Research Licence Interim Inspection Report

Project Title	Investigation into the role of sperm PLCzeta in human oocyte activation
Centre Name	IVF Wales
Centre Number	0049
Research licence Number	R0161
Centre Address	Department of Obstetrics and Gynaecology School of Medicine, Cardiff University, Heath Park, Cardiff, Wales CF14 4XN
Treatment centres donating to this research project	0049
Inspection date	4th March 2008
Licence Committee Date	18th June 2008
Inspector(s)	Andrew Leonard
Fee Paid - date	Fee paid
Person Responsible	Professor Karl Swann
Nominal Licensee	Nazar Amso
Licence expiry date	31/12/2009

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 1 March 2007 and 28 Feb 2007.

Brief Description of the Projects

R0161, **Investigation into the role of sperm PLCzeta in human oocyte activation**

Lay Summary
At fertilisation the sperm stimulates the egg to begin cell divisions and development. Studies in animals have suggested that the way the sperm does this is by introducing a special protein into the egg during the process of sperm-egg fusion. This protein is called PLCzeta. In our studies, we have injected messenger RNA for this protein into eggs that have failed to fertilise during IVF or ICSI. The RNA is then translated into PLCzeta protein within the egg. We have shown that this treatment can stimulate unfertilised human eggs to begin development. The biochemical effects of this protein inside the egg mimic those induced by the sperm at fertilisation. These results show us that PLCzeta can mimic the stimulatory effect of a sperm on egg development. This supports our proposal that the presence of PLCzeta in sperm is essential for normal fertilisation and development to take place. After injecting PLCzeta we found that some embryos could develop in culture up to blastocyst stage. Since these embryos were not fertilised by a sperm they are called parthenogenetic. Such parthenogenetic embryos are not viable and so cannot develop much further. However, the generation of parthenogenetic blastocysts from unused eggs may be useful in future as a source of embryonic stem cells that would not require the use of viable human embryos.

		R0161
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

The interim inspection was of Project R0161, Investigation into the role of sperm PLCzeta in human oocyte activation. Project R0161, previously R0147, has had a research licence to use oocytes to develop parthenogenic embryos since September 2003. The current licence is due to expire on 31 December 2009.

Project R0161 investigates the role of PLCzeta in human oocyte activation its objectives being:

i) to establish the effectiveness of PLCzeta in causing Ca2+ oscillations in human oocytes
ii) to establish the potential and optimal concentration of PLCzeta that is required to stimulate efficient preimplantation development of human oocytes
iii) to establish the relationship between the pattern of Ca2+ oscillations generated in human oocytes by PLCzeta and the degree of subsequent preimplantation development

It should be noted that expression of the sperm protein PLCzeta in human oocytes stimulates their activation and parthenogenic development up to the blastocyst stage. Further development can not occur due to the absence of male genetic material.

Research over the last 18 months has indicated that different patterns of Ca2+ oscillations are generated in human oocytes and that the pattern of oscillation depends upon the amount of PLCzeta protein that is expressed after injection of PLCzeta mRNA into the oocytes. Oocyte activation and embryo development also depend upon the expression level of PLCzeta; either too little or too much PLCzeta expression is not consistent with embryo development. Development to the blastocyst stage is only seen when PLCzeta expression in the oocyte is within a relatively narrow range. These observations are consistent with similar studies carried out with human PLCzeta luciferase in mouse oocytes (Yu et al. Human Reproduction. (2007) 23, 365-373).

The rate of progress on PLCzeta RNA injected oocytes was as expected however the centre also want to inject recombinant PLCzeta protein into oocytes. This work was delayed due to difficulties in producing the recombinant protein; experiments will be started in the next year. This will allow the centre to complete and publish work related to objective (i). Some work related to objectives (ii) and (iii) has yet to be completed, but will be performed when experiments are restarted using recombinant human PLCzeta protein. It is anticipated that recombinant protein will increase the success rate of parthenogenic development to the blastocyst stage. It will also facilitate investigation of changes in cytoplasmic bulk movements in parthenogenic embryos using digital particle image velocitometry. These movements appear to be related to the calcium oscillations induced in human oocytes by PKCzeta.

