Role of the embryology laboratory in the human embryonic stem cell line derivation process

José Luis Cortés · Fernando Cobo · Angela Helen Barnie ·
Purificación Catalina · Carmen Cabrera · Ana Nieto · Rosa Montes ·
Ángel Concha

Received: 21 July 2006 / Accepted: 10 October 2006 / Published online: 25 November 2006
© Springer Science+Business Media B.V. 2006

Abstract With the introduction of regenerative medicine and cell therapy programmes by means of human embryonic stem cells (hESC), several research centres have begun projects of derivation of hESC lines. In some stem cell banks, such as the Andalusian Stem Cell Bank, the law also permits the creation of these cell lines. Therefore, the recovery of cryopreserved embryos, their culture and the subsequent derivation to hESC lines requires a suitable embryology laboratory and specialized and highly qualified staff. Moreover, new techniques, from therapeutic nuclear transfer, need this type of laboratory and staff, too. Several International Associations have drawn up some guidelines for laboratories where embryos are manipulated and they reflect the physical space, the staff and the equipment needed in these kinds of laboratories. Nevertheless, we can see that these guidelines do not distinguish between IVF laboratories and other laboratories that obtain hESC lines, so it would be convenient to make a distinction. Following these guidelines, we have tried to draw up

concurrent aspects applicable to areas of embryology within stem cell banks. So, the design and the specific implementation programmes for these areas and other research centres with this area but which do not use IVF techniques is vital to develop embryonic cell lines in optimum conditions for future therapeutic applications, although maybe it is rather premature to standardize this type of research.

Keywords Cleanrooms · Embryology laboratory · Embryonic stem cell lines · International guidelines · IVF · Stem cell bank

Introduction

The transplant of cells of human origin is a sector of medicine, which shows increasing opportunities for the treatment of diseases, some of which until now have been incurable. Thus, with the introduction of regenerative medicine and cell therapy programmes using human stem cells, a great number of research centres have attempted the derivation of cell lines through the use of human embryonic stem cells (hESCs) (Reubinoff et al. 2000). Stem cell banks are the establishments entrusted with assuring the quality, traceability and safety of stem cell lines. This should be achieved with close attention to the standardisation of processes and the implementation of quality control

J. L. Cortés (✉) · F. Cobo · A. H. Barnie ·
P. Catalina · C. Cabrera · A. Nieto · R. Montes ·
Á. Concha
Andalusian Stem Cell Bank (Spanish Central Node),
Hospital Universitario Virgen de las Nieves, Avda
Fuerzas Armadas, 2, 18014 Granada, Spain
e-mail: josel.cortes.sspa@juntadeandalucia.es

programmes and methods, which reflect the best current practices. Furthermore, in some stem cell banks, such as ours, the law also permits the creation of these cell lines (Spanish Law 14/2006 2006). Therefore, the recovery of cryopreserved embryos, their culture and the subsequent derivation to hESC lines (Cowan et al. 2004), requires a suitable embryology laboratory or area, as well as specialized and highly qualified staff. Furthermore, the derivation of patient-specific hESC lines only for therapeutic clinics, using the nuclear transfer technique, should need this type of laboratory and a specifically dedicated team. In this context, the processes and procedures used to obtain hESC lines, must adhere to a series of safety rules and must follow strict work protocols (International Standard Organization 1999; Department of Health 2001; American Association of Tissue Banks 2002).

Several International Associations have drawn up some guidelines for laboratories where embryos are manipulated, reflecting the physical space, the staff and the equipment needed in these kinds of laboratories. Nevertheless, after the thorough analysis of these guidelines, we can observe that they do not distinguish between “in vitro” fertilization (IVF) laboratories and other embryology laboratories that obtain hESC lines using research proposals or cell therapy, so, it would be convenient to make a distinction, since some of the techniques used are different, as are the objectives. In this review, a comparative analysis of the common contents established by different guidelines has been carried out. Following these guidelines, we have tried to draw up both concurrent aspects which are applicable to areas of embryology within stem cell banks in the hope that it might serve as an example of good practice guidelines for the research community in these establishments in order to obtain cell lines using cryopreserved and cloned human embryos. There are some countries (United Kingdom, Singapore, etc.), where the use of fresh embryos in research is validated, entails embryos to be cultured to the blastocyst stage in a routine embryology laboratory and then handed over to the stem cell unit. Even, stem cell research centres of these countries would need an embry-

ology laboratory to derive cloned embryos, which will follow certain guidelines.

