Research Licence Renewal Inspection Report

Project Title	Human Gamete Activation, Interaction and Signalling
Centre Name	Research Licence R0172 covers the project at Centre 0119 Research Licence R0173 covers the project at the near-by Centre 0209
Centre Number	Centres 0119 and 0209
Research licence Number	R0172 and R0173
Centre Address	Centre 0119 = ChRS Research Laboratory – (04-Lab2-009), Assisted Conception Unit, Birmingham Women's Hospital, Egbaston, Birmingham, B15 2TG Centre 0209 = Laboratory WX1.25, Institute for Biomedical Research, The Medical School, University of Birmingham, B15 2TT
Treatment centres donating to this research project	0119, ACU, Birmingham Women's Hospital
Inspection date	19th August 2009
Licence Committee Date	18th November 2009
Inspector(s)	Andrew Leonard; Janet Kirkland
Fee Paid - date	Fee paid
Person Responsible	Dr Jackson Kirkman Brown
Nominal Licensee	Dr Sarah Connor
Licence expiry date	31st December 2009

About the Inspection:
The purpose of the inspection is to ensure that research is carried out in compliance with the HF&E Act 1990, Code of Practice, licence conditions and directions and that progress is made towards achieving the stated aims of the project.

The report is used to summarise the findings of the inspection highlighting areas of firm compliance and good practice, as well as areas where improvement may be required to meet regulatory standards. It is primarily written for the Licence Committee who makes 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 1st September 2008 and 19th August 2009.

Brief Description of the Projects

Project: **R0173: Human Gamete Activation, Interaction and Signalling**
Licensed since: 1st January 2006

The lay summary of the project has been modified in this renewal application and now reads:

Due to the technical and logistic difficulties of undertaking experimental work on the human egg almost nothing is known about what happens as a sperm moves through the outer egg coats to achieve fertilisation. We now have the technology available to begin to examine this in detail. We hope that the results will inform us about how sperm and eggs may 'talk to each other' and enable us to not only better understand how these things go wrong and may cause infertility but also to devise better future treatments and contraceptives.

In this project we will employ advanced fluorescent imaging (microscopy) techniques to examine in detail the events occurring as human sperm and eggs interact, particularly with reference to concentrations of calcium which we know form a vital part of the signalling that occurs. The data we hope to generate will give new insight into the very early events occurring in fertilisation, we hope that one early outcome for this data may be new treatments to help couples where the sperm lacks the crucial ability to 'activate' the egg and hence normal fertilisation cannot take place.

Research activities	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

Project R0173, Human Gamete Interaction and Signalling, has held a research licence since 1st January 2006 which is due to expire on 31st December 2009. The research licence was originally needed because the project aimed to investigate the early interaction of sperm and oocytes and molecular mechanisms within the two cells which support the resulting acrosome reaction. Thus the possibility existed that an embryo would be created, though this was controlled by limiting the observations to no more than 5 hours after the interaction of sperm and oocyte.

The research is effectively managed and is now well resourced. The Person Responsible (PR) is appropriately qualified and has taken actions to implement the recommendations of the previous inspection report. The PR has completed the PR Entry Programme. A new Nominal Licensee is included in the application; the inspectorate consider the applicant to be appropriately qualified and suitable for the role given her connection with the research project. The researchers have appropriate training and experience and are well qualified to continue the programme of research.

The premises and equipment are safe, clean and well maintained and are suitable for the intended research purposes.

Donation processes at the centre were considered appropriate and there has been no evidence of coercion in patient feedback or complaints at Centre 0119 or to the HFEA. Procedures are in place to ensure that patients are treated respectfully and are provided information and time to make an informed decision regarding their participation in the research project. A review of research consents in patient records, and embryologists witnessing checks thereof, revealed no discrepancies.

Patient information and consent forms were reviewed and were considered well presented and compliant with the HFEA Code of Practice, 8th edition except for the issue discussed below.

To date on this project, work has been carried out which has not involved licensed activities in that experiments have focussed on the responses in sperm to contact with the oocyte zona pellucida. Thus investigations have used 'failed to fertilise' oocytes (i.e. oocytes in which pronuclei have not appeared 48 hours or more after insemination), which have been enucleated by cytoplasmic removal to produce zona pellucida preparations, and sperm derived from research donors. Neither of these materials is considered HFEA-licensable however the researchers effectively treat them as such in their working practices. Research work commenced in April 2009 after a year suspension due to staffing issues and a change in focus to other non-licensable research goals, though the PR considered these goals needed to be addressed before the licensable research could be performed. Since April 2009, research materials from 21 patients have been collected. Many have donated cumulus masses and follicular fluid while 8 patients have donated 19 failed to fertilise or immature oocytes. Unfortunately, work then had to be suspended in June 2009 due to smoke damage from a fire in an adjacent corridor. Research work is now recommencing.

This renewal application describes future research work on the project which includes, in addition to the existing work in the licence: In vitro maturation of immature MI oocytes; Culture

for 7 days of the embryo products of oocyte-sperm interactions investigated, to assess developmental potential; Subsequent biopsy and molecular, including aneuploidy, assessment of those embryos. These activities require a HFEA research licence and will only begin if the renewal application is approved by the Licence Committee. The applicants wish to modify the title of research project R0173 to 'Human Gamete Activation, Interaction and Signalling', 'activation' being now included to reflect the greater emphasis on embryonic activation and its effect on development. It should be noted that the additional research work was recommended, in part, by the peer reviewer of the application in 2006. The peer reviewer of this application recommends acceptance of the proposal without changes.

The peer reviewer and inspectorate note that the applicants have not confined their research to the original project proposal but accept that the research performed was not licensable and was considered by the applicants to be essential. It provided them key knowledge and experience which allows them to now progress with the research proposed in this application, which includes that research previously planned on this research licence.

To ensure compliance with Code of Practice requirements:

- The inspectorate recommend the incident reporting SOP should include that incidents are reported in writing on the appropriate HFEA report form to incidents@hfea.gov.uk within 24 working hours, to ensure compliance with Direction 0011.
- The inspectorate note that while local ethical approval is in place for the research work common to the licence application in 2006 and this renewal application, the addition research work proposed in this application, e.g. IVM, embryo culture for 7 days and aneuploidy assessment, has not been approved by the local ethics committee, albeit an application is in progress. The PR assured the inspectorate that the new research work proposed will not, and lawfully can not, be performed until ethics committee approval for it is place. He is though seeking to include the additional research work in this licence reapplication so that the HFEA licensing process and ethics review process can progress in parallel. The inspectorate note the PR's obvious commitment to performing research within the regulatory and ethical framework in a compliant manner and his assertion that he would consider it unlawful and unethical to perform the additional research proposed in this application without ethical approval. The inspectorate recommends ethical approval is obtained for the additional work proposed, at the earliest opportunity and that the proposed additional work is not performed until that ethical approval is in place.
- The patient information provided at inspection had not been reviewed to include the new additional research proposed in this application. If used for consenting patients for the proposed research, it would not provide patients with 'all relevant information as is proper' as required by HFE Act (1990) as amended, Schedule 3, 3(1)b. This was discussed with the PR who informed the inspectorate that patient information is being updated and submitted to the local research ethics committee along with the application for the proposed additional research. The inspectorate recommend ethical approval is obtained for the revised patient information at the earliest opportunity and that the proposed additional work is not performed until that ethical approval is in place.

The inspectorate also make the following suggestions to assist the development of better practice:

- The personnel records for the Research Associate were reviewed. Evidence of induction activities and training were observed however it was noted that the worker had not undergone the hospital trust induction course. It is suggested that the Research Associate undergo this course to facilitate his ability to perform effectively in the hospital environment.
- It is suggested that the research tissue culture incubator in Centre 0209 is validated before use in research experiments
- The patient information clearly states that withdrawal of consent can be made at any time up to egg collection without it affecting future treatment and 'You must ensure that this has been documented and signed by a member of staff'. It is suggested that the patient information provides contact details for a named individual through whom this can be achieved, as well as relating that it can be discussed with any member of staff.

The inspectorate recommends renewal of the research licence for project R0173 for a period of 3 years. Due however to the delay in local ethics committee approval for the additional proposed research work and associated patient information, the inspectorate also recommend that a condition be placed on the licence that no research be performed until appropriate ethical approval and patient information is in place and has been supplied to and approved by the HFEA Executive.

