

**Centre:** Newcastle Fertility Centre at LIFE

**Centre No:** 0017

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**Research Licence:** R0152

### **Derivation of human embryonic stem cell lines using nuclear transfer and parthenogenetically activated oocytes**

#### **Lay Summary (submitted by the Centre)**

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.

#### **Abstract of the Licensed Research Project R0152 (submitted by the Centre)**

The aim of this research is to derive embryonic stem cell lines from oocytes activated after somatic cell nuclear transfer or parthenogenetically. This will be the first step towards the technology to enable embryonic stem (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.

#### *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" (*this is illegal in the UK*). However, in culture this embryo can give rise to 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 the 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 also be used to treat human diseases in which the genetic defect is well defined.

In addition, NT-ES cell lines could serve to establish *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 oocyte-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 lines from parthenogenetically activated nonhuman primate eggs (*Macaca fascicularis*) have been derived (Vrana *et al.*, 2003). These 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 the 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 the possibility to derive genetically compatible material for female egg donors/patients. Meanwhile, there are several reports which describe successful induction of parthenogenesis in human eggs suggesting that 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 parthenogenetically 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.

## **Experimental Details**

### *Source of oocytes*

It is known that mammalian oocytes spontaneously mature *in vitro*, following liberation from the follicle (reviewed in Hovatta, 2004) and human oocytes undergo normal cleavage following the addition of gonadotrophins to culture medium (Armstrong et al., 1991). The first children, triplets from oocytes matured *in vitro* after being taken from ovarian tissue were born in 1991 (Cha et al., 1991). *In vitro* maturation of human oocytes been further developed in the last few years and more children have been born from *in vitro* matured oocytes (for review see Hovatta, 2004).

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 oocytes to study TC. 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. Therefore, isolated immature human oocytes will be evaluated / classified and *in vitro* matured.

#### *Source of donor nuclei*

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

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

- Stem cell lines (*activity 1*)

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 (*activity 2*).

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.

#### *Parthenogenesis*

To derive parthenotes, oocytes will be artificially activated using different chemical, mechanical, and/or electrical stimuli and cultured until blastocyst stage.

Embryos created either through therapeutic cloning or by parthenogenesis will be used for the derivation of human embryonic stem cells lines and epigenetic studies.

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