Guidelines and guidances used

To carry out this study we have analysed the guidelines suggested by the following International Associations (Table 1): ESHRE 2000; CAP 2002; FSA 2002; ACE 2003; ASRM 2004; NAMS 2005; NAP 2005.

The majority of guidelines studied, except NAP, 2005, which assess all the aspects of the embryology laboratories, belong to associations dedicated to human assisted reproduction.

There are other guidelines related to hESCs, such as those of the National Institute of Health (NIH 2002), and the Indian Council of Medical Research (ICMR-DBT 2006), but they have been excluded from this assessment, because they do not include basic aspects that deal with embryology.

On the other hand, we have assessed the European Union Tissue and Cell Directive (2004/23/CE, 31st March) (European Union 2004), which establishes setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells in the European Union countries.

Aspects assessed in the study and methodology

The majority of the guidelines studied (ESHRE 2000; CAP 2002; FSA 2002; ACE 2003; ASRM 2004; NAMS 2005), except NAP 2005, show a similar approach in the treatment of the different sections analysed. For this study, the common and discordant aspects of the different guidelines have been assessed, but only concerning the aspects related to the area of embryology.

The aspects analysed, which we consider to be important, are the following (Table 2):

1. Organization of the laboratory and definition of services.
2. Laboratory staff of an area of embryology.
3. Laboratory space and design.
4. Equipment and procedure manuals.
5. Laboratory safety and infection control.

Table 1 Guidelines reviewed in this study

Research association	Abbreviation	Guideline	Web page
<i>Assisted reproduction techniques</i>			
European Society of Human Reproduction and Embryology	ESHRE	Guidelines for good practice in IVF laboratories, 2000	www.eshre.com
College of American Pathologist	CAP	Laboratory Improvement, 2002	www.cap.org
The Fertility Society of Australia	FSA	Reproductive Technology Accreditation Committee (RTAC). Code of practice for centres using assisted reproductive technology, 2002	www.fsa.au.com/rtac/
National Academy of Medical Sciences of India	NAMSI	National Guidelines for accreditation, supervision and regulation of ART clinics in India, 2005	www.icmr.nic.in
Association of Clinical Embryologists	ACE	Accreditation Standards and Guidelines for IVF Laboratories, 2003	http://ace.ivf.net/ace/
American Society for Reproductive Medicine	ASRM	Reviewed guidelines for human embryology and andrology laboratories, 2004	www.asrm.org
<i>Stem cell research</i>			
National Institute of Health	NIH	Guidance for investigators and institutional review boards regarding research involving human embryonic stem cells, germ cells, and stem cell-derived test articles, 2002	www.nap.edu/books
National Academies Press	NAP	Guidelines for Human Stem Cell Research, 2005	www.nap.edu/books
Indian Council of Medical Research	ICMR-DBT	Guidelines for Stem Cell Research and Therapy, 2006	www.icmr.nic.in

6. Quality control.

7. Legislative and ethical aspects.

It is obvious that some of the aspects analysed are common to both embryology laboratories dedicated to IVF, and those dedicated to deriving hESC lines. However, there are others, which are specific to each type of laboratory.

We have made a comparative assessment of the above mentioned aspects, showing similarities and differences, as others have done (Cohen et al. 2004). Also, we have made some recommendations for the embryology laboratories that derive hESC lines in each section.

Finally, we have assessed separately the new European Directive, analysing the impact of its application in a stem cell bank, which derives hESC lines.

Organization of the laboratory and definition of services

None of the guidelines reviewed make reference to the organization of these types of laboratories.

Perhaps, the ASRM 2004 is the most detailed as far as the organization of embryology laboratories is concerned, but it is focused exclusively on activities with reproductive ends (e.g. sperm preparation, insemination of oocytes, intracytoplasmic sperm injection [ICSI], embryo culture, embryo transfer, etc.).

