Research Licence Renewal 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	1st July 2009
Licence Committee Date	16th September 2009
Inspector(s)	Andrew Leonard Paula Nolan
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 26th March 2009 and the renewal inspection on 1st July 2009.

Brief Description of the Project

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

Lay Summary (unchanged from last renewal)
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 termed parthenogenic. Such parthenogenic embryos are not viable and cannot develop much further. However, the generation of parthenogenic 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

Project R0161, 'Investigation into the role of sperm PLCzeta in human oocyte activation, has been licensed since January 2005. The research is licensed for 'the creation of human embryos' to 'promote advances in the treatment of infertility' (*Human Fertilisation and Embryology Act 1990 Sch 2 3(2)(a))* and 'to increase knowledge about the development of embryos' (*Human Fertilisation and Embryology (Research Purposes) Regulations 2001 s2(a)).* The licence expires on 31st December 2009 and the centre wishes to renew it for a further 3 years for the same purpose and defined justifications.

The project uses failed to fertilise oocytes and oocytes harvested during follicular reduction in over-stimulated intrauterine insemination patients, obtained from IVF Wales (HFEA centre 0049). The research project is sited in the same hospital building as the treatment centre. Expression of the sperm protein PLCzeta in human oocytes stimulates activation and parthenogenic development as pseudo-embryos to the blastocyst stage. Further development cannot occur due to the absence of male genetic material.

The researchers have appropriate experience and are well qualified to continue the programme of research. The PR has completed the PR Entry Programme and has taken appropriate and timely action regarding the breaches, non-compliances and recommendations made in the reports on the inspections of the project in March 2008 and March 2009.

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.

Scientific practices and oocyte/pseudo-embryo usage in the project were considered by the peer reviewer to be appropriate for the objectives.

The projects renewed objectives are:

i) to establish the effectiveness of PLCzeta in causing Ca^{2+} 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, and compare this with other activation protocols.
iii) to establish the relationship between the pattern of Ca^{2+} oscillations generated in human oocytes by PLCzeta and the degree of subsequent preimplantation development.

Research carried out under the project licence has indicated that different patterns of Ca^{2+} oscillations are generated in human oocytes and that the pattern of oscillation depends upon the amount of PLCzeta protein expressed after injection of PLCzeta cRNA 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. Thus some aspects of objectives i) – iii) have been meet but further work is required to fully achieve them as well as objective iv), which has only recently been added to the project plan.

No oocytes have been used in the project since March 2008 due to:

a) The researchers wish to inject PLCzeta protein into oocytes and this requires the production recombinant human PLCzeta. Several groups worldwide are attempting this but have yet to be successful. The PR discussed how work on this goal is progressing in a local collaborator's laboratory.
b) The time taken to raise funding and to source the microscope system with which to perform Digital Particle Image Velocimetry (DPIV). The system is now in place and has been validated.
c) Recruitment to the project started again in April 2009, patients being supplied with research information and consent forms. The PR and NL are concerned however that no patients have yet consented. This was discussed on inspection and the PR discussed plans to improve recruitment. Usage in the next year is projected at 50 fresh oocytes and 200 failed to fertilise oocytes

No regulatory concerns were observed by the inspectorate on this renewal inspection. An appropriately completed application form has been sent to HFEA by the PR and the renewal licence fee has been paid. Peer review supports renewal of the project licence and the project has also been previously approved by an appropriately constituted Ethics Committee and that approval remains active for the term of the proposed licence.

It is recommended that the research licence for project R0161 be renewed for a 3 year term with no additional conditions applied.