The researchers have appropriate experience and are well qualified to continue the programme of research. The proposed premises and equipment are appropriate and procedures are in place to ensure that patients are treated respectfully and their consent is not breached. The donation to research procedure complies with the Code of Practice 7th edition. Oocyte usage in project R0161 this year was reported in the pre-inspection questionnaire. In summary, 34 fresh and 87 failed to fertilize oocytes were used, most having been donated from patients undergoing IVF/ICSI cycles at centre 0049. Some oocytes are donated from follicular reduction procedures performed on overstimulated IUI patients. No

embryos are donated to the project. Usage in the next year is projected at 50 fresh oocytes and 200 failed to fertilise oocytes.

There are minor points for consideration by Licence Committee:

- The Inspectorate noted that annual portable appliance testing (PAT) for electrical safety was one month overdue (last completed in December 2006). Code of Practice, 7th edition, Standards.6.3.2 includes 'The Centre shall provide a safe working environment for all staff'. That PAT testing has not been performed within the scheduled time is contrary to this Standard. The PR was advised to ensure that the Contractor to Cardiff University Medical School who provides the PAT service, should visit the Centre as soon as possible. The PR contacted the Health and Safety Office on the day of inspection to organise this.
- No procedure is in place for retrieval and appropriate disposal of oocytes in the event that patients withdraw consent. This is potentially contrary to the spirit of Code of Practice, 7th edition, Standards S.8.1.3: The Centre shall also have written procedures: (a) to ensure that embryos are only used in accordance with the Donors' consent. It is accepted by the inspectorate that oocytes are used within a few hours of transfer to the researchers, if not sooner, and consent is provided a long time before, ie prior to the treatment cycle starting. Thus it is highly unlikely that patients would choose to withdraw consent and there be enough to stop experimenting on their oocytes. The PR should however consider the possibility that this might occur and should document a procedure as to how IVF Wales and project R0161 staff could respond in these circumstances to ensure that oocytes are appropriately disposed of if patient consent for research is unexpectedly withdrawn.
- The patient information sheet was last updated in August 2006 and the consent form in May 2005. Thus both information sheet and consent form are 'out of date' as such documents should be subjected to review at least annually (as required by Code of Practice, 7th edition, S.5.2.5). The consent form also refers to an earlier version of the information sheet than that supplied and includes the provision for a researcher to sign and receive a copy. The involvement of the researcher in signing and receiving a copy of the consent form would reveal the patients identity, contrary to the Centre's described working practices and their Local Ethical Committee's requirement that no patient identifying information passes to the researchers. The letter sent to a participant's GP is also not version controlled or production or review dated, and contains inaccurate information as it refers to the Cardiff Assisted Reproduction Unit as opposed to IVF Wales, Centre 0049's new name. Some of these issues were discussed with the PR on inspection and he accepted that the information and consent form needed reviewing and said that this would be done prior to resumption of research work when PLCzeta protein is available. The inspectorate recommend that the Centre review all patient information and consent forms to correct errors highlighted above, and any others they may find, and do so at least annually to ensure they remain up to date.
- Patient information does not seem to provide contact details for somebody 'independent' of the research with whom patients can discuss donation. It provides details for a consultant clinician at Centre 0049 (2 sessions/week) who is also the Nominal Licensee of the research project. This situation has been in place for some years without comment from the HFEA. The inspectorate accept that the Nominal Licensee is 'distanced' from

the research project and in many ways is suitable for the advisory role, however it not clear the situation is strictly compliant with:

1. Code of Practice, 7th Edition, Standards 8.4.1: Where embryos are used for research, the Centre shall ensure that clinical and research roles are separated, so that individuals involved in advising Patients regarding clinical decisions about their licensed treatment are not involved in the research project to which Patients are considering donating embryos.
2. Code of Practice, 7th Edition, Standards 8.4.2: Where embryos are used for research, the Centre shall ensure that: (a) a designated individual who is not directly involved in the Donors' treatment is available to discuss the project of research and the possibility of donating material to the project with the Donors.

