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HUMAN FERTILISATION AND EMBRYOLOGY AUTHORITY

LICENSED EMBRYO RESEARCH CENTRE

INITIAL APPLICATION FOR A RESEARCH LICENCE

The completed form and all supporting documents should be returned together with the appropriate fee to:

The Human Fertilisation and Embryology Authority
Paxton House
30 Artillery Lane
London E1 7LS

Guidance notes for completing this application can be found at the end of this form

Form revised June. 2001

Details of the centre undertaking research

Name of centre/institution
(including department)
Newcastle Fertility Centre at Life and
Institute of Human Genetics, Newcastle University

Address:
Newcastle Fertility Centre at Life
Times Square
Newcastle upon Tyne
NE1 4EP

Address for correspondence (if different):

Tel No **0191 219 4640**

Tel No:

Fax No:**0191 219 4747**

Fax No:

e.mail address (if applicable): **a.p.murdoch@ncl.ac.uk**

Are the premises used for the provision of licensed treatment? (Please tick appropriate box below.)

Yes [X] No [ ]

If "yes" please give centre number: **0017**

Corporate information

2.1 Is the centre a NHS facility or a private operation? (Please tick appropriate box.)

NHS [X] Private [ ]

2.2 If private please give the following information:

i. **Limited Company**
Company Name:
Registration No:
Registered Offices:

ii. **Partnership**
Name of Partners:

iii. **Sole Trader**
Name of Owner:

Person Responsible

Name: **Professor Alison Murdoch**

Position: **Head of Department**

Nominal Licensee

Name: **Dr Miodrag Stojkovic**

Position: **Reader in Embryology and Stem Cell Biology**

Research History

Please briefly summarise the history of any HFEA licensed research carried out at your centre, highlighting any previous projects in the same area of research proposed in this application:
(Please include the research licence numbers. You may continue on a separate sheet if you wish.)

This work reflects an extension of our existing licence RO145 for the derivation of stem cells. This has already resulted in the successful derivation of an ES cell line. The study aims to generate an alternative source of stem cells which will have enhanced potential for therapy. Since this involves the sensitive issues of nuclear transfer, we are submitting it as a separate research project for your consideration.

Title of research project

6.1 Please give the full title of the project
Derivation of human embryonic stem cell lines using nuclear transfer and parthenogenetically activated oocytes

6.2 Please indicate the purpose(s) of research (as defined in the HFE&E Act 1990) the project falls under. You may tick more than one box.

a. to promote advances in the treatment of infertility ☐

b. to increase knowledge about the causes of congenital disease ☐

c. to increase knowledge about the causes of miscarriage ☐

d. to develop more effective techniques of contraception ☐

e. to develop methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation ☐

f. i. to increase knowledge about the development of embryos X

ii to increase knowledge about serious disease X

iii to enable any such knowledge to be applied in developing treatments for serious disease X

Lay Summary

7.1 The Authority requires a brief summary of the project in lay terms, which will be disclosed to the public upon request. Please provide a short paragraph that we may use for this purpose. N.B., all other information provided in the application remains confidential.

It is recognised that human embryonic stem cells offer a great potential for therapies for many diseases such as diabetes. These stem cells are derived from embryos which are created for IVF treatment but which are not suitable for treatment. If stem cell treatments are to reach their full potential we need to derive stem cell lines which are genetically similar to the recipient so they will not be rejected. This may require the application of techniques such as nuclear transfer and parthenogenic activation. Nuclear transfer involves the transfer of genetic material from adult skin cells to eggs which have had the cell's nucleus removed. Parthenogenic activation involves an egg being artificially stimulated by chemical or electronic means in order to make the egg start embryo development. The present application is to undertake some of the initial studies that are needed to understand methods that will develop this technology.

Duration of project

8.1 Please give the proposed date of commencement for the project.

As soon as licence granted

8.2 Please give the period of time for which you wish the licence to be granted.

Three years

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9. Usage of material

9.1 Please indicate in the box below the estimated numbers of oocytes and embryos you **expect** to use during the period of the licence. If more than one year has been requested please indicate the year usage in the appropriate boxes.

Material	Year 1	Year 2	Year 3
Fresh Oocytes	100	100	100
Frozen Oocytes			
Failed to fertilis Oocytes	900	900	900
Fresh Embryos			
Frozen Embryos			

10. Centre(s) providing oocytes/embryos

10.1 Please give the names of the centres that will be supplying materials for this project, together with an indication of the number of embryos and or oocytes they each will be providing.

