From the Department of Clinical Science, Intervention and Technology, Division of Obstetrics and Gynaecology, Karolinska Institutet, Stockholm, Sweden

OPTIMISATION OF HUMAN EMBRYONIC STEM CELL DERIVATION AND CULTURE- TOWARDS CLINICAL QUALITY

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i R64, Karolinska Universitetssjukhuset, Huddinge

Fredagen den 26 november, 2010, kl 8.15

av
Susanne Ström

Huvudhandledare:
Professor Outi Hovatta
Karolinska Institutet
Department of Clinical Science, Intervention and Technology
Division of Obstetrics and Gynaecology

Bihandledare:
Dr. José Insunza
Karolinska Institutet
Institutionen för Biovetenskaper och Näringslära
Avdelningen för Medicinsk Näringslära

Fakultetsopponent:
Dr. Jennifer Nichols
University of Cambridge
Wellcome Trust Centre for Stem Cell Research

Betygsnämnd:
Professor Anna Wedell
Karolinska Institutet
Institutionen för Molekylär Medicin och Kirurgi
Sektionen för Genetik

Professor Karin Forsberg-Nilsson
Uppsala Universitet
Institutionen för Genetik och Patologi

Professor Rune Toftgård
Karolinska Institutet
Institutionen för Biovetenskaper och Näringslära
Centrum för Bioteknik

Stockholm 2010

ABSTRACT

For clinical grade human embryonic stem cell (hESC) lines, a robust derivation and culture system without any substances having animal origin would be optimal. The general aims of these studies have been to gradually improve our hESC derivation and cultures.

The first step towards clinical quality was the use of human foreskin fibroblasts instead of mouse embryonic fibroblasts to support the undifferentiated growth of the pluripotent stem cells. This was followed by replacing foetal calf serum as a supplement in the culture medium with the commercially available Serum Replacement, first in the cultures and later also for the derivation of new hESC lines.

The immunosurgery generally used for isolation of the inner cell mass (ICM) involves animal serum and complement. We have been able to replace the surgical method with a mechanical procedure for the isolation of the inner cell mass, and this gives better results.

We have also evaluated whether the morphology of the embryos donated to stem cell research has an impact on derivation success. We have carried out statistical analyses on the early cleavage rate, morphological score of the embryo at cleavage stage and the score for the ICM and the trophectoderm at the time for isolation of the ICM. We have shown that there is no correlation between the morphology and derivation success. All embryos donated for stem cell research should be used for isolation in an attempt to derive new hESC lines. Even embryos with no visible ICM have generated pluripotent hESCs.

In the final study we have been able to culture hESCs on a human recombinant laminin, LN-511, for more than 20 passages (four months) in a well-defined medium devoid of any animal-derived components. The use of a well-defined system is most important in understanding the pluripotent state and being able to direct the differentiation in the desired direction for clinical applications in the future.

We have taken hESC research from a culture system that depended on several animal-derived components to a totally xeno-free system. We hope that these improved culture procedures can be used for the development of cell lineages for use in therapeutic purposes.

Key words: hESC, culture, derivation, pluripotent stem cells

ISBN [978-91-7457-047-2]