The centre should consider the above standards and ensure they are compliant with them

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- That patient information should provide for patients to vary or withdraw their consent and provide contact details for a named individual through whom this can be achieved, to be compliant with Code of Practice, 7th edition, S.8.3.1 and G.5.13.1(g). The project's patient information currently does not do this.
- That the consent form should specify precisely that the patient is consenting for the donation of their failed to fertilise oocytes to research project R0161, or words to this effect, in the case of IVF patients. This will ensure informed consent is taken appropriately. In the opinion of the Inspectorate, the project's existing consent form does not make this clear enough.

The inspectorate recommend continuation of the research licence for project R0161.

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 R0177

Principal investigator	Professor Karl Swann
Scientists	2
Collaborators	1
Support staff (receptionists, record managers, quality and risk managers etc)	Staff at centre 0049 recruit patients to the research project

Highlighted areas of firm compliance
The research centre is on the 5th floor of the University Hospital Wales within a zone of the building assigned to academic departments from the Cardiff University Medical School. The zone provides facilities for multiple research groups, including the research centre and one of their collaborators. The facilities and staff in this zone are part of Cardiff University, who provide Health and Safety, Human Resources and other support. Oocytes are donated to the project from IVF Wales (HFEA centre 0049) which is based on the 1st floor of the same hospital. The PR is the Project Head and has been PR since 2003. The PR in conversation showed an understanding of the regulatory requirements of the HFEA; the PR is not the PR of a Treatment and Storage Licence. The PR is a research Professor of Cardiff University with many years of research experience. The PR is assisted by a post-doctoral research scientist on the project. This post was vacant for some months in the last year but was filled in July 2007 with a candidate the PR considers is appropriate for the role. The Head of Embryology at Centre 0049, who has a role in coordinating between the research project and Centre 0049 but NO role in carrying out the research, was placed on the licence after the last inspection (Licence Committee held 13th Sept 2006). Resource management and project coordination is achieved through meetings between the PR and the post-doc, held as required. Meetings are also held with the embryologists and nurses of Centre 0049 from where oocytes are obtained. Minutes were seen of these meetings. Research data is reported back to Centre 0049 in research seminars by the PR and his post-doctoral worker. The PR liaises with his collaborator at Cardiff University Medical School regularly about the

project and is in daily contact. Collaboration is also required with groups in Oxford and Cambridge Universities; this is achieved by email and visits. A joint grant application to the Wellcome Trust has been submitted, indicating these communication pathways operate effectively.

BBSRC funding for the project is in place until the end of the current licence, by which time current objectives are expected to have been met. Funding for continuation of the research project has been applied for from the Wellcome Trust as the PR considers the project has potential for application in the IVF sector which requires further work.

Experiments undertaken are monitored via a computer database kept by the PR which was shown to the inspectorate. Experiments and their results are also documented in laboratory notebooks kept within the licensed premises. Research activities are controlled by written procedures, supplied prior to inspection, which ensure a consistent approach and that patient consent is collected appropriately. The centre has an incident reporting procedure which is compliant with the Code of Practise, 7th edition.

