). Chair's letter (03) 03 implies that centres have a working week from the end of the statutory storage period in which to perish the embryos. The inspectorate require the centre to ensure they adhere to the statutory storage limit, with the provisos encompassed in Chair's letter (03) 03.

Executive recommendations for Licence Committee

Note the single breach of the statutory storage limit for a batch of embryos

Areas not covered on this inspection

Donor and patient records at Centre 0052 and 0063

4. Patient information and consents

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

Summary of findings from inspection:

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

Summary
The PR has liaised with the centres in the preparation of patient information and consent forms to ensure the patient information and consenting process are compliant. Patient information and consent forms were reviewed by the inspectorate and considered fit for purpose with three exceptions: 1) The ability to withdraw consent at any time is clearly noted in the generic research information (form Clin 009) but the contact point and mechanism for doing so is not clearly stated in this document or specific project literature 2) Form Clin 009 does not discuss what to do if the patient needs further information, though contact details for the PR at centre 0062 are provided on the specific project information as a contact if further information is required. Form Clin 009 should contain details for contacts at the recruiting centres and/or at centre 0062, from whom further information can be obtained. Both recruiting centres have research nurses and nominated clinicians (mentioned in the Stem Cell Derivation information) who can provide information. 3) The IVF research consent form does not give the ability to consent to specific individual projects even though this is discussed in form Clin 009. There is discussion of such a consent form in the flow chart for the consenting procedure but it was not provided. It should be noted that the PR stated that centre 0062 had recently dealt with a situation of withdrawn consent in an appropriate manner, disposing of the embryos, therefore exception (1) above would appear to have not adversely impacted on patients consenting freedoms. A stem cell research patient information and consent form was provided by the centre as embryos for stem cell research derivation at other centres are first sent for metabolic analysis at centre 0062. It was considered fit for purpose
Summary of records audit
Records were not audited as the patient notes are at centres 0052 and 0063.
Issues for consideration
• The contact point and mechanism for withdrawing consent is not stated in the generic research information leaflet (form Clin 009) nor in the specific project information, though the possibility of withdrawing consent is discussed in form Clin 009, as required by Code of Practice, 7th Edition, G.5.13.1. • Form Clin 009 does not provide details of contacts at the recruiting centres and at centre 0062 from whom further information about the research projects can be obtained, as required by Code of Practice, 7th Edition, G.5.13.1 (h), albeit contact details for the PR of centre 0062 are provided on the specific project information sheets. • The IVF Research Consent Form does not provide the ability to limit consent to specific

individual projects or vary the consent, as required by Code of Practice, 7th Edition, G.5.13.1 (f,g). This ability is discussed in form Clin 009 but only research in general is consented to on the IVF Research Consent Form.
Executive recommendations for Licence Committee
Note the need for amendments to the patient information and consent forms.
Areas not covered on this inspection
The original patient files at Centre 0052 and 0063 were not inspected.

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
EMBRYO USAGE The PR stated that centre 0062 used approximately the expected number of fresh (478 from centre 0052) and frozen (203 from centre 0052; 99 from centre 0063) embryos between 01/10/2005 to 31/5/2007. On inspection, the PR determined from the embryo storage log that in the previous year (01/09/06 to 31/08/07), the centre used 528 fresh and 26 frozen embryos from centre 0052, and none from centre 0063. In comparison, between 01/10/04 and 20/09/05, the centre used 645 fresh and 682 frozen embryos. Usage next year will comprise approximately 400 fresh embryos and 100 frozen embryos. OTHER ITEMS A large set of standard operating procedures has been developed by the centre to regulate its activities, as discussed in (1) Organisation, above. It was noted that document control headers in the standard operating procedures did not include the future review date and some procedures had not been updated for approximately 2 years. The storage and removal of embryos from the research dewar is witnessed, evidenced by examining the embryo storage and experimental log. A procedure is in place for witnessing. It was noted that embryo disposal is not appropriately witnessed and the centre were advised to do so. The centre maximise the data output from the embryos they receive by performing metabolic studies first and then using the embryos for other research purposes, such as stem cell derivation at centre 0246 and 0252, or histochemistry/molecular biology in-house.
Summary of audit of stored and biopsied material
Material for research is stored in a locked dewar. An audit of stored material is performed annually though embryos are normally used within a few weeks of being placed in storage. This audit verifies all embryos have been used when stated and that witnessing procedures are functioning. A spot-check of the dewar was not performed as the PR stated that there were no embryos in storage at the time of inspection.
Renewed project objectives
Objectives at last inspection *Objective (i) The effect of cryopreservation on human embryo development and metabolism*. *Objective (ii) Nitric oxide signalling in early human embryo development.* **Objectives added in licence renewal application**