Report of Inspection findings

1. **Organisation**

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

Summary of findings from inspection
Evidence of:

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

Staff R0173

Principal investigator	Dr Jackson Kirkman Brown
Scientists	1 Senior Lecturer; 1 Lecturer; 3 post-doctoral research fellows; 4 PhD students;1 Research Associate; 1 embryo biopsy practitionner
Collaborators	0
Support staff (receptionists, record managers, quality and risk managers etc)	A dedicated research recruiter at Centre 0119 recruits patients to the research project

Highlighted areas of firm compliance
The research premises of Centre 0209 comprise a laboratory within the Institute of Biomedical Research, a research-dedicated building which is part of the University of Birmingham School of Medicine. The Institute is approximately 300 meters from the Assisted Conception Unit, Birmingham Women's Hospital (*i.e.* Centre 0119, the donating centre to project R0173). The facilities and staff within the Institute are part of the University of Birmingham, which provides health and safety, human resources, financial management, training and other infrastructural support. A research dedicated laboratory within Centre 0119 will also be used for some aspects of the proposed project (e.g. embryo culture and biopsy for aneuploidy assessment). This laboratory is also administered by the University of Birmingham School of Medicine. The researchers are well organised and have clear lines of communication and control. The Person Responsible (PR) is a Senior Lecturer within the School of Medicine with many years of research experience, and also the Director of Research and Development at the Centre for Human Reproductive Sciences in Centre 0119. The PR is the project head, has been PR since its inception on 1st January 2006, and has an understanding of HFEA regulatory requirement. The PR is not the PR of a Treatment and Storage Licence and has completed the PR entry programme. The proposed Nominal Licensee (NL) is a lecturer within the School of Medicine and again has considerable research experience and works on the project. The inspectorate consider the proposed NL to be appropriately qualified and a suitable appointment given the role she has within this research project.

All research staff are employed by the University of Birmingham and on recruitment undergo induction through the university for health and safety, fire safety, occupational health etc. They also undergo a local induction course which covers the laboratory and HFEA-regulated work, and the activities of Centre 0119. Induction is signed off in staff personnel files, which are stored in a locked cupboard in the PR's office in the Centre for Human Reproductive Sciences in Centre 0119. The PR said that staff training meets the requirements of funding bodies and the university and involves attendance at conferences, seminars and internal and external training programmes. Continual professional development (CPD) is also recorded in the staff personnel files stored in the PR's office in Centre 0119. The personnel file for the recently appointed Research Associate were reviewed with this worker and appropriate induction and training activities were discussed and seen to be documented.

The licensed research has, and will be, funded from multiple research grants, several of which last until 2012. The PR is confident that financial support for the 3 year term of the licence is in place, though further funding is being sought to support the research groups work in general as well as the licensed research activities.

Resource management and project coordination is achieved through weekly meetings between the PR, NL and researchers, albeit workers on other non-licensed projects also attend. These are not minuted but email updates are kept. It was recommended at the last inspection that minutes are kept of these meeting but the PR considers the email records to be adequate as he leads the research and is closely involved, with the NL, in its delivery. He is though planning in the near future to hold minuted meetings of those workers involved solely in HFEA licensed activity. Recent staff changes within the department will make this easier. Minuted meetings are held between the Research PR, a recently appointed Research Associate, and the embryologists and nurses at Centre 0119. Research data is also reported back to Centre 0119 in research seminars at team meetings. The Research Associate has been employed at Centre 0119 since April 2009 to facilitate patient recruitment to research.

Laboratory standard operating procedures (SOPs) are available for staff in an electronic central folder on a university maintained computer server, accessible to specified users. The PR considered the SOPs include all required laboratory methods. The SOPs are updated and added to as required and are document controlled. At the inspection in August 2008, it was required that a procedure for reporting serious adverse events to HFEA should be developed; this SOP was prepared and was seen at this inspection and was considered appropriate with one minor exception. Indeed the PR used this protocol to report appropriately a recent adverse incident at the centre.

Issues for consideration

- The personnel records for the Research Associate were reviewed. Evidence of induction activities and training were observed however it was noted that the worker had not undergone the hospital trust induction course. It is recommended that the Research Associate undergo this course at the first available opportunity.
- The incident reporting SOP should include that incidents are reported in writing to incidents@hfea.gov.uk within 24 working hours to ensure compliance with Direction 0011

Executive recommendations for Licence Committee
The Licence Committee is asked to endorse the recommendations made in relation to the areas for consideration cited above.
Areas not covered in by this inspection
All covered

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

Highlighted areas of firm compliance
The laboratory in Centre 0209 used for licensed activity is locked on a card key system, access being restricted to licensed personnel only. Currently, oocytes (enucleated by cytoplasmic removal and thus non-licensible) are used on the day of donation to prepare zona pellucida preparations. These are stored frozen in the laboratory in Centre 0209 but do not constitute licensed material. Indeed no licensed material or activities have occurred on this project for reasons discussed elsewhere in this report, though they will in the next licensing period. The laboratory in Centre 0209 was appropriately equipped, with a dedicated incubator, two air flow cabinets and two specialised microscopes with micromanipulation and microinjection equipment allowing vital observation of ion currents in oocytes/sperm using ion binding fluorescent dyes. Multiple wavelength channels can be monitored allowing several ion currents to be observed simultaneously within a sperm or oocyte, and data stored electronically. There is also an adjacent laboratory for molecular biological procedures. The proposed NL's office is close to the licensed laboratory in Centre 0209 and is key pad locked unless occupied. It contains research records and documentation in a locked filing cabinet. Security arrangements were appropriate. In addition, no patient identifying information is kept at Centre 0209 as all research samples are derived from Centre 0119, and paperwork which accompanies them to Centre 0209 contains a research subject number but no identifying information. The laboratory in Centre 0119 which will be used in the new work proposed in the renewal application is secured by a key pad locked door and restricted to licensed research staff. It contains two incubators, an air flow cabinet and microscope. It is proposed to also equip the laboratory with MINC incubators for culture at reduced oxygen concentrations. The laboratory will be used for in vitro maturation (IVM) of MI oocytes, their fertilisation with research donated sperm, and their culture for 7 days. The PR said that it is clearly stated in the culture procedure that culture can be for a maximum of 7 days only and under no circumstance can exceed 14 days post-fertilisation. Embryos will then be destroyed to produce preparations for aneuploidy and other research analysis. The PR's office is across the corridor from this laboratory. It contains documents pertaining to the research licence, some containing patient identifying information. These are stored securely in a locked filing cabinet. The office is also locked when unoccupied and is within the secure laboratory area of Centre 0119. The PR considers they have all equipment required for the project. Both laboratories are administered by the University of Birmingham School of Medicine who ensure they are safe, clean and well maintained. All apparatus is on service contracts organised centrally by a specified person; the PR considers that all equipment is well maintained. All portable appliance testing (PAT) was within the dates scheduled according to the University of

Birmingham protocol for PAT certification. The premises are risk assessed and randomly inspected by university health and safety staff. The university Biological Safety Officer also inspects the premises on a biennial cycle.

The licensed laboratory at Centre 0209 was in June 2009 damaged by smoke from a fire which arose after electrical malfunction of the air conditioning system in a service conduit in the access corridor to the laboratory. The incident was reported appropriately to the HFEA by the PR and licensed activity suspended. The room was decontaminated by a specialist company and all equipment replaced or cleaned, serviced and tested by specialist technicians, and PAT tested. The PR has been advised by manufacturers that equipment will operate normally after cleaning and servicing. Some smoke contaminated chemicals remain as evidence for insurance claim purposes. These have been sealed in plastic bags and stored in the molecular biology laboratory. Research activity has slowly restarted and, according to the PR and NL, will be at full speed a month after the inspection. The inspectorate consider the PR and NL's responses to this incident provide good evidence of effective management of the research project and associated resources, as well as compliance with HFEA incident reporting requirements.

On the renewed licence, the PR wishes storage of oocytes and storage of oocytes within ovarian tissue to be added as activities. These will be stored within a dedicated research dewar in the treatment and storage facility at Centre 0119. The cryostorage facilities were considered compliant with all HFEA regulations at the last inspection in August 2008 and have not been changed.