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 R0161

Principal investigator	Professor Karl Swann
Scientists	3
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. The facilities and staff in this zone are part of Cardiff University, which provides Health and Safety, Human Resources and other support to the licensed centre. 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, has been PR since 2003 and has completed the PR entry programme. The PR showed an understanding of HFEA regulatory requirements and is not the PR of a Treatment and Storage Licence. The PR is a professor of Cardiff University with many years of research experience. The PR has advised the HFEA of staff changes and provided CVs as required. New staff follow a university and a laboratory induction course. As part of the latter course, new staff are trained to apply research methods to mouse oocytes before they are allowed to experiment using donated human oocytes. Continual professional development opportunities are provided for staff (e.g. conference attendance; research methods courses; statistics courses) and were considered by the inspectorate to fulfil Code of Practice requirements. The Head of Laboratory at Centre 0049 and an embryologist at the same centre, who has a research doctorate from the laboratory in which project R0161 is performed, are also on the research licence. The PR, as recommended in the inspection report from March 2009, has taken action to ensure effective separation of treatment and research activities. This was also

discussed with the Head of Laboratory at Centre 0049, who assured the inspectorate that working practices have been adopted which ensure effective separation. For example, the Head of Laboratory and embryologist on the research licence have no role in taking patient consent for research, nor do they process gametes or embryos from patient who have consented for research.

Resource management and project coordination is achieved through meetings between the PR and research workers, held as required. Meetings are also held with the embryologists and nurses of Centre 0049. Minutes were seen of these meetings. Research data is reported back to Centre 0049 in research seminars given by the PR, the last time one having been held in January 2009 . The PR plans to present the research project again at an all staff meeting in the near future in order to raise the projects profile and hopefully to enhance recruitment. Research progress is also included in articles in the IVF Wales patient's newsletter.

The PR regularly liaises with his collaborator at Cardiff University Medical School regarding the synthesis of recombinant PLCzeta. 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 was submitted last year and proved successful, indicating these communication pathways operate effectively.

The project is now funded by grants from the Wellcome Trust and existing funding is in place for approximately 18 months. Further grant funding will be sought for the remaining period of the proposed licence.

Research activities are controlled by written procedures provided electronically to all researchers on arrival. All procedures are validated first using mouse oocytes. The centre has an incident reporting procedure which is compliant with the Code of Practice, 7th edition.

The project has been previously approved by an appropriately constituted research ethics committee. This approval was for the term of the project and is still in operation.

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 when unoccupied. 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 of oocytes/parthenogenic embryos. No long-term storage facilities are required as material is processed from fresh to a non-viable state within 5 days of receipt; licensed material is not subjected to long term storage. The laboratories were appropriately equipped, with a dedicated incubator and two specialised microscopes with micromanipulation and microinjection equipment, one in each laboratory. One of these microscopes has recently been upgraded with dual fluorescence and differential interference contrast (DIC) optics and specialised digital recording capabilities, to enable DPIV analysis of oocyte cytoplasmic movements to be performed. 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 he can service and repair the equipment more effectively and at lower cost than external service engineers. All equipment was seen to have been subjected to portable appliance testing (PAT) for electrical safety.
Issues for consideration
None
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 a patient consents, oocytes which fail to fertilise during IVF/ICSI treatment or are recovered from follicular reduction during IUI treatment, are donated to research. The donating centre considers oocytes to have failed to fertilise if no signs of fertilisation are apparent after overnight incubation with sperm, then again on inspection in the early afternoon following the day of insemination. This definition varies from the HFEA definition, which requires no signs of fertilisation 48 hours after insemination. Thus the material used in the research project may potentially be embryonic, albeit abnormally slow in fertilising, which is one reason why the HFEA licence is required. Work has been suspended since March 2008 due to problems with the production and supply of recombinant PLCzeta and the financing, sourcing and commissioning of the required microscope system with which to perform DPIV analysis. Research recruitment in Centre 0049 started again in April 2009 as the microscope system had been installed. The PR and NL are concerned however that no patients have yet consented. his was discussed on inspection and the PR discussed plans to improve recruitment. The procedures for donation are compliant with Code of Practice requirements. The PR has ensured that the transfer of oocytes from Centre 0049 to the research laboratory is easily accomplished. Donors are recruited by the clinical nursing staff, as the research nurse left in October 2008 and has not been replaced. This may be a factor in the lack of recruitment to the project since April 2009 Written and verbal research information is given to patients either by the Head of Laboratory, when they attend an evening information seminar, or by nursing staff at the patients' first consultation. At the next visit several weeks later, the nurses explain the research information if required, and assist the patients in completing 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 on request from either the Nominal Licensee of the research project (a consultant at Centre 0049), the Head of Laboratory, the scientist/embryologist or from a scientist in the Medical School whose contact details are on the patient information sheet. 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. These features also limit the possibility of coercion. Patient information is also balanced and non-coercive and no patient complaints have been received regarding coercion. Staff on the research licence have no role in taking patient consent to research.