Objective (i) The role of creatine phosphate and creatine kinase in early human embryo development
Objective (ii) the value of non-invasive Amino Acid Profiling (AAP) in diagnosing the health of human IVF embryos prior to transfer.
Objective (iii) Stem cell derivation

Work is on-going to satisfy these objectives

A new objective (iv) is *to investigate the relationship between Amino acid profiling and DNA damage in early human embryos*.

Summary of research undertaken

PR summary of work carried out so far:

Progress to date

1. Update on work reported in Licence Renewal Application:

Objective (i) The effect of cryopreservation on human embryo development and metabolism.
The draft paper by Stokes et al, referred to, has now been published: Stokes PJ, Hawkhead JA, Fawthrop RK, Picton HM, Sharma V, Leese HJ and Houghton FD (2007). Metabolism of human embryos following cryopreservation: Implications for the safety and selection of embryos for transfer in clinical IVF. *Human Reproduction* **22**: 829-35

A patent protecting the data has also been published: International Patent Application No PCT/GB2007/000277; CRYOPRESERVATION METHOD

Objective (ii) Nitric oxide signalling in early human embryo development.
This work has not yet been published

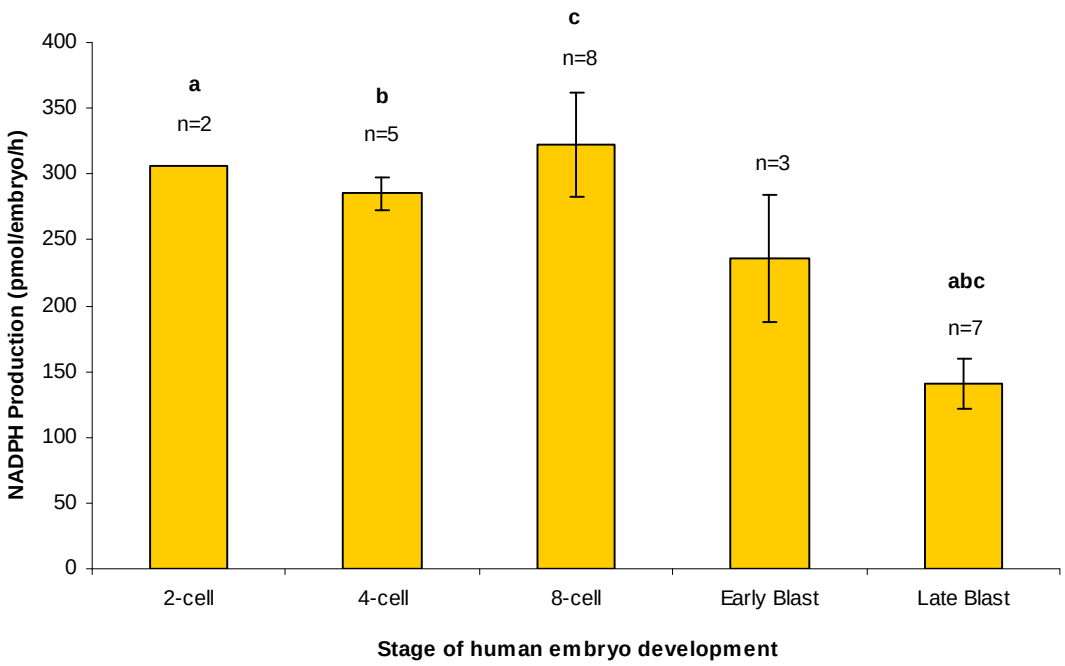
2. Objectives following the last Inspection

Objective (i) – The role of creatine phosphate and creatine kinase in early human embryo development.