Newcastle Fertility Centre at Life

11. Current Research Projects

11.1 If you currently hold a research licence please give the project number(s) and title(s) of current research projects below. If you do not currently hold a research licence, please proceed to section 12.

RO145 Epigenetic studies of preimplatnation embryos and derived stem cells
RO122 An investigation of the use of laser biopsied blastocysts of PGD

11.2 Please indicate in the boxes below the number of oocytes and embryos **received** and the number that were **used** during the past year, in **all your currently licensed research projects**. This allows audit of embryos received but found to be unsuitable for research. Please give the name(s) of the centre(s) who supplied the material and give the data for each supplying centre separately.

Dates: From: ____1st Jan 2003___ To: ____31 Dec 2003___

Centre Name: Newcastle Fertility Centre at Life

Total number of embryos received	Total number of embryos used	Total number of oocytes received	Total number of Oocytes used	Total number of embryos created for research
Fresh: 985 **Frozen:** 79	**Fresh:** 828 **Frozen:** 73	**Fresh:** 0 **Failed to Fertilise:** 0 **Frozen:** 0	**Fresh:** 0 **Failed to Fertilise:** 0 **Frozen:** 0	0

Please continue on a separate sheet if required

12. Abstract

Please give a summary of the work you propose to undertake

The Stem Cell Group in Newcastle holds a research licence RO145 to derive ES cells and has already successfully derived such a line. This application is an extension of that licence to provide an alternative source of embryos by nuclear transfer and parthenogenic activation of oocytes. One of our group (Dr Stojkovic) has had extensive experience in nuclear transfer in animals. Dr Herbert is an established authority in oocyte maturation. We wish to use these skills to study this alternative source of stem cells. If successful, these sources offer an important development for the potential of stem cell therapy.
Although we consider this research to be a natural scientific development of our existing licensed research given the long term aim of that project to achieve therapeutic stem cell technology, we have submitted this as a new application in recognition of the political sensitivity of the nuclear transfer procedure.

13. Objectives

Please state the aims and objectives of the project. If the project involves human embryonic stem cells, please provide justification for their use, e.g. could the same results be obtained using other material such as animal stem cells or adult stem cells?

The aim of this research is to derive embryonic stem cell lines from oocytes activated after somatic cell nuclear transfer or parthenogenically. This will be the first step towards the technology to enable ES cells to be derived which will be antigenically matched to the recipient.
We have already demonstrated that we are able to derive stem cells from human embryos. We have also demonstrated experience in nuclear transfer in animals and consider that we have sufficient knowledge to transfer this to the study of human cells. No further animal work is needed before research on human research starts.

14. Background

Please state how the project fits in to the current state of knowledge on this subject (maximum of 1500 words).

Derivation of hES cells using therapeutic cloning

Cloning, also referred to as nuclear transfer (NT), denotes the introduction of a nucleus from an adult donor cell into an enucleated oocyte to generate a cloned embryo. When transferred to the uterus of a female recipient, this embryo has the potential to grow into an infant that is a clone of the adult donor cell; a process termed "reproductive cloning". However, in culture this embryo can give rise to embryonic stem (ES) cells that have the potential to become almost any type of cell present in the adult body. Because ES cells derived by nuclear transfer are genetically identical to the donor and thus potentially useful for therapeutic applications, this process is called "therapeutic cloning" (TC).

Therapeutic cloning might substantially improve the treatment of many incurable diseases (Alzheimer, Parkinson, diabetes) since therapy for these diseases is currently limited by the availability or immunocompatibility of tissue transplants. The objective of TC is to produce pluripotent human embryonic stem (hES) cells that carry the nuclear genome of the patient and then induce them to differentiate into replacement cells (Rhind *et al.*, 2003), which offers end of the use of immunosuppressive therapy in cell transplantation.

In animals, cell transplantation using TC, nuclear transfer (NT) and derivation of NT-ES cells from NT-blastocysts has been successfully applied in parkinsonian mice (Barberi *et al.*, 2003). Furthermore, specific gene defects could be repaired using homologous recombination in the cultured ES cells. Following differentiation into the appropriate tissue, the 'repaired' ES cells would then be transplanted back to the patient (Rhind *et al.*, 2003). This strategy has already been successfully carried out in mice (Rideout *et al.*, 2002) and homologous recombination can be used in hES cells (Zwaka and Thomson, 2003). Therefore, TC could be also used to treat human diseases in which the genetic defect is well defined.