In our opinion, the area of embryology in charge of deriving hESC lines from cryopreserved and cloned human embryos, must be included in the organization chart of stem cell banks or other research centres, in such a way that it interacts directly with the rest of the areas, such as those of cell culture, microbiology and environmental control, cytogenetics, traceability, etc.

Laboratory staff of an area of embryology

The different research associations recommend the basic assignation of staff in these laboratories, and the functions of each one of them in their guidelines. The staff planning for an IVF laboratory is determined depending on the work volume of each centre (ESHRE 2000; CAP 2002),

Table 2 Comparative analysis of guidelines about embryology laboratories focused to assisted reproduction techniques

	ESHRE 2000	CAP 2002	FSA 2002	ACE 2003	ASRM 2004	NAMSI 2005
Organization of the laboratory and definition of services					X	
Laboratory staff	X	X	X	X	X	X
Director	X	X	X	X	X	X
Laboratory supervisor	X	X	X	X	X	X
Embryologist	X	X	X	X	X	X
Technical staff					X	
Laboratory space and design	X	X	X	X	X	X
Restricted access			X		X	
Separated from other activities that could interfere	X	X	X	X	X	X
“Wet area”	X		X	X	X	X
Storage space and adequate changing areas for staff		X		X		X
Equipment and procedure manuals	X	X	X	X	X	X
Adequate environmental level air filter	X	X	X	X	X	X
CO ₂ incubators with alarm systems	X	X	X	X	X	X
Biological safety cabinet (BSC)	X					
Horizontal laminar flow cabinets						X
Mechanical pipetting devices	X	X	X			
Temperature and pH devices to CO ₂ incubators	X	X	X	X	X	X
Energy provision system	X	X		X		X
Microscopes and other types of instruments	X	X	X	X	X	X
Micromanipulation methods	X	X	X	X	X	X
Procedure manuals	X	X	X	X	X	X
Laboratory safety and infection control	X	X	X	X	X	X
Quality control	X	X	X	X	X	
Legislative and ethical aspects	X	X	X	X	X	X

although, we can establish basic figures within the laboratory organization chart:

- The Director is the person responsible for the embryology laboratory. In the different guidelines, there is discussion about the degree and experience in IVF laboratory management of the director. All societies agree that the director must dominate all the techniques that concern this type of laboratory. He/She must have proof of previous experience and provide the rest of the staff with adequate training in all aspects connected with assisted reproduction and embryonic manipulation. In stem cell banks and other research centres with an area of embryology in their organization chart, the figure of the director could be replaced by the managing director of the centre and he/she could assume the aforementioned cited functions, knowledge in stem cell culture and

tissue banking, and the coordination of the other areas. Furthermore, we think the director should have experience in business management.

- Laboratory supervisor: some societies (FSA 2002; ASRM 2004) have recommended this figure, whose main mission would be the coordination of all tasks carried out inside this area. He/She must also participate in the accreditation of training courses related with the speciality and he/she must possess demonstrated experience in embryonic manipulation techniques. In centres that obtain hESC lines like the Andalusian Stem Cell Bank, this figure is replaced, and his tasks shared, by the person responsible for traceability, the quality assurance manager, or by the embryologist himself, who finally will be responsible for planning the work in this area

and the supervision of the laboratory technicians.

- Embryologist: All societies agree that this person would be responsible for doing all embryology laboratory planning. There is discussion about the graduate level of this figure, but we think that the embryologist must have a superior level degree in biomedical sciences and experience in mammalian embryology and “in vitro” culture techniques. Recently, two European Embryology Societies have suggested the recognition of the embryologist within the reproductive field (AGRBM 2005; Castilla et al 2006). These societies, one German and, the other Spanish, respectively, do not take into account that there could be embryologists who are not involved in reproduction. This person will be responsible for deriving cell lines using human cryopreserved or cloned embryos.
- Technical staff: We consider that the presence of those who assist the embryologist is essential. The ASRM 2004 suggests this figure, but only for satellite facilities. His/Her work would be support for the embryologist, as well as the person who would control supplies and provisions, culture media, etc. In time, the laboratory technician could train or specialize in some of the techniques related to the derivation of cell lines, always under the supervision of the embryologist who is responsible for the laboratory.