Issues for consideration

- It is suggested that the research tissue culture incubator in Centre 0209 is validated before use in research experiments

Executive recommendations for Licence Committee

None

Areas not covered in by this inspection

All covered

3. Donation of material

Desired outcome: Donors are recruited appropriately and any research carried out on their embryos is in accordance with their consent.

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
- Ensuring patient consent is not breached
- Donor and patient records
- Prevention of coercion of prospective donors

Highlighted areas of firm compliance
Research on the project was resumed in April 2009 having been suspended due to the absence of the research nurse and the PR having modified the research path to collect more data to inform the licensed research experiments. Donors are recruited from Centre 0119 by the Research Associate appointed in April 2009 according to a documented standard operating procedure for research recruitment. Written and verbal information regarding the research is given when the patients attend an information session (the clinical group meeting) to introduce them to the clinic, during which they are also given a tour of the laboratories and clinical facilities, and briefed by the research PR in his role as scientific lead within Centre 0119 about IVF treatment and research projects. They are advised that research donation will not have any influence on their clinical treatment. Patients have been investigated by the referring clinics by this stage so can sign some clinical consents and the HFEA registration forms at the end of the session. Patients also indicate whether they are interested in research donation on a tick box in their clinical consent forms but are told by the research PR that this is not a commitment. The suitability of those indicating interest in research donation is assessed on the basis of medical records and those considered suitable are provided further information, which gives details of a person to whom they can address additional questions. The patients next attend a consultation during which they sign HFEA treatment consent forms and discuss their treatment plan, after which they can discuss research consent with the Research Associate. The Research Associate has a defined list of information he provides the patients. Patients then undergo down-regulation and attend for ultrasound scanning, after which they discuss research consent with the Research Associate and sign consent forms if they choose to donate to research. Research consent is obtained before the treatment cycle is started, i.e. long before egg collection, but one to two months after they have received information about the research. Patients have therefore had an appropriate amount of time to consider research donation. Verbal and written information is also provided at times separately from the treatment process to avoid overwhelming patients with information. Indeed, patients are provided with multiple opportunities to obtain further information about the research from the Research Associate before consenting. The inspectorate consider patient information is balanced and non-coercive and note that no patient complaints have been received on this issue. The Research Associate is used solely for recruitment and information provision so is in

practice independent of the research. In discussion, he showed appropriate understanding of that independence and a sensitivity regarding the need to ensure consent is fully informed and given free of coercion of any kind. The Research Associate is briefed by the research PR as to the progress of the research. Indeed research progress is fed back to Centre 0119 staff in research seminars at all staff meetings.

The research recruitment protocol includes verification of research consent before and when samples are transferred to research. Prior to egg collection, patient records are signed by the research PR to indicate whether they contain a valid and complete research consent form. This signature is removed if a patient withdraws consent and staff at Centre 0119 know to discuss consent withdrawals with the Research Associate or research PR immediately, and to note withdrawal in patient records. The Research Associate in discussion described appropriate actions to be taken in the event that he was informed of a withdrawal of consent. In the clinical laboratory, research consented patients who fulfil the research donation criteria are pinpointed, the consent is again checked in patient records, then the clinical embryologists call the researchers to warn of an imminent donation. Excess mature or failed to fertilise (i.e. oocytes in which pronuclei have not appeared 48 hours or more after insemination) oocytes are then selected by the clinical embryologists for research donation. Researcher's have no role or influence in the clinical laboratory. Donated oocytes are then transferred to the researchers along with a handover sheet with patient details and research number, on which it is verified with a witness signature that research consent is in place. That research consent has been checked by the embryologists is noted by the Research Associate in the research donation log to verify it has been done.

The oocytes are transferred to the researchers in anonymised straws marked only with a research number while the handover sheets and research donation log book are stored securely in the research PR's office. All research material is anonymised from this point and no patient identifying information leaves the research PR's office. The Research Associate prepares instead a research usage form labelled with the anonymous research number, to accompany the sample and record subsequent manipulations. Anonymisation can be reversed, if necessary, via the log book stored securely in the PR's office.

Currently, oocytes are transferred from Centre 0119 to the laboratory at Centre 0209 in a portable incubator. They are there immediately placed in an incubator, for short term (< 2 hours) culture before use in experiments. To date these experiments have only used oocytes (i.e. oocytes in which pronuclei have not appeared 48 hours or more after insemination), to fertilise oocytes from which zona pellucida has been prepared. Indeed, such oocytes are enucleated at Centre 0119 before transfer to Centre 0209 and thus are not licensible. In the future, experiments will utilise viable oocytes for ion current imaging at Centre 0209. The licence renewal application also proposes new work including in vitro maturation, post fertilisation culture and embryo development assessment and aneuploidy screening. Licensed activity associated with this work will be performed in the research dedicated laboratory in Centre 0119. Oocyte transfer to this research arm was discussed with the PR. The transfer to research process will be the same as that used at present and will be facilitated by the Research Associate or PR, who will operate as the patient identifying information cut-out. The research laboratory within Centre 0119 is staffed by researchers, not clinical embryologists, isolating research from treatment effectively.

The researchers see no patient identifying information after anonymisation, nor does such

information leave Centre 0119. Experimental notes and data are, and will be, documented on computer hard-drives and laboratory notebooks, oocytes or embryos being referenced by their anonymised research code. Experimental notes and records are/will be kept within the secure research premises. When records are no longer required, they are stored in a locked cupboard in the PR's office in the Centre for Human Reproductive Sciences in Centre 0119.

At the inspection in 2008, it was required that the PR document exactly when oocytes are entering the research pathway and that research consents are verified to be in place at that time. On this inspection, evidence in SOPs, patient records and discussions with staff indicated that the point of entry into research has been clearly defined, as discussed above, and that consents are verified at this time and that this check is documented.

Patient consents for research and witnessing by embryologists of the consents being in place were reviewed in 5 sets of patient records held in Centre 0119. No discrepancies were noted in the consents or the embryologists' witnessing checks that the consents were in place

Issues for consideration
None
Executive recommendations for Licence Committee
None
Areas not covered in by this inspection
All covered

4. Patient information and consents

Desired outcome: Patients are provided with appropriate information which allows them 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

Highlighted areas of firm compliance
Patient information and consent forms for project R0173 were assessed by the inspectorate. They clearly explain that 'failed to fertilise' oocytes will be used in research and that these are of no use in treatment and therefore would not affect it. The information forms also discusses the facility for a patient to consent to supply 1 or 2 'good' oocytes, but only if 12 - 15 or >15 oocytes, respectively, are collected. This is considered by the centre, as discussed in patient information, to have only a minimal impact on the potential success rate of a fresh cycle, but the information clearly states that it may affect the number of embryos available for freezing. The researcher's have however currently and in the past collected only failed to fertilise oocytes for zona pellucida preparations, so the consent forms have the two sections blanked out which are appropriate for providing consent for research using mature 'good' oocytes from patients with 12 or more oocytes. At the inspection in 2008 it was noted that patient information did not provide contact details for somebody independent of the research with whom patients could discuss donation. It also did not inform patients that they can see a counsellor to discuss the implications of donation, as required by Code of Practice, 7th Edition, G.6.7.2 (a). The patient information provided at this inspection was seen to have been appropriately modified to address this issue. The patient information and consent forms for project R0173 were compliant with the Code of Practice, 8th edition, except where noted below.
Issues for consideration
• The information clearly states that withdrawal of consent can be made at any time up to egg collection without it affecting future treatment and 'You must ensure that this has been documented and signed by a member of staff'. It is suggested that the information provides contact details for a named individual through whom this can be achieved, as well as relating that it can be discussed with a member of staff. • The patient information provided at inspection had not been reviewed to include the new additional research proposed in this application. If used for consenting patients for the proposed research, it would not provide patients with 'all relevant information as is proper' as required by HFE Act (1990) as amended, Schedule 3, 3(1)b. This was discussed with the PR who informed the inspectorate that patient information is being updated and submitted to the local research ethics committee along with the application for the proposed additional research, as discussed in Section 5 below. The inspectorate recommend ethical approval is obtained for the revised patient information at the earliest opportunity and that the proposed additional research work is not performed until that information and ethical approval is in place.