In addition, NT-ES cell lines could serve in establishment of *in vitro* human disease models for basic research, drug discovery and toxicology. Drug discoverers use stem cells as a new resource for increasing confidence in the mechanism of action of new targets and the safety of modulating their activity (Street *et al.*, 2003).

Furthermore, derived NT-embryos and NT-ES cells offer excellent opportunity for studying the effect of oocytes derived mitochondrial proteins in somatic cells obtained by NT, the role of mtDNA and epigenetic mechanisms including cell reprogramming and non-controlled differentiation of hES cells.

Derivation of hES cells from parthenogenetically activated oocytes

One additional source for the derivation of hES cell lines are so called parthenotes or blastocysts which were recovered after artificial activation of oocytes. Previously ES cell line from parthenogenetically activated nonhuman primate egg (*Macaca fascicularis)* has been derived (Vrana *et al.*, 2003). This kind of stem cells could provide a potential source for autologous cell therapy in the female and the procedure can be used to identify factors important for successful activation of human oocytes.

Parthenogenesis, from the Greek word for "virgin birth," is an unusual mode of reproduction. Female aphids and turkeys, and certain female reptiles, are among the creatures that can reproduce this way. Their eggs can divide on their own as though they had been fertilised by a sperm, then go on to develop into embryos and offspring. Scientists in recent years have triggered parthenogenesis in the eggs of a few mammals, including rabbits and mice, but the resulting "embryo" has never developed beyond the early fetus stage in mammals.

However, this phenomenon was inspiration for the scientist to isolate the first stem cell lines from primate parthenotes, embryos grown from unfertilised eggs that. To create the parthenotes, the scientists from USA treated macaque eggs with chemicals that prevent eggs from ejecting half their chromosomes - as they do when fertilised (chemical, electrical or mechanical stimuli are necessary to mimic a sperm's arrival). Four of the 28 developed into blastocysts and a stable stem cell line from one of them was established. From these stem cells, the researchers developed a considerable variety of cells, including intestine, skeletal muscle, retina, hair follicles, cartilage, bone, nerve cells that secreted the brain chemical dopamine, the kind of cell that is gradually lost by Parkinson's patients and also spontaneously beating cells resembling heart cells. This offers possibility to derive genetically compatible material for a female egg donors/patients. Meanwhile, there are several reports which describe successful induction of parthenogenesis in human eggs suggests the goal of obtaining stem cells from human parthenotes is achievable.

We would like to use parthenogenesis and isolate stem cells from human parthenotes for two major reasons: I) to compare parthenogeneticaly derived stem cells with human embryonic stem cells derived from fertilised blastocysts and II) artificial activation of fused oocytes is necessary step in therapeutic cloning, therefore we would like to improve the efficiency of activation of human oocytes comparing different stimuli.

Nuclear transfer in human, will it work?

According to the data obtained from NT-animals there are several problems which might also appear during NT-procedure using human material. Many of the cloned animals are born with several different malformations including large offspring syndrome which appears due to the incorrect reprogramming and/or epigenetic/imprinting processes. Some scientists argue that primate embryos can never undergo successful NT, since meiotic spindle removal from recipient oocytes, which occurs during oocyte enucleation, appears to prevent embryo reconstruction (Simerly *et al.*, 2003). However, the majority of the NT-experts suggest that the major obstacle of the successful NT-procedure in human is only technical and logistic i.e. low number of donated oocytes (Birmingham, 2003).