Issues for consideration
None
Executive recommendations for Licence Committee
None
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
Manipulation of oocytes is carried out in two licensed laboratories within a medical school zone of the University Hospital of Wales. The facilities and staff in this zone are part of Cardiff University Medical School, who provide Health and Safety support. The laboratories are secure and restricted to licensed personnel. Licensed material only leaves the premises when non-viable (e.g. after fixation for staining and cell counting). Project records are stored in one licensed laboratory (laboratory books) and also in the PR's office (computer spreadsheet database) which is also part of the licensed premises. This office was also secure and is locked whenever the PR leaves it. Oocytes donated to research are transferred from Centre 0049 to the research centre in a portable incubator by the researchers. A dedicated incubator is used for short term culture storage of oocytes/parthenogenic embryos. No longer-term storage facilities are required as material is processed from fresh to a non-viable state. Viable licensed material is not subjected to long term storage. The laboratories were appropriately equipped, with a dedicated incubator in one, and two specialised microscopes with micromanipulation and microinjection equipment, one in each laboratory. Apparatus is on service contracts in general, however the microscopes and micromanipulation and microinjection equipment are serviced and repaired by the PR, who has considerable experience of this equipment; the PR considers that they can service and repair the equipment more effectively and at lower cost than external service engineers
Issues for consideration
The Inspectorate noted that annual portable appliance testing (PAT) for electrical safety was one month overdue (last completed in December 2006). Code of Practice, 7th edition, Standards.6.3.2 includes 'The Centre shall provide a safe working environment for all staff'. . That PAT testing has not been performed within the scheduled time is contrary to this Standard. The PR was advised to ensure that the Contractor to Cardiff University Medical School who provides the PAT service should visit the Centre as soon as possible. The PR contacted the Health and Safety Office on the day of inspection to organise this.
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
If the patient consents, oocytes which fail to fertilise during IVF/ICSI treatment and those recovered from follicular reduction during IUI treatment are donated to research. Work on this project has been suspended for 9 months due to problems with the production and supply of recombinant PLCzeta. In that time, Centre 0049 has changed its name and location within the University Hospital of Wales. The procedures for donation noted on the last inspection in 2006 are though all still in place and the PR has ensured that transfer of oocytes from Centre 0049 to the research project laboratory can be easily accomplished. Donors are recruited from Centre 0049 by a dedicated nurse. Written and verbal information regarding the research project is given to patients when they attend the patient information session on a Wednesday evening or at their first consultation for treatment scheduling. At the next visit several weeks later, the dedicated nurse explains the research patient information, if required, and assists the patients in completing the treatment consent forms and the research consent if they decide to donate to research. At subsequent visits a checklist is used to check if patients are aware of the project and if they would like to donate to research. Further information can be obtained by patients from the Nominal Licensee of the research project (a clinical consultant at Centre 0049) whose contact details are on the patient information sheet, or from embryologists or nurses at Centre 0049. These staff are briefed on the research project progress by the researchers in seminars to IVF Wales staff. Research progress is also included in articles in the IVF Wales patients newsletter. Research consent is obtained before the treatment cycle is started, i.e. long before egg collection, but several weeks after information is first supplied. Thus patients have adequate time in which to make their decision and to obtain further information, so consent is informed and considered. These features also limit the possibility of coercion. The inspectorate consider coercion is unlikely to occur for further reasons: Researchers do not encounter the patients; Patient information is balanced and non-coercive; No patient complaints have been received on this issue; The staff of IVF Wales are professional and patient feedback suggests sensitive to their patients needs and respectful of their dignity. Prior to transfer to the researchers, the embryologist releasing the oocytes checks that research consent has been signed by the patients and signs to this effect in a 'research donated oocytes' book held within Centre 0049, evidenced on inspection. The embryologist also notes the anonymised number by which the oocytes are henceforth identified as well as including a patient details sticker. The receiving researcher signs for receipt and verifies the oocyte identifier and that research consent was confirmed by the embryologist. The oocytes

are then transferred from the clinical laboratory to the research laboratory in a portable incubator by the researchers.

Patient records are all retained by Centre 0049 and no patient identifying information is transferred to the research centre. On arrival in the laboratory the oocytes are cultured in the incubator while they are logged onto the computer spreadsheet database. They are then used in experiments within the next few hours.

