CHRIS O'TOOLE
File R0152

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

PEER REVIEW FORM (New Applications)

Applicant: Newcastle Fertility Centre at Life and Institute of Human Genetics, Newcastle University

Centre number: 0017

Research project number:

Title of project: Derivation of human embryonic stem cell lines using nuclear transfer and parthenogenetically activated oocytes

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Referee:

Position held/ area of work:

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1. THE AREA OF RESEARCH

Do the proposals fall into one of the categories of research listed in the Act? If so, which one?

This research falls into category fi, fii and fiii.

2. IMPORTANCE

Do you consider that the proposals address important issues in the advancement of knowledge or treatment of infertility? If not, please state your reasons.

Therapeutic cloning (TC), as the researchers suggest, could be used to create models for human disease, and to reduce the possibility of immune rejection upon transfer of ES cell derived differentiated cells. The importance of circumventing immune rejection when using ES cells therapeutically cannot be underestimated. Furthermore, the creation of disease models that can be studied from the earliest stages of development may allow us a greater understanding of the mechanism of disease and how best to combat it. However, there is no likely direct benefit in the treatment of infertility.

3. ORIGINALITY

Has the work proposed been carried out before or not? If so, is there justification for repeating the experiments?

Work recently published in Science demonstrates that human SCNT is possible, and stem cells can successfully be derived from the resulting blastocysts. In their work entitled "Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst" [Science, March 12, 2004, 303(5664):1669-74], Hwang and associates describe the isolation and expansion of stem cell lines derived from embryos created by SCNT. The only notable difference between the previously published study and the current proposal being that Hwang et al. used autologous SCNT, while Stojkovic and Herbert propose to use non-autologous donor cells for SCNT. Given that the cell line reported in Science could have been from parthenogentic activation, it is important to repeat the work with genetically distinct nuclear donors.

4. JUSTIFICATION

Have experiments on animal models or other types of human cells reached a point at which the use of human embryos is justified?

Somatic cell nuclear transfer has been successfully accomplished in many animal species. Recently, Hwang and associates have accomplished this procedure in humans as well, and isolated ES cells from the resulting embryos, but just like Dolly, independent confirmation is essential.

4.a. CREATION OF EMBRYOS FOR RESEARCH

Does proposed work justify need for the creation of embryos specifically for research? Could researchers feasibly utilise other avenues in order to conduct the proposed work?

Unfortunately, the authors fail to comment on the practicality of alternate methods that might be used to achieve a similar result. The authors mention the usefulness of homologous recombination in altering SCNT produced human ES cell lines. Others have suggested that this procedure could potentially be used to eliminate histocompatibility issues and create disease models using existing stem cells lines. Additionally, it has been suggested that co-transplantation of differentiated ES cell derived cells with hematopoetic cells derived from the same ES cell lines could reduce or eliminate immune rejection in recipients. It is important for the authors to acknowledge this previous work, and state why they believe that TC would be a preferred method.

5. 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?

The methods for creating SCNT embryos are outlined in the grant application. The source of the oocytes and somatic cells is clear and detailed, and consent forms appear to be complete and exhaustive. There is little information detailing the handling of oocytes once collected. The authors indicate that they expect many of the gametes will be immature at acquisition, but provide no details regarding if or how these oocytes will be matured. Furthermore, there are no NT or embryo culture protocols included. Additionally, while the authors indicate that "different chemical, mechanical and/or electrical stimuli" will be used to activate the NT oocytes, no other detail is provided. While we concede that there is little human data on which to draw at this point, there is sufficient animal data to provide at least some more specific potential activation protocols. Overall, more detail would be useful.

6. 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?

Clearly, it is difficult to determine how many oocytes would be required to achieve SCNT, or how many embryos needed to ensure success isolating ES cell lines. However, the researchers have not clearly indicated what they would consider to be a success, how many stem cell lines they aim to produce if successful, or at what point the experiments would be terminated. The focus of this grant seems to be only the derivation of SCNT human embryos, as there is no information in the methods section detailing the isolation of stem cell lines from these embryos. Furthermore, there is no indication in the grant application of tests that would be performed on resulting stem cell lines to determine their normality or usefulness. The authors reference an alternate research programme 'research programme RO146', which may in fact contain this information -- however, this reviewer has no access to that document, and therefore cannot assess the validity of the proposed analysis for this portion of the project. If not included in the alternate research program tests, it would be critical that a specific test was included to ensure that resulting lines are of SCNT and not parthenogenic origin.

7. DURATION

Is the proposed duration of study appropriate?

The three-year duration indicated should be sufficient to accomplish this project.

8. THE APPLICANT

Do you know the applicants work personally or by repute? Does the team have the necessary qualifications and ability to carry out the proposed work?

The research team headed by Drs. Stojkovic and Herbert has demonstrated the scientific and technical ability to carry out the proposed experiments. Their combined background in embryology, in-vitro cell culture, nuclear transfer and stem cell technology inspires confidence that the proposed project could be successfully accomplished.

9. ANY OTHER COMMENTS

10. OVERALL ASSESSMENT

Please tick your recommendation of the proposed work:

-Reject for licence, flawed in scientific or technical approach

-Resubmit application, has potential but needs revision following feedback from reviewers

√ -Accept in current form

Licence should be granted in current form.