Therapeutic cloning is not reproductive cloning

NT and reproductive cloning have been achieved in a number of species, namely sheep, cows, goats, rabbits, cats, mice (reviewed in Mullins *et al.*, 2004), mule (Woods *et al.*, 2003), horse (Galli *et al.*, 2003) and rats (Zhou *et al.*, 2003). The majority of described anomalies and problems which occurs in NT-animals requires transfer of whole NT-embryos and are observed either in whole embryo or offspring. Reproductive cloning is an inefficient and error-prone process that results in the failure of most clones during development (Hochedlinger and Jaenisch, 2003). On the other hand, TC is fundamentally different from human reproductive cloning; therapeutic produces stem cells, not babies. In therapeutic cloning, the nucleus of a donors unfertilized egg is removed and replaced with the nucleus of a patient's own cells and derived NT-embrys are not transplanted into a womb. For the derivation of NT or parthenogenetic ES cell lines we need to isolate inner cell mass cells from NT or parthenogenetically derived blastocysts, trophectoderm which is necessary for implantation and development of embryo will be lysed using immunosurgery. In TC, there is no need to transfer recovered NT-embryos or parthenotes and reprogramming errors do not appear to interfere with TC (Hochedlinger and Jaenisch, 2003). The methodological differences between reproductive cloning and TC are presented in Figure 1 (adopted from Hochedlinger and Jaenisch, 2003).

Despite the abundant evidence for aberrant reprogramming and altered gene-expression profiles in NT-embryos and offspring, normal expression profiles have been described for many genes in some NT-embryos (metabolic enzymes lactate dehydrogenase, citrate synthesis and phosphofructokinse (Winger *et al.*, 2000), pluripotency factor *Oct4*, *gp130, TEC*-3 (van Stekelenburg-Hamers *et al.*, 1994) and RNA polymerase (Daniels *et al.*, 2000) in NT-bovine embryos, and β-*actin*–GFP in the pig (Koo *et al.*, 2000). It was demonstrated that reproductive cloning is less efficient than using NT to produce ES cells (Wakayama *et al.*, 1998; Wakayama and Yanagimachi, 1999; Munsie *et al.*, 2000; Wakayama *et al.*, 2001), which might indicate that competent cells are selected for ES cell culture (Hochedlinger and Jaenisch, 2003). In addition, disruption in epigenetic imprints does not necessarily interfere with the capacity of ES cells to make specific cell lineages. For instance, parthenogenetic primate ES cells can differentiate *in vitro* or *in vivo* into a range of cell types (Cibelli *et al.*, 2002) and NT-ES cells can be induced to develop into a range of cell types, including neurons (Wakayama *et al.*, 2001). Neuronal function of NT-ES cell-derived dopaminergic neurons was shown *in vitro* by electron microscopy, measurement of neurotransmitter release and transplantation of NT-ES cell-derived dopaminergic neurons corrected the phenotype of a mouse model of Parkinson disease, demonstrating an *in vivo* application of therapeutic cloning in neural disease (Barberi *et al.*, 2003).