Executive recommendations for Licence Committee
The Inspectorate recommend that the Licence Committee approve this renewal application but that renewal be subject to a condition on the licence that no research be performed until appropriate ethical approval and patient information is in place and has been supplied to and approved by the HFEA Executive.
Areas not covered in by this inspection
All covered

5. Scientific practice R0173, Human Gamete Interaction and Signalling

Desired outcome: Research is carried out in accordance with licence conditions and makes progress towards achieving stated aims

Summary of:

- Peer review

Summary

Activities on the licence: Licence R0173 was original granted to include the activity 'creation of embryos in vitro for use in research'. The licence renewal is requested to include the activities, 'the storage of oocytes', 'the storage of oocytes in ovarian tissue', 'the creation of embryos in vitro for use in research', and 'the use of donated embryos in research'.

Other activities: IVM of Immature Oocytes; Assisted Oocyte Activation; Sperm-Cumulus Oocyte Complex Signalling; After they have been used in research some eggs/embryos will also be checked for aneuploidy using standard PGS techniques which may include FISH or microarray 'chip' techniques.

Licence renewal is requested for the purposes of:

- promoting advances in the treatment of infertility (*HFE Act 1990 as amended, Sch 2, 3A(2)(d))*
- increasing knowledge about the causes of miscarriages (*HFE Act 1990 as amended, Sch 2, 3A(2)(e))*
- developing methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation (*HFE Act 1990 as amended, Sch 2, 3A(2)(g))*
- increasing knowledge about the development of embryos (*HFE Act 1990 as amended, Sch 2, 3A(2)(h))*

Embryo/oocyte usage in last year: 'Only eggs surplus to treatment have been supplied to the projects. All of these eggs have been from Centre 0119. We had a pause in supply whilst waiting for new funding for an independent research nurse/associate to take consents. This has now been filled and we have been consenting material, within the current reporting year, since April 2009

'So far this year we have had 21 patients consented for the project, of which many have donated cumulus masses and follicular fluid. Eight patients have donated 19 failed to fertilise or immature oocytes. All of these have been frozen in media to leave non-viable as the material in this batch was being collected for purification of zona pellucida protein.'

'Work to date on this project has concentrated upon use of 'non-licensed' material (i.e. eggs unused in treatment e.g. MI and GV and failed fertilisation eggs) for zona pellucida characterisation and studies. Currently if being exposed to sperm the zona would have all cytoplasm removed - this experiment therefore does not require a licence.'

Proposed usage in the next year:

Material	Expected usage
Fresh Eggs*	80 (some will be immature)
Frozen Eggs	0
Failed to Fertilise Eggs	80
Fresh Embryos	<160 (product of fertilisation of oocytes donated to the project
Frozen Embryos	0

Summary of audit of stored and biopsied material

No licensed materials are stored

Renewed project objectives and methods, as stated in the renewal application

Summary

The objective of this proposal is to elucidate the events that occur during human sperm and egg interaction at a cellular and molecular level, including methods for achieving improved oocyte activation/fertilisation rates.

Aims

These aims are not mutually exclusive and many may be accomplished at once in single experiments:

- Evaluation of techniques to artificially activate human oocytes, particularly around therapeutic assisted activation focused upon those which would meet and comply with EUTCD requirements.
- Characterisation of the Ca^{2+} responses, motility and other physiological changes induced in human sperm by cumulus oophorus and zona interaction and penetration in zona-enclosed human oocytes.
- Characterisation of any focal or global signalling events generated in cumulus cells due to interaction or presence of human spermatozoa.
- Precise monitoring of the human fertilisation event and understanding of how any calcium signalling events occurring within the sperm may relate to those initiated in the oocyte.
- Examination of whether signals initiated in the periphery of the cumulus, possibly induced by interaction with spermatozoa, propagate to the oocyte itself.
- Accurate examination of the early fertilisation events in the fresh human oocyte.
- Effects of aneuploid state of the oocyte upon any of these signals or their subsequent events.

Methods

A key aspect of our proposed research and experimental plan is to perform multiple experiments where possible on individual eggs. This maximises the useful research outcome from the limited and important material available.

Briefly, fresh oocytes, obtained via informed consent will be maintained in IVF conditions on a microscopy-imaging system. Immature oocytes will first be subject to in-vitro maturation protocol before being used in the same experiments. The events occurring in sperm interacting with the egg, cumulus/corona cells, and the oocyte itself will all individually or in unison be examined by fluorimetric probe techniques as will responses in the oocyte both to

artificial and natural fertilisation. Resultant material will be subjected to aneuploidy characterisation.

IVM of Immature Oocytes

We hope to perform in-vitro maturation of immature oocytes (GV, MI) to MII for use in the research projects.

Oocytes donated to research by patients undergoing ART treatment tend to be either oocytes which have failed to fertilise or are immature (GV, MI stage). Whilst 'failed fert' oocytes can provide material for some research projects e.g. a source of zona pellucida, they are not suitable for much of our research involving the study of fertilisation and/or egg activation. Since the number of donated metaphase II oocytes which have not been exposed to sperm is very small, the development of a reliable method of in vitro maturation (IVM) of oocytes to metaphase II would greatly increase the utility of the donated material we receive. Such methodologies are clinically important too and offer the hope of increased success of ART treatment, particularly for women who respond poorly to ovarian stimulation such as PCOS sufferers and may allow the use of milder stimulation regimes reducing the risk of ovarian hyperstimulation syndrome although as yet no randomised clinical trials have been conducted (Siristaditis et al, 2009).

Many protocols exist for the IVM of human oocytes (reviewed in Picton, 2002). The majority of oocytes that we receive will have been stripped for ICSI. Since the crucial role of the cumulus cells in oocyte maturation and attainment of developmental competence is well established (Staigmiller and Moor, 1984) we will use a co-culture system with homologous cumulus cells recovered either during egg collection or hyalurindase treatment. We will use a culture media for oocyte maturation which was formulated to mimic the components of human follicular and tubal fluid [Wynn et al., 1998] supplemented with recombinant gonadotrophins and HSA which several studies have shown to be essential to achieve high levels of oocyte maturation and subsequent embryo development (see Picton, 2002). It is also well established that the oxygen tension of the mammalian female reproductive tract is much lower than in the atmosphere (Mastroianni and Jones, 1965, Maas et al., 1976) and a number of studies in animals have shown that both oocyte maturation and embryo development are improved by culture at low oxygen tensions (reviewed in Hashimoto, 2009). We would therefore mature the oocytes at 37°C, 5% O_2 for 48 hours.

Assisted Oocyte Activation

Our initial aim with the matured oocytes or any of those which have failed to fertilise is to be to image and examine the effects of Assisted Oocyte Activation upon the egg to evaluate the use of a modified technique (not requiring A23187 and therefore appropriate under EUTD) for assisting in cases of activation failure.

There are currently many couples where after the sperm has entered the egg, either unassisted or through ICSI, the egg fails to be 'activated'. Failure of 'oocyte activation' means either that embryos are never produced and hence the couple cannot be helped by assisted reproductive technology, or that only very few embryos are produced within a given ICSI treatment cycle.

A specific sperm phenotype where oocyte activation failures are known to occur is globozoospermia (e.g. Kilani et al., 2004). In globozoospermia and other cases a sperm-

based activation problem has been proven by similar results when injecting the sperm into a mouse oocyte (MOAT, Heindryckx et al., 2004), this is clearly not legal under UK legislation. The zona-free "hamster test" which is legal does not test for oocyte activation rather it tests for ability of sperm to decondense.

For many years it has been known that the process of oocyte activation relates to a process whereby entry of the sperm triggers several oscillations in the level of intracellular calcium ions ($[Ca^{2+}]_i$), which occur over a number of hours, that regulate resumption of meiosis in the oocyte, subsequent syngamy and formation of the embryo (reviewed in: Machaca, 2007; Ducibella and Fissore, 2008; Swain and Pool, 2008). In fact these processes can be achieved even without the presence of a sperm through parthenogenesis to create embryos for stem cell generation and other research uses (reviewed in Paffoni et al., 2008).

In many couples where oocyte activation fails this is likely to be due to either the lack, or reduced levels of the soluble messenger from the sperm, insensitivity of the oocyte to this messenger or a combination of the two.

Much research surrounding egg-activation to date has focused upon the elegant work of the teams involving the UK teams of Karl Swann and John Parrington on sperm phospholipase C zeta (PLCz, reviewed in Swann and Yu, 2008) and examination of men where lack of PLCz causes failure of oocyte activation (Yoon *et al*., 2008). The research from these teams and others has highlighted just how important failure of the oocyte to activate is, though many more oocytes may also be failing to activate due to other factors. One approach would clearly be to manufacture and add PLCz at the necessary purity to the oocyte, though this involves injecting a manufactured compound directly into the egg.