Five sets of material were traced back from the laboratory experimental database to the patient notes, via the 'research donated oocytes' book. Notes for two patients were in long term storage. Notes for three patients were readily available and were inspected. Appropriate consents were present in each case.

Issues for consideration

No procedure is in place for retrieval and appropriate disposal of oocytes in the event that patients withdraw consent. This is potentially contrary to the spirit of Code of Practice, 7th edition, Standards S.8.1.3: The Centre shall also have written procedures: (a) to ensure that embryos are only used in accordance with the Donors' consent. It is accepted by the inspectorate that oocytes are used within a few hours of transfer, if not sooner, and consent is provided prior to the treatment cycle starting. Thus it is highly unlikely that patients would choose to withdraw consent and there be enough to stop experimenting on their oocytes. The PR should however consider the possibility that this might occur and should document a procedure as to how IVF Wales and project R0161 staff could respond in these circumstances to ensure that oocytes are appropriately disposed of if patient consent for research is unexpectedly withdrawn.

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 R0161 were reviewed by the inspectorate. They were compliant with the Code of Practice, 7th edition, except for the specific issues raised below. They clearly explain that for IVF patients, only failed to fertilise oocytes would be considered for use in project R0161. The consent for research is checked by nursing staff and embryologists several times during the treatment cycle before oocytes are donated to research; a checklist ensures the consent is checked at the time of donation by the embryologist.
Issues for consideration
1) The patient information sheet was last updated in August 2006 and the consent form in May 2005. Thus both information sheet and consent form are 'out of date' as such documents should be subjected to review at least annually (as required by Code of Practice, 7th edition, S.5.2.5). The consent form also refers to an earlier version of the information sheet than that supplied and includes the provision for a researcher to sign and receive a copy. The involvement of the researcher in signing the and receiving a copy of the consent form would reveal the patients identity, contrary to the Centre's described working practices and their Local Ethical Committee's requirement that no patient identifying information passes to the researchers. The letter sent to a participant's GP is also not version controlled or production or review dated, and contains inaccurate information as it refers to the Cardiff Assisted Reproduction Unit as opposed to IVF Wales, Centre 0049's new name. Some of these issues were discussed with the PR on inspection and he accepted that the information and consent form needed reviewing and said that this would be done prior to resumption of research work when PLCzeta protein is available. 2) Patient information does not seem to provide contact details for somebody independent of the research with whom patients can discuss donation. It provides details for a consultant clinician at Centre 0049 (2 sessions per week) who is also the Nominal Licensee of the research project. This situation has been in place for some years without comment from the HFEA. The inspectorate accept that the Nominal Licensee is 'distanced' from the research project and in many ways is suitable for the advisory role, however it not clear the situation is strictly compliant with: Code of Practice, 7th Edition, Standards 8.4.1: Where embryos are used for research, the Centre shall ensure that clinical and research roles are separated, so that individuals involved in advising Patients regarding clinical decisions about their licensed treatment are not involved in the research project to which Patients are considering donating

embryos.

Code of Practice, 7th Edition, Standards 8.4.2: Where embryos are used for research, the Centre shall ensure that: (a) a designated individual who is not directly involved in the Donors' treatment is available to discuss the project of research and the possibility of donating material to the project with the Donors.

The centre should consider the above standards and ensure they are compliant with them

3) Patient information does not include provision for patients to vary or withdraw their consent up to the point that oocytes are passed over to the researchers, as required by Code of Practice, 7th Edition, S.8.3.1 and G.5.13.1 (g), or provide contact details for a named individual through whom this can be achieved.

4) The consent form does not specifically state that the patients are consenting to the donation of any failed to fertilise oocytes generated in their IVF/ICSI treatment being used in Research Project R0161.

The PR accepted on inspection that the patient information and consent form needed reviewing and said that this would be done prior to resumption of research work when PLCzeta protein is available.

Executive recommendations for Licence Committee

1) That the Licence Committee consider the situation outlined in consideration (2) above, whereby the patient information provides only contact details for a consultant clinician at Centre 0049 from whom further information can be obtained, and that this person is also the Nominal Licensee of the research project.