Laboratory space and design

All guidelines studied, related to human reproduction (ESHRE 2000; CAP 2002; FSA 2002; NAMSI 2005; ACE 2003; ASRM 2004) coincide in pointing out the importance that the laboratory should have restricted access and be situated in a place where people and materials do not often circulate and which is separated from other activities that could interfere. Furthermore, all guidelines assessed show the need for a separate office space provided for record keeping, data entry, and related administrative functions. Also, a general “wet area” (i.e., medium preparation,

equipment, sterilization, etc.) is necessary. It is also necessary to count on storage space and adequate changing areas for staff, too. All these places could be common for the rest of the research areas, not only specific for the area of embryology. In our opinion, the most important matter is the design of the rooms where the derivation of hESC lines is carried out. Each laboratory has an inherent special feature in the structure of the centre where it is located, therefore, we consider vital that the manipulation and derivation of hESC lines be carried out in cleanrooms. These installations possess some special characteristics which are ideal for this type of procedure; rooms with air filtration using high efficiency particulate air (HEPA) filters that provide a specific positive pressure within the rooms and, which with a management system monitor, the number of environment particulates, the pressure, the humidity and the light intensity, etc. The work in these types of installations will minimize, without doubt, the cell line contamination risks, so, we can obtain sterile cell products with sufficient quality for human transplants (Pharmaceutical Inspection Co-operation Scheme 2002).

Equipment and procedure manuals

According to the guidelines studied, the societies understand that the sterile conditions in the embryology laboratory are an essential measure to reduce the risk of contamination of biological material, either by the presence of infectious biological particles or volatile organic substances. Therefore, an adequate environmental level air filter and an optimal feature of all instruments within the rooms, was specifically considered at first in the EU regulations, but this request has been eliminated from the Directive Annexes (European Union 2004).

All guidelines consulted establish that the CO₂ incubators must possess alarm systems, as well as CO₂ and external air gauges to carry out an exhaustive follow up of these parameters in the incubator.

As regards the type of biological safety cabinet (BSC) recommended in these laboratories, only the ESHRE 2000 advises the use of BSC, whereas

other societies, like NAMS [2005](#), advise the use of horizontal laminar flow cabinets with a thermostatically controlled heating plate.

The majority of guidelines recommend the use of mechanical pipetting devices, advising against mouth pipetting. The temperature control is a constant in the embryology laboratory, thus, all guidelines consulted recommend the use of devices to maintain the temperature and pH of the CO₂ incubators and control systems of the machines (microscopes, slide warmers, heating block, isolator, etc.).

The existence of an energy provision system, when there is an emergency or electric main fault, is a matter which societies like ESHRE [2000](#), CAP [2002](#), NAMS [2005](#) and ACE [2003](#) recommend in their guidelines to minimize the energy fault risks.

The need for microscopes and other types of instruments in the embryology laboratory is understood by all research societies.

With respect to procedure manuals, all societies greatly emphasize this section, showing that all procedures should be reflected in written protocols. In this way, they advise the creation of a code of procedures and good practices.

In our centre, the embryology laboratory is where the derivation of hESC lines is carried out, using cryopreserved embryos as well as nuclear transfer techniques. It has, of course, modern technical equipment and, due to its special features, has to fulfil the essential requirements described below.

It is important to point out that the work in our embryology laboratory is carried out in extreme aseptic conditions. The environmental air is filtered with F, G and HEPA filters, which can trap dust particles and environmental contamination. The existence of positive pressure in cleanrooms contributes to reducing the risk of contamination of the manipulated embryos (Pharmaceutical Inspection Co-operation Scheme [2002](#)). The human embryo handling must be done, from our point of view, in BSC, which contributes hugely to reducing the contamination possibilities. Also, the lighting conditions of the cleanrooms, when the embryos are outside the incubator, must be adequate (Noda et al. [1994](#)).