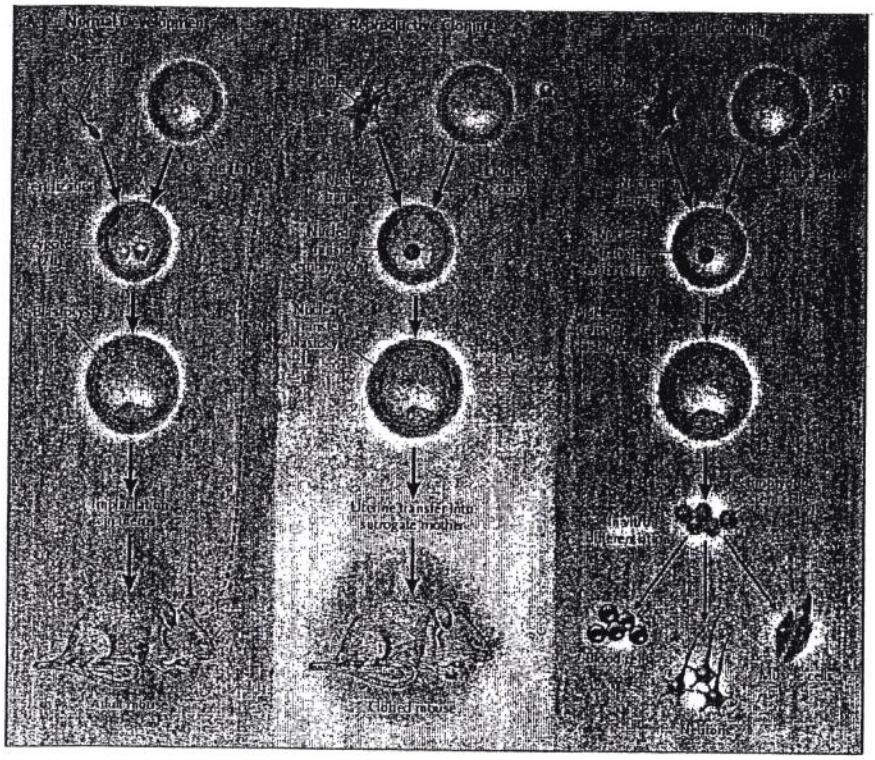

Figure 1. Comparison of normal development with development during reproductive cloning and therapeutic cloning. During normal development (Panel A), sperm cell futilises a zygote that undergoes cleavage to become a blastocyst embryo. Blastocysts are implanted in the uterus and ultimately give rise to an animal. During reproductive cloning (Panel B), the diploid nucleus of an adult donor cell is introduced into an enucleated oocyte, which after artificial activation divides into a cloned blastocyst. On transfer into surrogate mothers, a few of the cloned blastocysts will give rise to a clone. In contrast, therapeutic cloning (Panel C) requires the explanation of cloned blastocysts in culture to yield a line of embryonic stem cells that can potentially differentiate* in vitro *into any type of cell for therapeutic purposes.

We agree that the major obstacles in TC and derivation of human NT-ES cells are technical and methodical. However, many technical difficulties and obstacles in NT-procedure have been already overcome and were pivotal to the success of efforts to clone numerous animal species. For instance, the recent success in cloning of rats resulted from changing the methodology of the spontaneous activation of the oocyte that is known to occur in this species (Zhou *et al.*, 2003). Furthermore, embryos of Rhesus monkey have been cloned using somatic and embryonic cells (Mitalipov *et al.*, 2002) and offspring has been born using embryonic cells as donor cells (Meng *et al.*, 1997; Mitalipov *et al.*, 2002). In the human, early stages of human NT-embryos were recovered using somatic cells and parthenogenetically activated oocytes developed to the blastocysts (Cibelli *et al.*, 2001). In addition, the derivation of human NT-ES cells by the transfer of human somatic nuclei into rabbit oocytes was previously reported (Chen *et al.*, 2003). Recovered NT-ES cells maintained the capability of sustained growth in an undifferentiated state, form embryoid bodies and give rise to different cell types such as neuron and muscle (Chen *et al.*, 2003).

Stem cell Research Group in Newcastle.

This project involves collaboration between researchers based at the University Institute of Human Genetics (5* rated in RAE2001) and the Newcastle Fertility Centre at Life headed by Professor Murdoch. The two units are adjacent at the International Centre for Life (ICFL) in Newcastle upon Tyne. There is already a strong emphasis in mammalian developmental genetics/embryology at the ICFL with 12 academic staff whose primary focus is in this area. Following the recent establishment of the academic

posts for Drs M Stojkovic and Lako, the University of Newcastle is committed to strengthening the stem cell research environment: an additional Readership and Chair in Stem Cell Biology will be advertised this year.

Our team has very strong background in derivation and culture of hES cells which previously resulted in derivation of one new fully characterised hES cell line (Stojkovic *et al.*, submitted). In addition, our team has extensive experience in NT-technique in animals (Steinborn *et al.*, 2000; Zakhartchenko *et al.*, 1997; Zakhartchenko *et al.*, 1999; Hiendleder *et al.*, 2003; Hofmann et al., 2003; Santos *et al.*, 2003) including activation, physiology of mammalian oocytes and embryos (Herbert *et al.*, 2003; Laurincik *et al.*, 2003; Stojkovic *et al.*, 2002; Stojkovic *et al.*, 2003). We have already established collaborations with other workers in this field. We are working with Babraham Institute in Cambridge, one of the leading institutes in the epigenetic research. This collaboration already resulted in publication of manuscripts in high-ranking journals which describe epigenetic pattern of NT and IVF embryos (Dean *et al.*, 2001; Santos *et al.*, 2003). At the moment we are evaluating the epigenetic pattern of human IVF and ICSI embryos. Dr. Stojkovic is participating in the "Nuclear Transfer and Stem Cells" seminar which will be held in Chinchón, Madrid in Marhc 2004. Our stem cell group has developed close links with other groups. We have founded a North East Stem Cell Network and there are strong ties with stem cell researchers at the University of Durham (Dr. Nick Hole and Dr. Lyle Armstrong continue to collaborate with his previous colleague, Dr. Lako; and Dr. Stefan Przyborski, who carried out the teratomas validation of our hES cell line's pluripotency, continues to collaborate closely with our hES cell group). In addition, we have developed an interaction network with clinical researchers in the highly regarded Faculty of Medicine (where all eight RAE2001 submissions were graded 5 or 5*), with strong clinical focus groups for applications of stem cell research in diabetes, heart disease and liver disease. In addition to developing close links with stem cell researchers elsewhere in the UK we have been proactive in organising links to meet up with hES cell researchers from overseas in order to foster closer collaboration: we invited Lars Hamberger from the Goteborg group to Newcastle, have visited Singapore to meet with Ariff Bongso and are scheduled to visit Eva Sykova's group in Prague/Brno in February 2004.