That the Licence Committee consider endorsing the following recommendations related to the items for consideration above:

Consideration 1): That the Centre review all patient information and consent forms to correct errors highlighted above, and any others they may find, and do so at least annually to ensure they remain up to date.

Consideration 3): That patient information should provide for patients to vary or withdraw their consent and provide contact details for a named individual through whom this can be achieved, to be compliant with Code of Practice, 7th edition, S.8.3.1 and G.5.13.1 (g).

Consideration 4): That the consent form should specify that the patient is consenting for the donation of their failed to fertilise oocytes to research project R0161, or words to this effect, to ensure informed consent is appropriately taken.

Areas not covered in by this inspection

All covered

5. Scientific practice R0161, **Investigation into the role of sperm PLCzeta in human oocyte activation**

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

Summary of:

- Peer review

Summary

Licence granted for the purposes of:
The project was originally licensed under purposes laid down in Schedule 2 of the Human Fertilisation and Embryology Act 1990;
3(2)(a) to promote advances in the treatment of infertility

And under the purposes laid down in the Human Fertilisation & Embryology (Research Purposes) Regulations 2001;
2(2)(a) increasing knowledge about the development of embryos

Usage and expected usage in next year:
Most recent data for a 12 month period: From 27/06/2006 to 31/12/2007

Eggs	**Fresh**	**Failed to fertilise**	**Frozen**
Total number received	34	87	0
Total number used	34	87	0

No embryos have been supplied to the project, fresh or frozen, and none (except parthenogenic) created in this time period.

The PR stated 'Over an 18 months period since the last report/renewal we have used about half the planned number of oocytes. This is because we reduced the use of human oocytes in the second part of 2007 because we had achieved an initial aim of the work. Further progress requires the results of laboratory preparation work that is still ongoing (see below).

Estimated usage in the next year:

Material	**Expected usage**
Fresh Eggs*	50
Frozen Eggs	0
Failed to Fertilise Eggs	200
Fresh Embryos	0
Frozen Embryos	0

Summary of audit of stored and biopsied material

No licensed materials are stored

Renewed project objectives

Human oocytes have been imaged with photon imaging cameras using luminescence and fluorescence probes. In future the Centre plan to use a higher resolution camera based on a microscope system with intermittent fluorescence excitation. Specifically, oocytes will be imaged with a cooled CCD camera system that can simultaneously record both fluorescence images (to measure intracellular Ca2+) and differential interference contrast (DIC) images, which will be analysed for mass cytoplasmic movements using DPIV (see Appendix F). The centre are currently applying for funds to upgrade the microscope system. When the imaging system is upgraded, the centre will carry out experiments involving PLCzeta injection into human oocytes, and will also analysis the developmental response of the parthenogenetic embryos up to 6 days post-injection.

Renewed objectives
The above plan is within the remit of the previously submitted renewal proposal since it essentially involves just a technical change in the way data is collected and analysed. Our objectives are the same as stated previously. To reiterate;

i) to establish the effectiveness of PLCzeta in causing Ca2+ oscillations in human oocytes
ii) to establish the potential and optimal concentration of PLCzeta that is required to stimulate efficient preimplantation development of human oocytes
iii) to establish the relationship between the pattern of Ca2+ oscillations generated in human oocytes by PLCzeta and the degree of subsequent preimplantation development

One reason for recording both intracellular Ca2+ and DIC images of oocytes is that there is evidence in mouse oocytes that Ca2+ increases are accompanied by slight movements in the ooplasm (spasm). If this occurs in human oocytes it would give us another way of assessing whether Ca2+ changes are occurring in human oocytes and how the ooplasm is responding. These potential cytoplasmic movements will be correlated with other oocyte activation events, such as pronuclear formation and cleavage. This work will help to achieve objective iii) above and will be performed in collaboration with groups at Oxford and Cambridge Universities.