In most of these cases of failed oocyte activation, artificial activation surpasses this barrier. For many years one accepted method of oocyte activation has been a calcium ionphore, A23187. This equilibrates the low intracellular $[Ca^{2+}]_i$ levels with the higher external media concentration, artificially mimicking the first calcium oscillation. This has been used successfully worldwide in treating many couples and is generally known as Assisted Oocyte Activation by ionophore (e.g. Rybouchkin et al., 1997; Kim et al., 2001; Eldar-Geva et al., 2003; Murase et al., 2004; Heindryckx et al., 2004; Tejera et al., 2008; Kyono et al., 2008).

The problem with the ionophore technique, within EU regulation is that A23187 is marked as 'not for in-vitro diagnostic use' , and is marketed only in reagent grade as an antibiotic produced during fermentation by the bacteria Streptomyces chartreusensis; it also has other potentially undesirable non-specific side-effects such as uncoupling oxidative phosphorylation and inhibiting mitochondrial ATP production. Various authors have noted that A23187 techniques involve 'insufficiently tested and potentially harmful drugs' (Tesarik et al., 2002). Despite this as reported above it has gained wide-usage in AOA and apparently healthy children have been born (Rybouchkin et al., 1997; Kim et al., 2001; Eldar-Geva et al., 2003; Murase et al., 2004; Tejera et al., 2008; Kyono et al., 2008).

Within Europe, and for worldwide implementation, it is desirable to use a method of oocyte activation that can employ pure chemicals of known or testable grade where available information would indicate a minimum of side-effects or risk. A method of oocyte activation that mimics that produced by A23187, is application of strontium ions in a calcium free media, as the human body treats strontium in a very similar way to calcium, which it can mimic.

Strontium chloride, the active chemical currently has broad usage across many toothpastes where it is used to reduce 'sensitivity' being introduced at levels up to 10%.

Strontium-Assisted Oocyte Activation (Sr-AOA) has now been used successfully by a number of Japanese clinics, with healthy live-births resulting (Yanagida et al., 2006; Kyono et al., 2008). It therefore appears to offer a safer alternative for AOA than ionophore methods.

Patients who would have Sr-AOA as part of treatment would in all likelihood always have received at least one prior treatment cycle with less than 50% fertilisation rate of normal MII oocytes, or have a severe acrosomal deformity condition such as globozoospermia, where failure to fertilise would be expected and therefore Sr-AOA would be immediately offered. Currently the only option for these patients is to either undergo the severe risks and costs associated with many repeated cycles of ART treatment, hoping that an occasional fertilisation may lead to a pregnancy; or resort to travel abroad to unregulated clinics for the A23187 procedure which we believe has higher associated risks.

In the research proposed we would assess the efficacy of the Sr-AOA both by attempting subsequent activation of failed fertilisation mature oocytes and by fertilising matured oocytes. In some cases we will monitor intracellular calcium oscillations, in others we will just perform the procedure and assess embryo development. In eggs just left to activate without monitoring we will follow up by assessing the ploidy of the subsequent embryo. It will be very interesting and important for us also to do the procedure on MII unfertilised oocytes resulting from IVF failed-fertilisation. This should allow us to assess how many are failed activation as opposed to being due to sperm failure to penetrate and fuse. As aneuploidy assessments will be made we will consent patients for genetic testing but with a clear provision that no results will be fed-back.

Sperm-Cumulus Oocyte Complex Signalling

When the above, slightly simpler work is complete, we will move on to the research outlined in the earlier licence. Briefly:

In 1985 Hull and colleagues identified sperm dysfunction as the single most common cause of human infertility, accounting for one quarter of the study population (Hull *et al,* 1985). The explosion of treatment abilities (ICSI) in clinical male infertility has not been accompanied by significant advances in understanding of the cellular and biochemical processes that underlie sperm function, let alone the lesions that cause sperm dysfunction.

Our group have developed interests in the fundamental processes around motility regulation (Smith et al., 2009); Responses to physiological ligands, particularly progesterone (reviewed in Correia et al., 2007) and nitric oxide (Machado-Oliveira et al., 2008); acrosome reaction (AR – secretion of the acrosome contents) and the associated signalling and cellular interactions (Harper et al., 2006). Essentially we are aiming to examine events from when sperm are deposited in the cervix through to when they meet the oocyte, alongside maturation signals in the egg.

In particular relation to this licence, AR of the sperm is a prerequisite for sperm-egg binding and is consequently a crucial event in fertilisation. AR *in vivo* is thought to be induced by binding of the spermatozoon to proteins of the zona pellucida (ZP) which surrounds the egg. Progesterone (PG) will also induce AR but is a less effective agonist and probably functions *in*

vivo to prime ZP-induced AR (Roldan *et al,* 1994). Studies on animals have shown that AR is accompanied by a complex of cellular signalling (Ward and Kopf, 1993; De Jonge, 1994; Aitken, 1997; Breitbart and Spungin, 1997), but is essentially a Ca^{2+}-mediated secretory event, dependent upon ligand-activated influx of Ca^{2+} through membrane channels (Florman *et al,* 1998; Darszon *et al,* 1999).

Ca^{2+} influx and AR in human spermatozoa.

Due to the difficulty of obtaining human ZP in sufficient quantity, the vast majority of studies on $[Ca^{2+}]_i$ signalling and AR in human spermatozoa have used the readily available agonist progesterone (e.g. Kirkman-Brown *et al,* 2000, 2002, 2004). AR in human spermatozoa can be induced by intact or solubilised human ZP and recombinant human ZP-derived protein 3 (Cross *et al,* 1988; Bielfeld *et al,* 1994; Liu and Baker, 1994; van Duin *et al,* 1994; Franken *et al,* 1996; Chapman *et al,* 1998). This process appears to involve Ca^{2+}-influx (Bielfeld *et al,* 1994). Ca^{2+}-signalling in the mouse may be a good model for the human but there is almost no evidence to this effect. Still nothing is known of these events in the human with intact cumulus oocyte complexes.

We have performed the first Ca^{2+}-imaging work on freely motile cells in contact with female tract and have data in preparation (see attached poster, pdf below, presented at the 2009 Physiological Society Annual Meeting by Connolly *et al*., 2009). These techniques and abilities underpin ongoing work on sperm cumulus cell interaction and will facilitate the enclosed oocyte work. Our related work on effects of nitric oxide and progesterone co-exposure of sperm is based around effects occurring in the cumulus, we have now characterised the calcium signalling and tail beat effects as well as proving that native cumulus can S-nitrosylate human sperm proteins (Machado-Oliveira et al., 2008).

We performed the first successful and repeatable patch-clamp experiments on human spermatozoa (Gu *et al;* 2004) and are slowly assembling evidence of the channels observed (Jiménez-González et al., 2007). Although we have previously detected message for T channels ($alpha_{1G}$ and $alpha_{1H}$) in human testis, which appear to be localised in germ cells (Jagannathan *et al,* 2000) there is, as yet, little evidence for such expression and none for involvement of these channels in the response to ZP. Furthermore, the molecules involved in sperm-zona interaction may differ between human and mouse since the mouse has only three zona pellucida-derived proteins whereas a fourth is present in other species including humans (Hughes and Barratt, 1999; Lefièvre *et al,* 2004).

In 2002 intriguing evidence has emerged that mature mouse cumulus-enclosed oocytes have gap junctions and signalling occurring between each other (Arellano *et al,* 2002). If this can also occur in the human, it raises the possibility that human sperm penetrating through the cumulus may induce signals that travel to the oocyte, or that the early events after sperm egg fusion propagate a signal back to the cumulus which may have a physiological role in the block to polyspermy. We intend to examine this in detail, for further relevant method detail see publications from the group and attached publications.

Expertise in our laboratory.

Our laboratory has extensive experience in the study of cell signalling, particularly Ca^{2+} signalling. I have worked for twelve years on the characteristics and functions of Ca^{2+} channels in human spermatozoa. This work has involved extensive use of imaging

techniques.