An integral member of ICFL is PEALS (Policy, Ethics and Life Sciences) Research Institute. PEALS which includes social scientists, philosophers, theologians, scientists and artists, is led by Professor Erica Haimes, an acknowledged expert on the social and ethical aspects of reproduction and genetic technologies. PEALS hosted a meeting in Durham of the House of Lords Select Committee in 2000 at which they gave evidence for their deliberations about stem cell research and TC. Their subsequent report resulted in a change in the HFEA Act. Professor Haimes has a joint Wellcome Trust grant with Professor Murdoch to investigate patients perspectives of the donation of embryos for research for PGD and stem cell therapies. Professor Murdoch has an MRC grant to provide staff to ensure against potential conflicts of interest between clinical or research needs.

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Methodology/experimental design/analysis of results

Please state how this work is to be carried out. Please state clearly how you intend to dispose of the embryos after the research is completed. If human embryonic stem cells are to be used, please indicate clearly the fate of the stem cells throughout the project, and how they are to be disposed of after completion of the work.

Experimental design

Source of oocytes.

Newcastle Fertility Centre at Life performs approximately 600 IVF/ICSI treatment cycles per year. About 30% of the oocytes fail to fertilise and we wish to use these to study TC. In most cases these will be identified by the absence of 2 pronuclei on day 1 after insemination and will be allocated to research on that day. If no normal embryos have been identified in that treatment cycle, the oocytes will be retained until day 2 to allow transfer if cleavage has occurred.

In addition, some oocytes are retrieved during the follicle reduction procedure after superovulation which has produced too many follicles. Oocytes from both these sources would normally be discarded if not used for research.

During routine hysterectomy +/- oophorectomy procedures, we have access to normal ovarian tissue. For several years now we have recovered oocytes from these ovaries as part of other approved research. Most of these are immature gametes.

Source of donor nuclei

Oocytes will be used as cytoplast donors. They will be enucleated. For karyoplast we will use different somatic cell types.

The nuclei to be used for transfer will be from three sources.

- **Stem cell lines**
  We will use nuclei from cells from our existing derived ES cell line. The donors of the embryo from which the line was derived consented for further studies using these cells despite the fact that they would not be individually informed of the details of the study.
- **A woman undergoing a gynaecological procedure.**
  A 1cm skin biopsy will be taken from a woman undergoing a routine gynaecological operation. It is anticipated that sufficient cells will be obtained from one biopsy only.
- **A patient with Type 1 diabetes.**
  A 1cm skin biopsy will be taken from one patient who has Type 1 diabetes. This will allow us to develop a stem cell line from a patient with a specific disease.

After NT, the cell will be activated using different chemical, mechanical, and/or electrical stimuli. Methods which we have previously found to be successful in animal studies will be used. Since there is no previous published data relating to the methods required to activate human cells, precise details of the experimental methods cannot be given at this stage.

Parthenogenesis.

To derive parthenotes, oocytes will be artificially activated using different chemical, mechanical, and/or electrical stimuli and cultured until blastocyst stage.
After NT or parthenogenetic activation, cells will be studied to assess cleavage ability. All dividing cells will be then included in the research programme RO146 for the derivation of new ES cell lines. This project includes the study of epigenetic patterns in embryos and derived ES cell lines.

Activated oocytes which do not develop to blastocysts will be discarded according to the routine practice in the clinical embryology laboratories. Any derived stem cell lines will be deposited with the UK Stem Cell Bank and will be subject to their Code of Practice.

Consent procedure
All couples booking IVF/ICSI treatment at this unit are told at their initial consultation about the use to which all their embryos will be put. This includes the potential use of surplus embryos for research. If they agree to be approached at a later time to consider donation, this is documented in the notes. Those who agree are given written information about the research 4 days before the decision is required. This is the procedure which has been agreed under licence RO145 and approved by the local Ethics Committee.