Another measure to minimize the possibility of the embryonic contamination greatly would be the

use of disposable materials, although this depends on the economic possibilities of each laboratory. Furthermore, our Centre works according to the Guide to Good Manufacturing Practice (GMP), which provides supplementary guidance on the application of the principles and guidelines of GMP to sterile medicinal products (EC Guide to Good Manufacturing Practice [2003](#); Sharp [2005](#)).

To carry out the derivation of hESC lines, a very specific technique and equipment infrastructure is necessary. The development of micromanipulation methods and their connection to coupling in inverted microscopes have been determinant to be able to derive hESC lines from embryos, above all in nuclear transfer techniques. Also, laser technology is used for assisted hatching. This technique consists of making the embryonic membrane weaker to make the exit of the future blastocyst easier and so favour the derivation (Antinori et al. [1996](#)). Some groups suggest the use of laser as a new mechanical method of derivation of hESC lines, destroying the trophoblast and isolating the inner cell mass (ICM) (Tanaka et al. [2006](#)).

Laboratory safety and infection control

All guidelines reviewed make reference to the need for exhaustive laboratory safety and infectious risk control in addition to the installations and the staff. The ESHRE 2000 points out that all techniques carried out in an embryology laboratory include the biological material handling, and this means a potential danger of transmission of illness to the laboratory staff. So, the vaccination of the staff against hepatitis B is suggested. On the other hand, a screening of the couples who donate embryos should be carried out to rule out the presence of transmissible infectious agents. Other kinds of protective measures may be necessary like some secondary contention elements (laboratory clothing, non-powdered gloves, masks, etc.) (ESHRE [2000](#)).

The procedures and safety policy in the laboratory must include the whole staff and must be reviewed annually by the laboratory director. The protocols must include precautions with respect to fire and electric problems, for example internal and external disasters (FSA [2002](#); ASRM [2004](#)).

With respect to this, it is important to create and develop a biosafety manual according to the Workplace Occupational Health, Safety and Environmental Guidelines (FSA 2002; Cobo et al. 2006a, b).

Our bank has drawn up general safety guidances in stem cell bank installations. In them, we have identified the main risks in these types of installations and we offer some indications and safety answers which should be applied in the area of embryology (Cobo et al. 2006a, b), for hESC lines derived for research purposes as well as those derived for possible future therapeutic applications.

On the other hand, the introduction of a microbiology and environmental control programme is necessary with both a section dedicated to overall quality control and also infectious risk and safety control measures (Cobo et al. 2005, 2006a, b).

Obviously, many human and animal pathogens, which can be isolated in cell cultures suppose a risk for the cell lines, staff, as well as the patients who receive the cells. Any microbiological contamination of the material donated (e.g., embryos, bone marrow) or introduced during the derivation process could have a multiplication potential and could be a serious danger for the recipients.

It is good practice to apply a routine particle monitoring using a continued monitoring system (e.g., Particle Measuring System) in this type of laboratory (Cobo et al. 2006a, b), in order to reduce risk of introduction of pathogen microorganisms into the cell cultures.

Quality control

Some guidelines (ESHRE 2000; CAP 2002; FSA 2002; ACE 2003; ASRM 2004) point out the need to devise a procedure manual, which has to be known by the whole staff and reviewed annually. It should deal with important aspects including the equipment calibration, corrective action documentation, infection and contamination control, adverse event register, safety copies of all computerized documents and an emergency monitoring such as alarm control of the machines (FSA 2002; ASRM 2004). ESHRE 2000 suggests quality control based on the assessment of the results.

The Andalusian Stem Cell Bank has implemented a Quality Management System to obtain the certification and later the accreditation according to ISO 9001:2000 and ISO 17025:2005. So, the Bank is working on its general and specific procedures and processes, standard operational procedures, forms and registers all included in a data base and to be followed in order to assure the quality of the product/service we intend to offer.

Legislative and ethical aspects

Since each guideline is produced in a different place of the world, not much reference is made to these legislative and ethical aspects because the legislation is different depending on the geographic location. However, all guidelines make reference to the necessary requirements to obtain the accreditation. The most significant of them is the need to draw up an informed consent of the donor couples whenever something about the donated biological material is to be decided. The majority of the guidelines also make reference to the creation of a committee which, recurrently, should treat possible improvements on a legislative and ethical level.