During the past six years since my return from a Postdoctoral Fellowship at UMASS, Worcester in Professor Harvey Florman's laboratory, I have been working in the Assisted Conception Unit at Birmingham Women's Hospital. During this time I have promoted to my current level of Science Lead and gained a great deal of experience and expertise in the biology of human gametes. I have also been promoted to the level of Hon. Senior Lecturer in the University of Birmingham and have my own independent research group. Through the ACU at Birmingham Women's Hospital (a HFEA-registered centre) we have access to samples of human spermatozoa and eggs and expertise in their preparation, purification and storage.

Our laboratories have extensive imaging technology available funded by current and past research support and have just secured a further £500k investment in Laboratory Facilities from the Advantage West Midlands Science City initiative.

The result is that, within our laboratories, we have the ideal range of experience, expertise and skills to undertake this work. The proposed project is to gather data of central importance for our understanding of human fertilisation. The fact that this work has never been undertaken is a reflection of the difficulty involved in carrying out such studies on the human. We believe there is currently no other laboratory in the world in a position to undertake this type of work.

Oocyte preparation

Intracellular calcium changes will be measured with a calcium dye (e.g. Fura red-AM, Molecular Probes, Eugene, OR). Stock solutions of 1 mmol l^{-1} dissolved in DMSO plus 5% (w/v) pluronic F127 (Molecular Probes) will be used to load the oocytes, with a final concentration of 4μmol (e.g. Fura red-AM l^{-1}) for 10 min. Loading conditions may require much further optimisation according to the results obtained and effects of the presence of cumulus mass, as to date very little published work has been undertaken on human oocytes and these approximations are based on data from the mouse system. We have already got a considerable amount of date for sperm interacting with female tract cells and cumulus cells and will be using this to inform our work accordingly.

The loading medium may also be supplemented with sulfinpyrazone in certain experiments, which helps to prevent compartmentalisation and extrusion of the dye (Lawrence *et al*., 1998).

Sperm Preparation

200 μl aliquots of capacitated spermatozoa at 6×10^6 cells·ml^{-1} are loaded with (for example) Oregon Green bis-(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid (BAPTA 1)-AM (12 mM final concentration in DMSO dispersed with pluronic F-127) for 1h at 37 ℃ and 6% CO_2. Sperm are then washed to remove all excess dye. This method has been extensively validated in our laboratory and does not affect sperm function or viability.

Imaging

Ca^{2+} measurements will be carried out on our bespoke imaging systems. The systems include possibility of z-series acquisition; transmitted light as well as epifluorescence shuttering and filter wheels; environmental control (humidity, temperature and environmental

gases); and state of the art 3D reconstruction software.

Control of Patient Samples and Biological Materials

We previously understood that as we wished to follow events occurring immediately after the sperm enters the oocyte that there was a necessity to prove that an embryo could never be formed. Hence we chose to specify that the oocyte would always be destroyed within 5 hours of being exposed to sperm. This is because the pronuclei do not appear before 5 to 6 hours post-insemination and take a further 10 hours to lie close together (see Human Gametes and Conceptuses, Lucinda Veeck, 1998; Chapter 4, p34-35). Hence destruction before 5 hours post-insemination ensures that progression to the pronuclear stage cannot occur. Our current ethical approval and information is based around this. However, we ask that in future, subject to an amendment to our ethical approval we also be allowed to carry a number of these oocytes through to study embryological development. This assessment of potential for blastocyst development was requested by one of the referees of the original HFEA licence application in 2006 and would underpin any aneuploidy assessments made upon embryos from assisted oocyte activation.

To monitor times for oocytes only allowed to survive 5h post sperm exposure, two independent digital timers will be run alongside the experiments, one which sets an alarm at 4 hours 30 mins, the other at 4 hours 50mins; in addition real-times will be recorded from a clock in the imaging room and logged in the experimental laboratory book.

Our standard SOP for destruction of the oocytes is:

1. The droplet complete with oocyte is mixed with a greater than or equal amount of water allowing for lysis of the oocyte.

2. An excess of decontaminant / bleach is added to ensure all cells are killed.

Summary of research undertaken

As described in the renewal application:

Lay summary

The Birmingham research team focus upon trying to understand the pre-requisites for natural fertilisation – how a sperm and egg normally meet in the human body – and how this can be optimised for assisted reproduction and, or contraceptive developments. We have recently focused upon understanding how the fluid environment in the female tract, in which sperm 'swim' modifies their behaviour to something more like a 'crawl'. Alongside this work members of our team are characterising certain novel chemicals that affect how and the directionality of sperm swimming and effects of prominent signals in the female tract on sperm selection.

All of these data provide the bedrock for us to begin examination of the actual events happening when the sperm and egg come in close proximity to one another and what signals may then occur. Initial data from our laboratory has shown that calcium signals occur in the cells in the walls of the womb and tubes when they are exposed to sperm, we believe the same may well happen when the cumulus surrounding the egg contacts sperm, which in turn may ready the egg for fertilisation. These are the next experiments we intend to tackle.

A) How the work undertaken relates to the objectives.
Due to grant funding we decided to concentrate upon working out the signals occurring when sperm interacted with the female tract, cumulus cells and the characterisation of the zona pellucida proteins in the first instance. This work has all been funded and publication of the data is complete for some sections and in progress for others. We have identified and selected two recent key papers from our group to illustrate this below:

Paper 1 - Research around effects of nitric oxide and progesterone, as released by the cumulus around the oocyte on human sperm has clarified some events during the initial stages of sperm-COC interaction

Machado-Oliveira G, Lefièvre L, Ford C, Herrero MB, Barratt C, Connolly TJ, Nash K, Morales-Garcia A, Kirkman-Brown J, Publicover S. Mobilisation of Ca2+ stores and flagellar regulation in human sperm by S-nitrosylation: a role for NO synthesised in the female reproductive tract. Development. 2008 Nov;135(22):3677-86. PubMed PMID: 18842814.

Abstract
Generation of NO by nitric oxide synthase (NOS) is implicated in gamete interaction and fertilisation. Exposure of human spermatozoa to NO donors caused mobilisation of stored Ca2+ by a mechanism that did not require activation of guanylate cyclase but was mimicked by S-nitroso-glutathione (GSNO; an S-nitrosylating agent). Application of dithiothreitol, to reduce protein –SNO groups, rapidly reversed the actions of NO and GSNO on [Ca2+]i. The effects of NO, GSNO and dithiothreitol on sperm protein Snitrosylation, assessed using the biotin switch method, closely paralleled their actions on [Ca2+]i. Immunofluorescent staining revealed constitutive and inducible NOS in human oviduct and cumulus (the cellular layer investing the oocyte). 4,5- diaminofluorescein (DAF) staining demonstrated production of NO by these tissues. Incubation of human sperm with oviduct explants induced sperm protein S-nitrosylation resembling that induced by NO donors and GSNO. Progesterone (a product of cumulus cells) also mobilises stored Ca2+ in human sperm. Pre-treatment of sperm with NO greatly enhanced the effect of progesterone on [Ca2+]i, resulting in a prolonged increase in flagellar excursion. We conclude that NO regulates mobilisation of stored Ca2+ in human sperm by protein S-nitrosylation, that this action is synergistic with that of progesterone and that this synergism is potentially highly significant in gamete interactions leading to fertilisation.

Paper 2 – Investigation of the way sperm swim in a viscous environment is actually key to our understanding not only of how sperm swim in the hyaluronate matrix of the cumulus mass, but also how and which sperm can navigate through the female tract to the site of fertilisation. This work is moving towards fundamental changes in the dogma around sperm energetics and chemotaxis. We have an active programme in this area, a key recent paper being:

Smith DJ, Gaffney EA, Gadêlha H, Kapur N, Kirkman-Brown JC. Bend propagation in the flagella of migrating human sperm, and its modulation by viscosity. Cell Motil Cytoskeleton. 2009 Apr;66(4):220-36. PubMed PMID: 19243024.