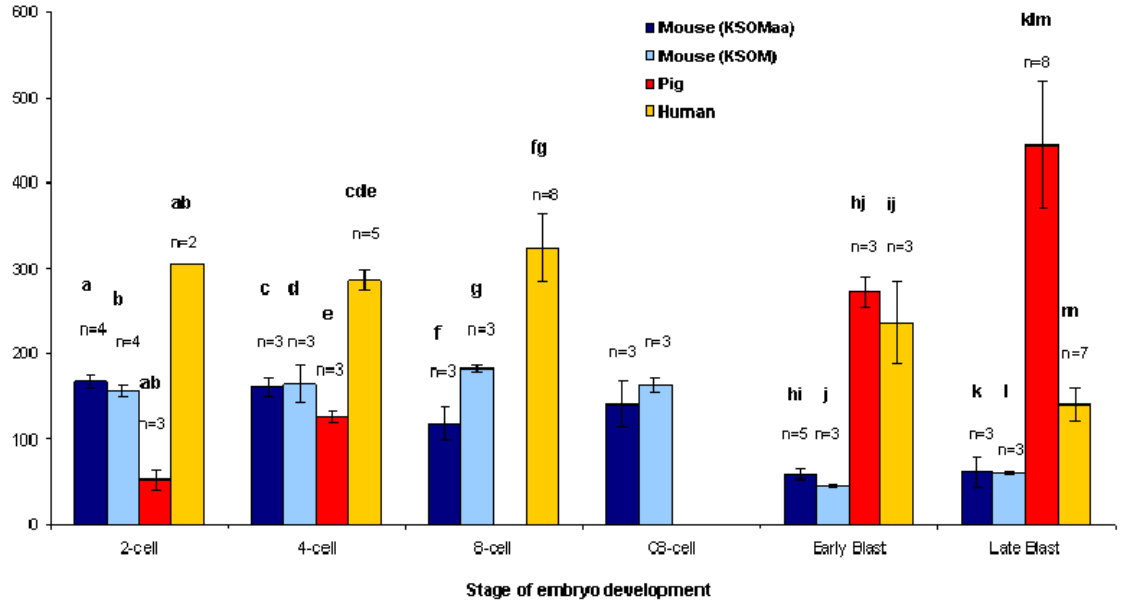
Research in mouse and pig embryos carried out in the York laboratory indicate a role for creatine phosphate in energy metabolism during preimplantation development. Creatine phosphate acts as a short-term store of energy; it may be converted into ATP by reaction with ADP, catalysed by the reversible enzyme creatine kinase, and can be thought of as a system for 'buffering' the ATP concentration in the cell.

The work represents a logical extension of on-going research in the York laboratory to study preimplantation embryo metabolism in relation to embryo function. We were drawn to the creatine phosphate system partly through consistently finding a high consumption of the amino acid arginine at all stages of preimplantation development; in the mouse, cow, pig and human; one of the fates of arginine is to be converted into creatine.

Measurements of the biochemical activity of the enzyme creatine kinase were made on surplus human embryos from the 2-cell to blastocyst stages. Some technical problems were encountered in extracting the embryos prior to measuring enzyme activity, but the following data were eventually obtained:



The data were compared with those for mouse and pig early embryos:



Biochemical activity profiles (pmol/embryo/hour) of Creatine Kinase during mouse, pig and human preimplantation embryo development in vitro.