Staff

Please list all the staff who will be involved in the proposed research.

Name	Profession/position	Qualifications/experience UKCC Pin No for nurses
Dr Miodrag Stojkovic	Scientist/Reader in Stem Cell Biology	
Dr Mary Herbert	Embryologist/ Scientific Director	
Dr Majlinda Lako	Scientist/Lecturer in Stem Cell Biology	
Professor Tom Strachan	Molecular Geneticist/Head of Dept. Institute of Human Genetics	
Professor Alison Murdoch	Gynaecologist/Head of Dept. Newcastle Fertility Centre	
Dr Meenakshi Choudhary	Research Fellow	
Mrs Petra Stojkovic	Research technician.	

Staff at Newcastle Fertility Centre at Life will be involved in the clinical aspect of the treatments.

Local research ethics committee

17.1 Please state the role of the ethics committee
Local Research Ethics Committee for Newcastle University and Newcastle Hospital NHS Trust.

17.2 Please list the chairman and membership of the ethics committee
It is not possible to give this information yet as the application may be seen by one of several local ethics committees according to local procedures. The information will be available with the approval when obtained.
Chairman

Members

Funding

Please supply copies of the objectives and protocols sections of any funding application made, excluding financial details. **This information is for HFEA use only and will not be sent out to Peer Review.**

Funding
University of Newcastle upon Tyne already granted Dr. M Stojkovic funds to purchase necessary equipment for micromanipulation of oocytes/embryos and TC. Thanks to other series of major infrastructural investments funded by JIF, SRIF and the One North East Regional Development Agency, we have excellent provision for molecular and cell biology facilities, including a first class mouse genetics facility, microarray and proteomics facilities confocal and laser microdissection microscopy, FACS analysis, automated sequencing/genotyping, robotic liquid handling and sample preparation facilities, and a bioinformatics suite managed by a Senior Research Fellow in Bioinformatics. Along with genome instability, stem cell biology is a major research theme of the Life Knowledge Park which has been supported already by a total of £3.5 million from DoH/DTI and by £1.4 million from One North East. A bid for a further £4.9 million investment from One North East has recently been approved. This provides additional new stem cell laboratories occupying 500 square metres plus additional equipment (£300K) for stem cell/human embryology research.
Further developments are being established to enable the derivation of hES lines which will be of clinical grade and thus suitable for therapeutic use. We have been awarded an MRC grant to improve *in vitro* culture using animal-free conditions. In combination with TC technique this will ensure that the new derived hES cell lines will be available for therapeutic application as well as basic research.

Supporting documentation

In order to process this application it is essential that the Authority be provided with a full set of appropriate supporting documentation as appendices to this application form. Please label each set of information with the appendix number indicated.

Appendix A: CVs of all staff engaged directly in the research project

Appendix B: Patient information relating to the proposed project

Appendix C: Relevant clinical and laboratory protocols

Appendix D: Consent forms regarding the use of oocytes/embryos for this project

Appendix E: Up to three most recent relevant publications

Appendix D: Consent forms regarding the use of oocytes/embryos for this project

Appendix E: Up to three most recent relevant publications

DECLARATIONS

Persons signing this application should note that Section 18 of the HFE Act 1990 states that " a licence committee may revoke a licence if it is satisfied that any information given for the purposes of application for the grant of the licence was in any material respect false or misleading." They should also note that under Section 41.3 provision of false or misleading information, knowingly or in a reckless manner is a criminal offence.

Person responsible

The information provided on this form is to the best of my knowledge true and accurate. I accept that should the application be approved for licensing, I will be required to jointly prepare, sign and submit a report of the work undertaken at yearly intervals from the date of licensing. I agree to act as the Person Responsible.

Signed ..... Date 23.2.04

Nominal licensee

The information provided on this form is to the best of my knowledge true and accurate. I accept that should the application be approved for licensing, I will be required to jointly prepare, sign and submit a report of the work undertaken at yearly intervals from the date of licensing. I agree to act as the nominal licensee.

Signed Miodrag Stojkovic Date 24/2/04

Chairman of Research Ethics Committee

The information provided in this form is to the best of my knowledge true and accurate. The Committee has given approval for this project.

Signed Jane Lomax Date 6 April 04