Abstract
A pre-requisite for sexual reproduction is successful unification of the male and female gametes; in externally-fertilising echinoderms the male gamete is brought into close proximity to the female gamete through chemotaxis, the associated signalling and flagellar beat

changes being elegantly characterised in several species. In the human, sperm traverse a relatively high-viscosity mucus coating the tract surfaces, there being a tantalising possible role for chemotaxis. To understand human sperm migration and guidance, studies must therefore employ similar viscous in vitro environments. High frame rate digital imaging is used for the first time to characterise the flagellar movement of migrating sperm in low and high viscosities. While qualitative features have been reported previously, we show in precise spatial and temporal detail waveform evolution along the flagellum. In low viscosity the flagellum continuously moves out of the focal plane, compromising the measurement of true curvature, nonetheless the presence of torsion can be inferred. In high viscosities curvature can be accurately determined and we show how waves propagate at approximately constant speed. Progressing waves increase in curvature approximately linearly except for a sharper increase over a distance approximately 20–27 μm from the head/midpiece junction. Curvature modulation, likely influenced by the outer dense fibres, creates the characteristic waveforms of high viscosity swimming, with remarkably effective cell progression against greatly increased resistance, even in high viscosity liquids. Assessment of motility in physiological viscosities will be essential in future basic research, studies of chemotaxis and novel diagnostics.

It was also stated in the research report in 2008:
'It is thought that many cases of infertility relate to as yet unknown causes to do with the interaction and recognition of gametes. Although some work has been done in the mouse, this is a poor model for the human, as many of the proteins shown to be important in the mouse fertilisation system do not exist in humans. Hence to properly understand the events underlying human fertilisation it is critical to examine them with human cells.

'Upon being ovulated the human oocyte is surrounded by a large mass of steroid producing cells (the cumulus oophorus). Work already undertaken in our laboratory has carefully examined the effects of the major steroid produced by this system (progesterone) upon human sperm and their calcium signalling. However, to further understand the events occurring as the sperm penetrates through the cumulus mass we need to examine the living and complex system.

'It is also important to resolve whether acrosome reaction, a prerequisite for fertilisation, occurs as cells pass through the cumulus or when they reach the zona glycoprotein coat of the egg. The site of this event will help us more clearly identify the likely major agonist and whether the role of other agonists is to remove sperm by making them respond 'too early'.

'We are in a unique situation where we can combine experience and expertise in calcium imaging within male human gametes with an accurate examination of how they interact with the oocyte. A further advantage is that with dual-labelling of the gametes we also hope to be able to examine whether contact with sperm induces signalling events within the cumulus, that pass to the oocyte signalling imminent fertilisation. Or whether as the egg is fertilised signals propagate back out through the cumulus - as potentially this may also act to prevent polyspermy.'

B) Research undertaken to date.
See above and our publications list below.

C) Results
See above and publications listed below

D) If progress was slower than anticipated, the reasons for this.
See above and publications listed below

It was also stated in the research report in 2008
'Progress on constituent parts of the research project has been rapid however we have as yet not performed experiments using licensed material. This is because further progress is required to effectively characterise proteins involved in sperm-zona interaction. Only then can experiments involving the creation of embryos, i.e. licensable activity, be undertaken in which variables will be limited and controlled such that effective conclusions can be drawn from the results. This will ensure that best use is made of available licensed material.

We envisage that in the near future we will be able to generate far better and more detailed data in our experiments. This has meant that in the last year, deferral of experiments creating embryos was desirable.'

E) If work originally proposed was not carried out, the reason for this.
See above, also we now envisage moving on, with our more-complete set of knowledge and questions to undertake the work in the study.

F) Publications which have arisen from work under the licence.
Publications across this area from our group since 2006:

Costello S, Michelangeli F, Nash K, Lefievre L, Morris J, Machado-Oliveira G, Barratt C, Kirkman-Brown J, Publicover S. Ca2+ stores in sperm: their identities and functions. Reproduction. 2009 Jun 19. PubMed PMID:19542252.

Smith DJ, Gaffney EA, Gadêlha H, Kapur N, Kirkman-Brown JC. Bend propagation in the flagella of migrating human sperm, and its modulation by viscosity. Cell Motil Cytoskeleton. 2009 Apr;66(4):220-36. PubMed PMID: 19243024.

Machado-Oliveira G, Lefièvre L, Ford C, Herrero MB, Barratt C, Connolly TJ, Nash K, Morales-Garcia A, Kirkman-Brown J, Publicover S. Mobilisation of Ca2+ stores and flagellar regulation in human sperm by S-nitrosylation: a role for NO synthesised in the female reproductive tract. Development. 2008 Nov;135(22):3677-86. Epub 2008 Oct 8. PubMed PMID: 18842814.

Lefièvre L, Chen Y, Conner SJ, Scott JL, Publicover SJ, Ford WC, Barratt CL. Human spermatozoa contain multiple targets for protein S-nitrosylation: an alternative mechanism of the modulation of sperm function by nitric oxide? Proteomics. 2007 Sep;7(17):3066-84. PubMed PMID: 17683036.

Jiménez-González MC, Gu Y, Kirkman-Brown J, Barratt CL, Publicover S. Patch-clamp 'mapping' of ion channel activity in human sperm reveals regionalisation and co-localisation into mixed clusters. J Cell Physiol. 2007 Dec;213(3):801-8. PubMed PMID: 17516540.

Ellis PJ, Furlong RA, Conner SJ, Kirkman-Brown J, Afnan M, Barratt C, Griffin DK, Affara NA. Coordinated transcriptional regulation patterns associated with infertility phenotypes in men. J Med Genet. 2007 Aug;44(8):498-508. Epub 2007 May 11. PubMed PMID: 17496197.

Meng F, To W, Kirkman-Brown J, Kumar P, Gu Y. Calcium oscillations induced by ATP in human umbilical cord smooth muscle cells. J Cell Physiol. 2007 Oct;213(1):79-87. PubMed PMID: 17477379.

Bedu-Addo K, Barratt CL, Kirkman-Brown JC, Publicover SJ. Patterns of [Ca2+](i) mobilization and cell response in human spermatozoa exposed to progesterone. Dev Biol. 2007 Feb 1;302(1):324-32. Epub 2006 Sep 28. PubMed PMID: 17054937.

Harper CV, Barratt CL, Publicover SJ, Kirkman-Brown JC. Kinetics of the progesterone-induced acrosome reaction and its relation to intracellular calcium responses in individual human spermatozoa. Biol Reprod. 2006 Dec;75(6):933-9. Epub 2006 Sep 6. PubMed PMID: 16957023.

Björndahl L, Kirkman-Brown J, Hart G, Rattle S, Barratt CL. Development of a novel home sperm test. Hum Reprod. 2006 Jan;21(1):145-9. Epub 2005 Nov 2. PubMed PMID: 16267078.

Key Reviews:

Bedu-Addo K, Costello S, Harper C, Machado-Oliveira G, Lefievre L, Ford C, Barratt C, Publicover S. Mobilisation of stored calcium in the neck region of human sperm--a mechanism for regulation of flagellar activity. Int J Dev Biol. 2008;52(5-6):615-26. Review. PubMed PMID: 18649275.

Conner SJ, Lefièvre L, Kirkman-Brown J, Michelangeli F, Jimenez-Gonzalez C, Machado-Oliveira GS, Pixton KL, Brewis IA, Barratt CL, Publicover SJ. Understanding the physiology of pre-fertilisation events in the human spermatozoa--a necessary prerequisite to developing rational therapy. Soc Reprod Fertil Suppl. 2007;63:237-55. Review. PubMed PMID: 17566277.

Lefièvre L, Bedu-Addo K, Conner SJ, Machado-Oliveira GS, Chen Y, Kirkman-Brown JC, Afnan MA, Publicover SJ, Ford WC, Barratt CL. Counting sperm does not add up any more: time for a new equation? Reproduction. 2007 Apr;133(4):675-84. Review. PubMed PMID: 17504912.

Correia JN, Conner SJ, Kirkman-Brown JC. Non-genomic steroid actions in human spermatozoa. "Persistent tickling from a laden environment". Semin Reprod Med. 2007 May;25(3):208-19. Review. PubMed PMID: 17447210.

Barratt CL, Kirkman-Brown J. Man-made versus female-made environment—will the real capacitation please stand up? Hum Reprod Update. 2006 Jan-Feb;12(1):1-2. PubMed PMID: 16354709.

Peer review comments

The peer reviewer concurred with the applicants indication of which defined purposes the project addressed, i.e

- promoting advances in the treatment of infertility (*Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(a))*
- increasing knowledge about the causes of miscarriages (*Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(c))*
- developing methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation (*Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(e))*
- increasing knowledge about the development of embryos (*Human Fertilisation and Embryology (Research Purposes) Regulations 2001 s2(a))*

The peer reviewer considered the application contained sufficient information to assess the work carried out in the preceding year.