There were obvious differences in enzyme profiles between species but overall, the data suggest a key role for creatine phosphate in energy metabolism during preimplantation development. In the case

of the human, the creatine kinase system appears to provide more buffering for ATP during the cleavage stages (when ATP generation is low) compared with the blastocyst (when ATP generation is high). The data also suggest that supplementation of culture media with creatine might be of benefit.

Objective (ii) – the value of non-invasive Amino Acid Profiling (AAP) in diagnosing the health of human IVF embryos prior to transfer.
This 400 patient trial commenced, but had to be suspended due to problems with the quality of the culture medium (the presence of particles – observed by the Embryologists). Interim analysis of the data confirmed our earlier studies (Brison et al 2004: Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover: Human Reproduction 19, 2319-2324) and showed that the depletion or appearance of certain amino acids were predictive of the ability of embryos to give rise to a pregnancy. It is hoped to recommence the trial shortly [The PR said on inspection the trial will restart in January 2008]

Objective (iii) Stem cell derivation
Provision of surplus human embryos, following non-invasive amino acid profiling (AAP) to the Institute for Stem Cell Research (Edinburgh) and the Institute for Stem Cell Research, University of Cambridge. 36 embryos were amino acid-profiled and sent to Edinburgh, but none gave rise to embryonic stem cells.

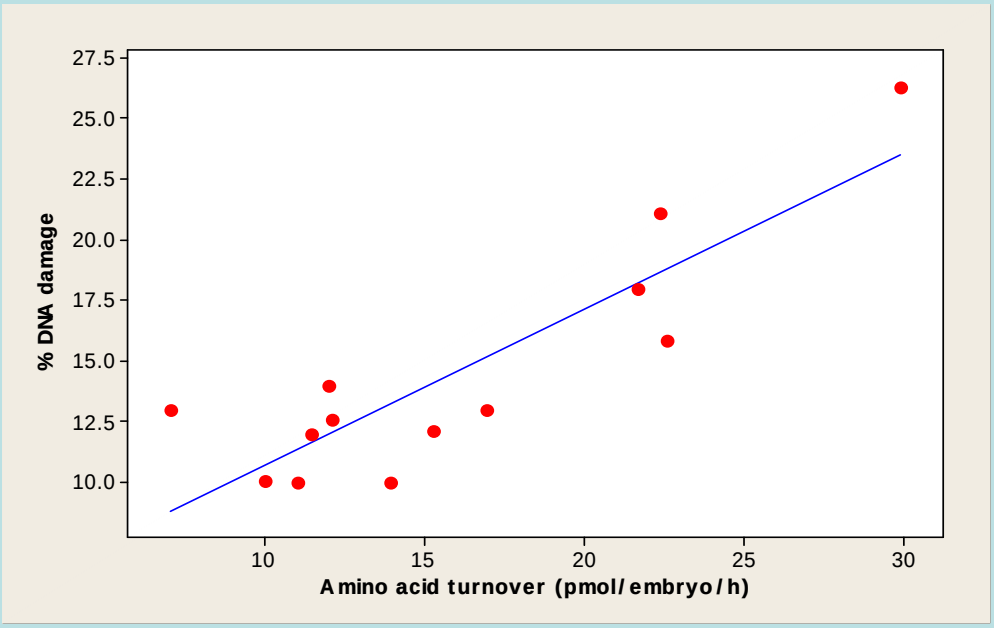
If progress was slower than anticipated, the reasons for this.
The work listed as 'Additional Studies' in the Licence Renewal to discover whether there was a distance apart at which the development of early human embryos was optimal is still on-going

If work originally proposed was not carried out, the reason for this.
Attempts to measure the creatine and creatine phosphate content of single mouse embryos were unsuccessful and the proposal to measure these compounds in human embryos were therefore not pursued.

Future work
Work will continue to pursue Objectives (ii) and (iii) above, ie:
(ii) to establish the value of non-invasive Amino Acid Profiling (AAP) in diagnosing the health of human IVF embryos prior to transfer
(iii) to provide surplus human embryos, following non-invasive amino acid profiling (AAP) to the Institute for Stem Cell Research, University of Cambridge.