Prior to transfer to the researchers, the releasing embryologist checks that research consent has been signed by the patients and signs to this effect in a 'research donated oocytes' log within Centre 0049, which was seen by the inspectorate on the day of inspection. The embryologist also notes in the log the anonymised number by which the oocytes are henceforth identified and patient details. The receiving researcher signs for receipt and verifies the oocyte identifier and that research consent was confirmed by the embryologist. The oocytes are transferred from IVF Wales to the research laboratory in a portable incubator by the researchers; no patient identifying information is transferred. On arrival, the oocytes are placed in the incubator while they are logged into paper and computer based records systems. Oocytes are used in imaging experiments within 1-2 hours, the experiments lasting for a maximum of 24 hours, though they may be cultured for a further maximum of 5 days to assess their developmental potential.
Issues for consideration
None
Executive recommendations for Licence Committee
None
Areas not covered in by this inspection
Consents in patient records for research donation were not checked during this inspection since no activity has occurred since March 2008. At the inspection in March 2008, consents were seen to be in order in the three sets of patient records inspected.

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. The PR has updated the consent forms and information to account for a regulatory issue raised in the report of the March 2009 inspection. The documents were considered by the inspectorate to be now compliant with the Code of Practice, 7th edition. 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 again by the embryologist at the time of research donation.
Issues for consideration
None
Executive recommendations for Licence Committee
None.
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 the period 01/06/2008 to 01/06/2009

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

No oocytes have been supplied to the project, fresh or frozen, and no embryos have been created in this time period.

Estimated usage in the next year:

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

Summary of audit of stored and biopsied material

No licensed materials are stored

Renewed project objectives

The PR stated

'We consider that the current proposals are within the remit of previously submitted proposal since they essentially involve technical changes in the way data is collected or analysed. Our objectives are essentially the same as stated previously. To re-iterate;

i) to establish the effectiveness of PLCzeta in causing Ca^{2+} 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, and compare this with other activation protocols.
iii) to establish the relationship between the pattern of Ca^{2+} oscillations generated in human oocytes by PLCzeta and the degree of subsequent preimplantation development.

Methods

'Human oocytes are microinjected with either PLCzeta cRNA or recombinant PLCzeta protein. The microinjection may be in conjunction with a fluorescent dyes such as Oregon Green BAPTA dextran. They are then imaged on a fluorescent microscope for a period of 2-10 hours. In some cases oocytes will be injected with a luciferase tagged version of PLCzeta (Swann et al. 2009; Yu et al. 2008), and then imaged with a photon counting camera. These methods and some of our initial results were published from similar work under an HFEA licence held at University College London (Rogers et al. 2004). We have also published a paper, and written a Chapter on the methods we use on mouse oocytes (Yu et al. 2008; Swann et al. 2009). These are methods that we have used previously, and will continue to use on human oocytes. All relevant methods are in the papers that are in Appendix B.'

'There are a few areas that we will concentrate upon in the next few years. Firstly we will try to make recombinant human PLCzeta protein to inject into oocytes. This has proved technically demanding and it is worth noting that no research group has yet made an active recombinant human PLCzeta (as opposed to mouse PLCzeta). However, we are constantly working in collaboration with Prof Lai's team at the School of Medicine in Cardiff University to make the recombinant human PLCzeta. In the next few years we also intend to carry out more studies that make direct comparison between activation rates using PLCzeta in comparison with other parthenogenetic activation protocols. Other activation methods will include treatment with Ca^{2+} ionophore or Sr^{2+} ions. For this work we will follow established and published protocols where Ca^{2+} ionophore is added at 5 micromolar concentrations for a period of 5 minutes. This is followed by treatment for 3 hours in 6-DMAP (Paffoni et al. 2005). Sr^{2+} has not been widely reported to be an effective activation treatment for human oocytes but we will try treating human oocytes with medium containing 10mM Sr^{2+} for 2-4 hours, which is a method we have in regular use the laboratory for mouse eggs (Rogers et al. 2006). In some cases parthenogenetic embryos will be cultured in vitro for up to 5 days and cell numbers examined as described in Yu et al. 2008.'

