HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

RESPONSE TO PEER REVIEW

Applicant: Professor Ian Wilmut

Centre number: 0202

Research project number: R0158

Title of project: Derivation of Human Embryo Stem Cells by Cell Nuclear Replacement for Technology Development and the Study of Motor Neuron Diseases

Questions were raised in three different areas as reproduced below along with our responses.

1. METHODOLOGY

Do you consider that the objectives are clearly defined and the methods proposed are likely to yield relevant and clear results? If not, what are the problems?

Peer 1

In general the objectives and methods are clearly defined. It would be helpful to know whether patients with MND from whom donor cells will be taken will have defined susceptibility, as inferred by genetic linkage analysis. This would be advantageous as the MND phenotype may be the end result of disparate cellular processes in different genetic forms of the disorder. How many patients with inferred mutation at each susceptible locus will be recruited? Are the investigators confident that the donors are available?

Response

We are confident that cells from patients with a variety of different genotypes will be available for this research. In the current application only patients with a definite genetic form of ALS will be recruited. That is, they are from kindreds with an autosomal dominant form of ALS due to a single gene defect.

Traditional linkage studies in ALS are problematic as the very short survival means that collecting DNA from a large number of individuals in a single kindred is difficult. As a result very few kindreds with autosomal dominant ALS have been linked:
ALS 3 One large Italian kindred fibroblast cell lines available through collaboration;
ALS 6 three kindreds linked, fibroblast cell lines from one individual in a kindred linked to chromosome 16 is available,
ALS 7 One USA kindred fibroblast cell lines not currently available.

We have fibroblast cell lines are available on three individuals from a further FALS kindred which is negative for SOD1 and VAPB mutations and not linked to any of the known loci. The kindred will be screened for linkage early in 2005.

We have a small number of other kindreds with two affected individuals who are alive and can be recruited. Other subjects who are index cases will also be used.

It is our intention to perform CNR on fibroblasts from the same kindred and from several kindreds linked to the same locus (eg: ALS 6). Control cell lines will also be established and cell lines generated from affected individuals and controls will be studied to determine differences in their gene expression and proteomic profiles to map out pathogenic pathways. Once the process has been optimised we would then be in a position to use CNR from apparently unrelated kindreds to look for disease associated changes that are common to affected individuals. This has the potential to identify groups of kindreds with similar pathogenic patterns and may help to identify the underlying gene defect.

2. ANALYSIS OF RESULTS

Are the numbers of gametes/embryos to be used realistic and are the statistical methods to be used appropriate to give meaningful results? If not, can you suggest alternatives?

Peer 1

Details of statistical methods to be used are not detailed in the application. I do not have the expertise required to advise on whether such detail is needed and would suggest specific biostatistical advice is sought. The numbers of gametes to be used suggest that some statistical detail might reasonably be requested of the applicants.

Response

The Institute has a group of statisticians who are available for advice. In a personal capacity, one of the group is on the Ethics committee which considered this application and so is aware of our proposals. We would seek her advice as we always have done in relation to our research. If the application is approved we will seek to identify other potential collaborating Centres in order to increase the number of oocytes available. We have not wished to do so until approval had been obtained for the proposed research.

Peer 2

Questions to the applicant:

- On the previous work they obtained 61 fresh eggs from the New Royal Infirmary of Edinburgh. 58 were used, 3 are unaccounted. Please explain the fate of these 3 eggs.

Response

These 3 eggs had degenerated in culture.

- How epigenetics will affect the outcome of this work?

Response

We recognise that, following the use of cell nuclear replacement, there may be epigenetic effects upon gene expression in the embryo stem cells and their

derivatives. It is for this reason that we propose to study sequentially first embryo development and then the cell lines in considerable detail as indicated in the application. We will be able to compare the cells with others derived in the same laboratory from donated embryos. While there is extensive evidence of epigenetic change recent reports have also shown that mouse embryo stem cells derived by cell nuclear replacement are able to contribute to chimeras with normal frequency (.

- On page 28 the applicant says that only human embryos will be used when they have at least half the normal number of cells a human embryo has: What is considered a 'normal' number of cells? What method will be used for counting?

Response
As the methods for human cell nuclear replacement are developed for the first time at Roslin Institute we will characterise the first embryos produced in several ways as indicated: -

The first embryos developing after cell nuclear replacement will be assessed for the normality of their development, as reflected by their capacity to undergo cleavage, compaction, and cavitation. In particular, blastocysts may be examined for the number of cells and the proportion allocated to the inner cell mass, the morphology of cell nuclei and for the occurrence of apoptosis.

As part of that process we propose to determine the number of cells in the trophectoderm and inner cell mass using a method for the differential counting of cells in mammalian blastocysts. This method has been used with human embryos (see Hardy et al, (1989) Development 107, 597-604; Hardy et al, (2004) Biology of Reproduction 68, 1165-1169). We have used the procedure in the Institute to determine cell numbers in livestock embryos. The number of cells reported in human blastocysts following in vitro fertilisation and embryo culture is very variable with a mean around 70 cells on day 6 (Hardy et al, 2003).

- Applicant will use embryo culture medium that is currently been used in IVF clinics. They should consider using medium optimised for nuclear transfer human embryos as the one recently described by Hwang et al. Science 2004.

Response
This is an excellent suggestion. We are already in communication with Hwang and hope to benefit from his experience. In the same vein we will seek to exchange information with all other groups who perform cell nuclear replacement in human.