Summary of research undertaken

1) *How the work undertaken relates to the objectives.*
A key aim in the research proposal is to quantify and calibrate the amount of PLCzeta required to trigger development up to the blastocyst stage. Research over the last 18 months has been designed to answer this specific question and the PR considers they have reached a reasonable conclusion with regards to how much PLCzeta protein is needed to achieve good development of human embryos. This estimate has a caveat in that it is based on data derived from PLCzeta RNA injected oocytes in which luminescence derived from co-expressed luciferase is used to estimate PLCzeta expression.

2) *Research undertaken to date.*
The human form of PLCzeta, in the form of cRNA, has been injected into human oocytes. The PLCzeta is tagged with luciferase so that levels of PLCzeta protein generated can be measured by the luminescence from the co-expressed luciferase using photon imaging

cameras. These cameras are also used to measure fluorescence derived from Ca^{2+} fluorophores to estimate cytoplasmic Ca^{2+} oscillations in the oocytes. In some experiments the parthenogenic embryo development of PLCzeta injected oocytes has been monitored after in vitro culture up to 6 days.

3) *Results*
The project has determined that different patterns of Ca^{2+} oscillations are generated in human oocytes and that the pattern of oscillations depends to some extent upon the amount of PLCzeta that is expressed. Oocyte activation and embryo development depends upon the expression level of PLCzeta; either too little, or too much PLCzeta expression is not consistent with embryo development. The development of PLCzeta injected oocytes to the blastocyst stage is only seen when PLCzeta expression is within a relatively narrow range of expression. These observations in human oocytes is consistent with similar studies carried out with human PLCzeta luciferase in mouse oocytes (Yu et al. Human Reproduction. (2007) 23, 365-373).

4) *If progress was slower than anticipated, the reasons for this.*
Progress on work injecting PLCzeta RNA was as expected. Work using PLCzeta protein was delayed due to difficulties in producing the recombinant protein. It is envisaged that these difficulties will be overcome by summer 2008

5) *If work originally proposed was not carried out, the reason for this.*
It was planned to inject recombinant PLCzeta protein as well as the cRNA encoding for PLCzeta, but it has proved technically demanding to produce pure human recombinant protein. Work using human oocytes has been postponed in the last 6 months awaiting progress in generating the recombinant protein. It is anticipated that PLCzeta experiments with recombinant protein will be started in summer/autumn 2008. This will allow the centre to complete and publish their work on PLCzeta and human oocyte activation within the current term of the licence.

Parthenogenetically produced blastocysts have yet to be analysed. This work will be performed when experiments are restarted using recombinant human PLCzeta, since it is anticipated that this will increase the success rate of development to the blastocyst stage.

6) *Please list references to any publications which have arisen from work under the licence.*
Publication in preparation 'PLCzeta dependence of preimplantation development in activated human oocytes' Yu, Y, Saunders, CM , Lai, F, Swann, K.

Peer review comments (if applicable)

Peer review was not required for this interim inspection. Peer review at last renewal (ie in 2006) can be summarised thus:

The embryos are produced in this project by parthenogenesis, i.e. in the absence of a male gamete. They have limited developmental competence and can not develop into a foetus.

The project specifically looks at the earliest events in the process of oocyte activation and as such require the production of embryos in vitro. The potential results are very important and will increase understanding of the mechanisms of fertilization, reasons for failed fertilization and methods of generating stem cells from parthenogentically activated eggs.

Plans for future work are a continuation of a long term programme of work by this group involving the use of techniques that have been established in animal models but which must be applied to human oocytes to answer the specific questions addressed by the project.

This application represents a continuation of a programme of research that has produced some significant advances in the field of fertilization and activation, fully justifying previous use of human gametes with every reason to expect that it will produce further valuable data.