A new objective (iv) is *to investigate the relationship between Amino acid profiling and DNA damage in early human embryos*.

Background:
We have measured the depletion/appearance of a mixture of amino acids by embryos from a variety of animals: mouse, cow, pig and human. These findings, and others, have been used to formulate a hypothesis which proposes that the viability of early mammalian embryos is associated with a metabolism that is "quiet" rather than "active" (Leese HJ, 2002:*BioEssays* **24**:845-849). This hypothesis has been extended in a further paper - Baumann CG, Morris DM, Sreenan JM and Leese HJ (2007) The quiet embryo hypothesis: Molecular characteristics favouring viability (*Molecular Reproduction and Development*, In Press) - where it is proposed that embryos with the highest metabolism have elevated requirements for nutrients (such as amino acids) due to the need to repair increased molecular and cellular damage. We have obtained support for this idea in early pig embryos where there is a strong relationship between amino acid profile and DNA damage:



Sturmey and Leese, unpublished

We now seek to discover whether this is the case for early human embryos.

Methods:
Single human embryos will be incubated for up to 24 hours to determine their 'Amino acid profile'. DNA damage will then be assessed in individual blastomeres using a modified Comet assay, which shows single and double strand breaks.

Discussion
The PR feels that the research will provide valuable information which relates to the question 'what makes a good embryo'? Our hypothesis is that a good embryo needs to expend less energy rectifying damage to the genome, transcriptome and proteome. If this is the case, it will provide numerous markers of embryo health; at the molecular and cellular levels – against which improvements in IVF technologies may be tested.

Peer review comments (if applicable)
NONE

Issues for consideration
1. Embryo disposal is not witnessed 2. Some standard operating procedures were dated from 2005

Executive recommendations for Licence Committee
NONE

Areas not covered on this inspection
NONE

Report compiled by:

Name: Andy Leonard

Designation: Inspector

Date: 23rd October 2007

Appendix A: Centre Staff interviewed

The PR, NL and 2 other staff members

Appendix B: Licence history for previous 3 years

Licence	Status	Type	Start date	Expiry date
R0067/7/a	Current	Research Project	01/02/2007	31/01/2010
R0067/6/b	Expired	Research Project	01/02/2004	31/01/2007
R0067/5/b	Expired	Research Project	16/01/2001	31/01/2004
R0067/4/a	Expired	Research Project	15/01/1998	15/01/2001

No conditions or recommendations on Licences.

Appendix C: Response of Person Responsible to Inspection Report

Centre Number: 0062

Name of PR: Professor Henry Leese
Date of Inspection: 12/09/06

Date of Response:

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

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).

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

28 November 2007
21 Bloomsbury Street London WC1B 3HF

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MINUTES Item 4

Research Project R0067: Biochemistry of early human embryos
University of York (0062)
Interim Inspection

Members:
Emily Jackson – Chair, Lay Member
Richard Harries, Lay Member
Clare Brown, Lay Member
Maybeth Jamieson, Consultant Embryologist, Glasgow Royal Infirmary
Neva Haites, Professor of Medical Genetics, University of Aberdeen

In Attendance:
Trish Davies, Director of Regulation/ Deputy Chief Executive
Chris O'Toole, Head of Research Regulation
Claudia Lally, Committee Secretary

Observing:
David Gomez, legal adviser to the HFEA

Providing Clinical Advice:
William Ledger, Professor of Obstetrics and Gynaecology, University of Sheffield

Providing Legal Advice:
Graham Miles, Morgan Cole Solicitors

Conflicts 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 (34 pages)
- no papers were tabled.

1. The Committee considered the report of the interim inspection and agreed that on the basis of the inspection report they had no concerns about the research project and were content for the licence to continue.

Signed..... Date 2.1.08
Emily Jackson (Chair)