'In addition to these studies we plan to use a new method of imaging the activation response to PLCzeta injection. So far we have been imaging human oocytes with photon imaging cameras using luminescence and fluorescence probes. In future we plan to make use of a microscope system that will allow us to record both fluorescence and differential interference contrast (DIC) images from human oocytes. The DIC images are then analysed by a technique called Digital Particle Image Velocimetry (DPIV). This relevant part of the project is in collaboration with Dr Adrian Thomas and Dr Chris Graham (Dept Zoology, Oxford University). The data containing images of eggs will be sent to Oxford by secure electronic transfer (FTP protocol). The methods and justification for this work is described in relevant parts of a Grant Application that we have been awarded by the Wellcome Trust (See Appendix B). After analysis for DPIV of human oocytes we will then carry out the same set of experiments involving PLCzeta injection into human oocytes, with analysis of the developmental response by following further development in culture (as above). The DPIV

related work does not require that we use recombinant PLCzeta protein so we plan to start this work soon using cRNA injection to express PLCzeta in human oocytes and trigger Ca^{2+} oscillations.'

'Following all of our experiments the oocytes/embryos will be destroyed by lysis in ethanol. The material is then disposed of in clinical waste bags.'

'Discussion
One of the key objectives is to correlate the Ca^{2+} oscillations induced by PLCzeta with activation rates and developmental outcome. This can be done by imaging Ca^{2+} levels in embryos directly and then seeing how the embryos develop in vitro. Future studies that compare PLCzeta induced activation with other methods of activation will be useful in assessing how important the Ca^{2+} oscillations are in later development. They will also provide data to show what methods of activation can be best used by other groups to activate human oocytes. If we can make, and use, recombinant human PLCzeta to trigger activation this should prove to be a simpler method for other research groups to use as part of protocols for activating human oocytes.'

'Whilst we are experienced in imaging Ca^{2+} or PLCzeta luciferase expression, these methods cannot be easily adopted by others in the field. Our previous work on human oocytes (Rogers et al. 2004) and work on mouse ocoytes (Yu et al. 2008) has emphasized the need to introduce a moderate amount of PLCzeta into eggs to obtain good development. To check the amount of PLCzeta that we introduce we are able to measure Ca^{2+} and/or image PLCzeta luciferase expression. Other groups are unlikely to have access to such equipment. It is also sometimes the case that imaging Ca^{2+} with fluorescent dyes itself impairs development. The new method that we will use to monitor Ca^{2+} transients involves DIC imaging combined with DPIV analysis of movements in the oocyte cytoplasm. This method can detect the presence of Ca^{2+} oscillations in mouse oocytes (see preliminary data in Appendix B, Wellcome Trust Grant proposal). DIC imaging and a suitable CCD camera are more readily available. So if the DPIV method can be shown to work in human oocytes, it will be a simple and less invasive way of assessing the presence of PLCzeta induced Ca^{2+} oscillations. If this method can be shown to be successful in monitoring Ca^{2+} oscillations in PLCzeta activated human oocytes, it could also be developed in the future as a method for assessing the 'best' embryo for transfer after IVF or ICSI (see Appendix B, Wellcome Trust Grant proposal).'