The peer reviewer considered that reasonable progress had been made in the previous year and stated: 'The previously proposed programme of work has been somewhat diverted, since the group felt that the available data on human oocyte and sperm activation and interaction was inadequate, requiring expansion prior to embarking upon meaningful further research that requires a licence. The quality and relevance of research emerging from this group that has been preferentially performed is demonstrated by the large number of high profile publications and the very thorough and detailed account of the current status and logical progression for future research detailed in the application.'

The peer reviewer considered that the applicants had not confined their activities to the work described in the previous application for acceptable reasons and stated: No licensed activities have been conducted for this project so far, for the reasons described above; therefore the number of embryos/gametes used has not been in accordance with the original proposal.

The peer reviewer considered that the use of embryos was justified in the future research work and that the plans for the future are likely to achieve decisive results towards the aims of the application. The peer reviewer stated: 'This group has thoroughly considered the studies required to advance knowledge and research in this field and has an in depth knowledge of the literature. The use of human embryos for the proposed project is justified in order to test the downstream effects of specific oocyte and sperm activation protocols on subsequent embryo viability. The outstanding performance of this group will ensure that they are best placed to achieve decisive results in their intended research.'

The peer reviewer also considered that creation of embryos in the project for research purposes was justified and stated: 'To test definitively the success of sperm or oocyte activation for improved in vitro fertilization it is necessary that the downstream influences on embryo development are monitored. This group is extremely well placed to achieve the best possible results from the proposed research.'

The peer reviewer noted cell nuclear replacement and stem cell derivation were not proposed activities in the application

The peer reviewer finally noted 'This is a very thorough and informative application from a leading group in the field. The group is very well placed to achieve success in the proposed research.'

The peer reviewer recommended acceptance of the application in its current form

Issues for consideration

The peer reviewer and inspectorate note that the applicants have not confined their research to the original project proposal but accept that the research performed was not licensable and was considered by the applicants to be essential to future licensed research experimentation. Furthermore the applicants consider that the research performed provided key knowledge and experience which allows them to now progress with the research proposed in this application, which includes that research previously planned on this research licence.

The inspectorate note that while local ethical approval is in place for the research work common to the licence application in 2006 and this renewal application, the addition work

proposed in this application, e.g. IVM, embryo culture for 7 days and aneuploidy assessment, has not been approved by the local ethics committee, albeit an application is in progress. The PR assured the inspectorate that the new research work proposed will not, and lawfully can not, be performed until ethics committee approval for it is place. He is though seeking to include the additional research work in this licence reapplication so that the HFEA licensing process and ethics review process can progress in parallel. The inspectorate note the PR's obvious commitment to performing research within the regulatory and ethical framework in a compliant manner and his assertion that he would consider it unlawful and unethical to perform the additional research proposed without ethical approval. The inspectorate recommends ethical approval is obtained for the additional research work at the earliest opportunity and that it is not performed until that ethical approval is in place.
Executive recommendations for Licence Committee
The Inspectorate recommend that the Licence Committee approve this renewal application but that renewal be subject to a condition on the licence that no research be performed until appropriate ethical approval is in place and has been supplied to the HFEA Executive.
Areas not covered on this inspection
All covered

Report compiled by:

Name Andrew Leonard

Designation HFEA inspector

Date 3rd October 2009

Appendix A: Centre Staff interviewed

PR, NL and Research Associate

Appendix B: Licence history

Licence	Status	Type	Active From	Expiry Date
R0173/1/a	Active	Research Project	01/01/2006	31/12/2009

Appendix C:

RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number 0119

Name of PR Jackson Kirkman-Brown

Date of Inspection 19th August 2009

Date of Response 27th October 2009

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

We are revising our incident reporting SOP to reflect that this should now be undertaken via email to incident.reporting@hfea.gov.uk using the latest form available online.

If approval is granted for the additional growing of embryos on through to blastocyst we will immediately apply for the relevant ethical approval with appropriate patient information. In terms of patient information, the new sheets would also include a named contact individual for withdrawal of consent.

Since the date of the inspection all fire-damaged chemicals etc have been disposed of and new clean replacements are in place.

Both the PR and new NL are grateful for the helpful guidance from the inspection team around various regulatory issues during the inspection.

2. Correction of factual inaccuracies

Please let us know of any factual corrections that you believe need to be made (NB we will make any alterations to the report where there are factual inaccuracies. Any other comments about the inspection report will be appended to the report).

None

Research Licence Committee Meeting

18 November 2009
21 Bloomsbury Street London WC1B 3HF

MINUTES Item 2

Research Project RO173: Human Gamete Activation, Interaction and Signalling (0209) (0119)
Research Licence Renewal Inspection Report

Members of the Committee:	In Attendance:
Emily Jackson, Lay Member – Chair	Joanne Anton – Minute taker
Richard Harries, Lay Member	Maria Cesay - Observer
Neva Haites, Professor of Medical Genetics, University of Aberdeen	Providing Legal Advice to the Committee: Graham Miles, Morgan Cole

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:

- Inspection report
- Anonymised peer review
- Renewal application form
- Email for PR submitting the renewal application form
- CV for the new Licence Holder – Dr Sarah Connor
- Licence Committee minutes initially for project R0160 on 25th November 2005, then for the same project, renumbered to R0173, on 16th September 2008
- Publications x 2
- no papers were tabled.

1. The Committee noted the inspection report findings and the Person Responsible's response, as appended at page 31 of the inspection report. The Committee noted the Person Responsible's intention to revise their incident reporting SOP to reflect that this should now be undertaken via email. The Committee also noted that if approval is granted for the additional growing of embryos on through to blastocyst; that the Person Responsible will immediately apply for ethical approval with appropriate information. The Committee also noted that since the date of inspection all fire-damaged chemicals and materials have been disposed of and have been replaced.

2. The Committee noted the following 3 suggestions for best practice as outlined in the inspection report which have not yet been addressed by the Person Responsible:

- The personnel records for the Research Associate were reviewed. Evidence of induction activities and training were observed, however it was noted that the worker had not undergone the hospital trust induction course. It is suggested that the Research Associate undergo this course to facilitate his ability to perform effectively in the hospital environment.
- It is suggested that the research tissue culture incubator in Centre 0209 is validated before use in research experiments
- The patient information clearly states that withdrawal of consent can be made at any time up to egg collection without it affecting future treatment. It is suggested that the patient information sheet provides contact details for a named individual through whom this can be achieved, as well as stating that patient information can be discussed with any member of staff.

The Committee's Decision

3. The Committee applied the licensing decision tree in consideration of the application.

4. The Committee identified the activities to be authorised by the licence as the storage of oocytes; the storage of oocytes in ovarian tissue; the creation of embryos for use in research and the use of donated embryos in research. The Committee agreed that they were satisfied that these activities are not prohibited under the Human Fertilisation and Embryology Act 1990 (as amended).

5. In considering stage 18(a) of the decision tree which requires that the activity is necessary and desirable for the purposes specified in paragraph 3A(2) of Schedule 2 to the Act, the Committee considered the purpose of the research project in relation to these requirements.

6. The Committee agreed that the activities are necessary or desirable for the following specified purposes:

   - Promoting advances in the treatment of infertility *Human Fertilisation and Embryology Act 1990, as amended Sch 2 3A (2)(d))*
   - Increasing knowledge about the causes of miscarriages *Human Fertilisation and Embryology Act 1990, as amended Sch 2 3A (2)(e)*
   - Developing methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation *Human Fertilisation and Embryology Act 1990as amended Sch 2 3A (2)(g)*

- to increase knowledge about the development of embryos
  Human Fertilisation and Embryology Act 1990, as amended Sch 2 3A2(h)

7. The Committee agreed that the proposed use of human embryos is necessary for the purposes of the research in order to test the downstream effects of specific oocyte and sperm activation protocols on subsequent embryo viability.

8. The Committee noted that the applicant has not provided evidence of ethics committee approval, as required by Stage 18g(i) of the licensing decision tree.

9. The Committee decided to grant a 3 year licence subject to an additional licence condition: that no research must be performed until appropriate ethics committee approval is in place and evidence of this has been provided to the HFEA executive.

Signed..... Date 9.12.09
Emily Jackson (Chair)