Issues for consideration
NONE
Executive recommendations for Licence Committee
NONE
Areas not covered on this inspection
NONE

Report compiled by:

Name Andrew Leonard

Designation HFEA inspector

Date 1st APRIL 2008

Appendix A: Centre Staff interviewed

PR and NL

Appendix B: Licence history

Licence	Status	Type	Active From	Expiry Date
R0161/2/a	Active	Research Project	01/01/2006	31/12/2009
R0161/1/a	Expired	Research Project	10/01/2005	31/12/2006
R0147/1/a	Expired	Research Project	30/09/2003	31/08/2004

The research project number changed as the project moved to centre 0049 in November 2004.

Appendix C:

RESPONSE OF PERSON RESPONSIBLE TO INSPECTION REPORT

Centre Number 0049

Name of PR Prof Karl Swann

Date of Inspection 4th March 2008

Date of Response 28th April 2008

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

With regards to the patient information sheets, we will print off new copies of the information sheet and consent forms when the work is renewed. When this is done we shall use new forms updated with the new name of the clinic. We shall also add the name of the research project (R0161) and provide a contact person. Mr Anthony Griffiths has agreed to act as an independent person who can be contacted by patients. The contact phone number is for a secretary who can contact Mr Griffiths. Draft new versions of the patient information sheet are in an accompanying email as part of this response, with changes highlighted for illustrative purposes. Please note that I am trying to keep the information sheet to just 2 pages. We also include the new consent forms that have had the line for a researcher to sign removed. All these forms would, of course, be printed on NHS Trust head paper.

With regards to the issue of patients withdrawing their consent after oocytes have been donated, we now have discussed this scenario and have agreed that the embryologist would call the laboratory or visit it in person to convey the message as soon as possible. The scientists would then destroy the oocyte in the manner used for all oocytes and embryos. Lyndon Myles, the embryologists and researchers will all be briefed and have access to a copy of the document (attached) to indicate what steps to take. We have also indicated on the new information sheets that patients can withdraw their consent and that this should be done by contacting a member of the embryology team.

With regards to portable appliance testing (PAT) the Cardiff University Health and Safety office have been notified of the concerns. The School of Medicine will shortly implement a new policy for PAT and there will shortly designate and train individuals who will perform the tests on an annual basis.

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).

Research Licence Committee Meeting

18 June 2008
21 Bloomsbury Street London WC1B 3HF

MINUTES Item 2

Research Project R0161: Investigation into the role of sperm PLCzeta in human oocytes activation
Based at IVF Wales (0049)
Interim Inspection

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

In Attendance:
Chris O'Toole, Head of Research Regulation
Claudia Lally, Committee Secretary

Providing Legal Advice:
Mary Timms, Field Fisher Waterhouse

Conflicts of Interest: members of the Committee declared that they had no conflicts of interest in relation to this item.

The following tabled papers were considered by the Committee:

- papers for the Committee (40 pages)
- no papers were tabled.

1. The papers for this interim inspection report were presented by Andrew Leonard, HFEA Inspector. Dr Leonard informed the Committee that this project investigates the role of PLCzeta in human oocytes activation. Over the past 18 months the research has indicated that different patterns of Ca2+ oscillations are generated in human oocytes and the pattern of oscillation depends upon the amount of PLCzeta protein that is expressed after injection of PLCzeta mRNA into the oocytes. Dr Leonard reported that a total of 34 fresh and 87 failed to fertilize oocytes were used, most having been donated from patients undergoing IVF/ICSI at centre 0049.

2. Dr Leonard summarised the findings of the inspection report, drawing the Committee's attention to the points for consideration at pages 4 to 5 of the report. He reported that the Person Responsible has responded appropriately to all of

these issues and has agreed to redraft the consent forms and send them in to the Executive.

3. Dr Leonard stated that the Executive support the continuation of the centre's licence.

The Committee's Decision

4. The Committee noted that new consent forms and patient information will be drafted and submitted to the Executive who will then check the documents for clarity and specificity.

5. The Committee decided that the licence should continue with no additional conditions.

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