'References
Paffoni,A., Tiziana,A.L., Brevini,D., Somiggliana,E., Restelli,L.,Gandolfi,F., Ragni,G. (2007) In vitro development of human oocytes after parthenogenetic activation or intracytoplasmic sperm injection. Fert. & Steril. 87, 77-82

Rogers,N.T., Hobson,E., Pickering,S., Lai,F.A., Braude,P. and Swann,K. (2004) PLCζ causes Ca^{2+} oscillations and parthenogenetic activation of human oocytes. Reproduction. 128, 697-702

Rogers, N.T., Halet, G., Piao,Y., Carroll, J., Ko, M.S.H. and Swann, K. (2006) The absence of a Ca^{2+} signal during mouse egg activation can affect parthenogenetic preimplantation development, gene expression patterns, and blastocyst quality. Reproduction 132, 45-57

Swann,K. Campbell,K., Yu,Y., Saunders,C. and Lai,F.A. (2009) Use of luciferase chimera to monitor PLC□ expression in mouse eggs. Methods in Molecular Biology. 518, 17-29

Yu, Y., Saunder,C.M., Lai,F.A. and Swann, K. (2008) Preimplantation development of mouse oocytes activated by different levels of human phospholipase Czeta. Human Reproduction 23, 365-373

Summary of research undertaken

In response to questions in the renewal application, the PR stated:

'1) How the work undertaken relates to the objectives of the original application.
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. Our research over the last 5 years has been designed to answer this specific question. We consider that we have reached a reasonable conclusion with regards to how much PLCzeta protein (introduced by cRNA injection) is need to achieve good development of human embryos. This work is not yet published since we want to combine it with studies using recombinant PLCzeta protein.'

'2) Research undertaken to date.
We have injected the human form of PLCzeta in the form of cRNA into human oocytes. The PLCzeta is tagged with luciferase so that we measure how much PLCzeta protein is generated. After injection of PLCzeta-luc RNA we have used photon imaging cameras to monitor Ca^{2+} oscillations and luciferase expression. In some experiments the parthenogenetic embryo development of PLCzeta injected oocytes has also been monitored after in vitro culture.'

'3) Results
We have found 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. We have also found that 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. This data in human oocytes is consistent with similar studies carried out with human PLCzeta luciferase in mouse oocytes (Yu et al. 2008 see Appendix B).'

'4) If progress was slower than anticipated, the reasons for this
Progress on work injecting PLCzeta RNA was as expected. Work using PLCzeta protein has been delayed due to technical problems in making human recombinant PLCzeta.'

'5) If work originally proposed was not carried out, the reason for this.
We had planned to inject recombinant PLCzeta protein as well as the cRNA encoding for PLCzeta. It is proving technically demanding to produce pure human recombinant protein. We have postponed work using human oocytes in the last year since we are awaiting progress in generating the recombinant protein. We anticipate restarting PLCzeta experiments with recombinant protein soon. This will allow us to complete and publish our work on PLCzeta and human oocyte activation. We have not yet carried out the analysis of blastocysts. We intend to carry out this work when we restart experiments using recombinant human PLCzeta since we anticipate that this will give us a higher success rate of development to the blastocyst stage.'

Peer review comments (if applicable)

The peer reviewer considered they had enough information to assess the work and recommended approval of the application in the current form. They noted that the application was being made to 'to promote advances in the treatment of infertility' and 'to increase knowledge about the development of embryos.' The peer reviewer also stated:

'**PROGRESS:** Previous work by this group has concentrated on investigating the role of PLCzeta in activation of cleavage by means of injection of luciferase tagged cRNA. From

this work they concluded that cleavage is activated in response to a specific amount of protein synthesis. In order to have better control over the amount of protein available in the egg, they now wish to administer recombinant protein directly instead of RNA. This will allow them to measure more accurately the calcium fluctuations that they believe to be associated with PLCzeta-mediated activation of cleavage and subsequent development to the blastocyst stage. Unfortunately, there have been problems with synthesis of the human form of this protein, so that to date these experiments have not been possible. Collaborations are ongoing to rectify this problem.'

'**LIMITATIONS:** The aims of the project remain as described in the original application, but no embryos/gametes have so far been used for the reason outlined above.'

'**JUSTIFIABLE:** The use of human embryos is completely justified for this proposed work. Defining the amount of PLCzeta required to direct development to the blastocyst stage and understanding the mechanism of activation in relation to calcium oscillations is likely to enable improved embryo development following IVF.'

'The embryos to be created for this project will be parthenogenic, and therefore not ultimately viable. They will be created only from surplus oocytes, either those that fail to fertilise after attempted IVF or from follicle reduction during AI. For the reason described above the creation of parthenotes is thoroughly justified.'

'**ANY OTHER COMMENTS:** This is nicely presented proposal that clearly describes a programme of work that may be of immense benefit to IVF efficiency. This group has an excellent track record in the field, established collaborations and secured funding to enable the project to flourish.'

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

Report compiled by:

Name Dr. Andrew Leonard

Designation HFEA inspector

Date 15th July 2009

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 1st July 2009

Date of Response 07/08/09

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

I am satisfied that the report reflects the nature of our research project and its management. I do not wish to raise any specific issues.

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).

The report is factually accurate.

HFEA Research Licence Committee Meeting

16 September 2009

21 Bloomsbury Street London WC1B 3HF

Minutes – Item 4

IVF Wales (0049; R0161) -- Renewal

Members of the Committee:	Committee Secretary:
Emily Jackson (lay) – Chair Richard Harries (lay) Hossam Abdalla (clinician)	Kristen Veblen Legal Adviser: Stephen Hocking, Beachcroft
Apologies: David Archard (lay) Lesley Regan (clinician)	

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

The following papers were considered by the Committee:

- papers for licence committee (91 pages)
- no tabled papers

The Committee also had before it:

- HFEA Protocol for the Conduct of Licence Committee Meetings and Hearings
- 7th edition of the HFEA Code of Practice
- Human Fertilisation and Embryology Act 1990 (as amended)
- HFEA (Licence Committees and Appeals) Regulations 1991 (SI 1991/1889)
- Decision Trees for Granting and Renewing Licences and Considering Requests to Vary a Licence; and
- Guidance for members of Authority and Committees on the handling of conflicts of interest approved by the Authority on 21 January 2009.

1. The Committee considered the papers, which included the inspection report with the response of the Person Responsible (PR), application form, publications, peer review and minutes of previous Licence Committees.

2. The Committee noted that the research project, an investigation into the role of sperm PLCzeta in human oocyte activation, had been licensed since January 2005 and that the current licence would expire on 31 December 2009.

3. The Committee noted that the renewal inspection had taken place on 1 July 2009 and that the PR had responded to the report on 7 August 2009.

4. The Committee noted that the report indicated that the PR had updated the patient consent and information sheets to account for a regulatory issue raised in the report of the March 2009 inspection and that the inspectorate now considered these documents compliant. The Committee was satisfied by the PR's action on this issue.

5. The Committee also noted the peer reviewer's positive view of this project.

6. The Committee identified the activity to be authorised by a licence as creation of embryos *in vitro*. The Committee agreed that they were satisfied that this activity was not prohibited under the HFE Act 1990 (as amended).

7. The Committee decided that this activity was necessary and desirable for the following purposes:
   - Promoting advances in the treatment of infertility
     HFE Act 1990 (as amended) 2 3(2)(a)
   - increasing knowledge about the development of embryos
     HFE Regulations 2001 2(a)

8. The Committee decided that it was satisfied that the proposed use of embryos was necessary for the purpose of this research. In making this decision, the Committee took into account that quantifying and calibrating the amount of PLCzeta required to trigger development up to the blastocyst stage, and understanding the mechanism of activation in relation to calcium oscillation was likely to enable improved embryo development following IVF.

9. The Committee noted that the patient information had been amended by the PR, as outlined in the report, to be fully compliant, and given this, the Committee agreed that it was satisfied that these forms were fit for purpose.

10. The Committee considered itself satisfied that it was appropriate to grant a licence, noting that the appropriate fee had been paid and that it was in receipt of an application.

11. The Committee agreed that it continued to be satisfied as to the character, qualifications and experience of the Nominal Licensee. Also, the Committee agreed that it also continued to be satisfied as to the character, qualifications and experience of the Person Responsible as required for the supervision of the activities to be discharged under Section 17 of the HFE Act 1990 (as amended).

12. Further, the Committee agreed that it continued to be satisfied that the premises for which the licence was to be renewed continued to be suitable for the proposed activities.

13. The Committee, in light of the recommendation of the Executive, decided to grant a licence for a period of three years, with no additional conditions.

Signed..... Date 25.9.09.
Emily Jackson (Chair)