In Spain, currently, the Law 14/2006, May 26th, about Assisted Reproduction Techniques deals with the legal order on health care and research, above all in regenerative medicine (e.g., stem cell research, somatic cell nuclear transfer, etc).

Andalusia has been pioneer in this type of research regulation. The Andalusian Parliament passed the Law 7/2003, on October 20th 2003, with the regulation for research using human embryos, non-viable for IVF, which have been stored in liquid nitrogen for more than 5 years. Furthermore, a Spanish Royal Decree (2132/2004) with the establishment of the requirements and procedures to seek the development of research projects with stem cells obtained using spare embryos was published on October 29th 2004.

At a European level, a directive which establishes setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells has recently been published. This is the previously aforementioned Directive 2004/23/CE, March 31st 2004 (European Union 2004),

and we comment its significance in the last point of this review.

NAP 2005, as previously indicated, is focused on the hESC research. Therefore, its legislative and ethical recommendations are more significant because they are focused on the procurement of gametes, blastocysts or cells for hESC generation, and derivation of hESC lines. Furthermore, it includes a section on the banking and distribution of these cell lines, the medical and pharmaceutical application, and the international collaboration that this type of research requires. Finally, they also make a proposal to establish the “Institutional Embryonic Stem Cell Research Oversight Committee”.

Assessment of the new European directive

On March 31st 2004, the European Parliament issued Directive 2004/23/EC, which was published in the Official Journal of the European Union on April 7th 2004 (European Union 2004). This Directive arose with the intention to unify the diversity of standards across European countries that regulate the extensive exportation/importation of certain tissues, the need to increase both availability of donated tissues and the effective use of those tissues available, and to avoid errors arising from the multiple coding and classification systems in use across Europe. It applies to all human tissues and cells, including adult and embryonic stem cells, but excludes blood and blood products as well as human organs. It is interesting that the European Directive is the most suitable for the needs of an embryology laboratory, which derives hESC lines. Furthermore, Mortimer (2005) has criticized the directive, because it tries to include in the same normative both embryology laboratories of reproduction centres and tissue banks and research centres which derive cell lines or research with them. In this sense, he suggests that stem cells need more care than reproductive cells, and also suggests that some aspects indicated in the directive are difficult to carry out in an embryology laboratory dedicated to reproduction, but which are not difficult to carry out in an embryology laboratory which derives hESC lines. Some of the

aspects which make these types of laboratories different would be the need for a cleanroom or not, the sample procurement methods, handling and processing of samples, packaging, labelling and storage, traceability, the different methods used in each laboratory, as well as the different factors, such as intrinsic and extrinsic factors which affect both types of laboratory (e.g., culture media, temperature, culture gas/atmosphere, air quality, embryonic stress during the culture, etc.). Therefore, from our centre we also consider that a distinction should be made between both.

Recently, the Official Journal of the European Union has published the Commission Directive 2006/17/EC, of February 8th 2006, implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.

Therefore, the Directive considers the need to guarantee the safety of all types of handling and research. May 2007 is the final date for EU countries to draw up this Directive.

Conclusions

So far, the embryology laboratory has focused its activity on IVF techniques. Nevertheless, after the discovery of embryonic stem cells and their contribution to the damaged tissue regeneration, there are numerous research centres and tissue banks which have introduced hESC lines programmes using cryopreserved embryos, so, a new dimension and new perspectives are provided to the embryology laboratory. In some countries, like Spain, the legislation permits the creation of hESC lines using spare cryopreserved embryos from IVF cycles, or cloned embryos using the nuclear transfer technique, only for a therapeutic use. This work is preferentially being done in research laboratories, but also can be done in cell banks, like the Andalusian Stem Cell Bank. In other countries, like the United Kingdom, the legislation permits the use of fresh embryos. But in others, the legislation has banned the use of embryos to derive hESC lines (e.g., Italy).

The derivation of hESC lines and the viability maintenance requires meticulous techniques and

aseptic processes (Fig. 1). This is critical in the derivation of hESC lines due to the limited number of available embryos and the still low efficiency success rate observed to date (Hoffman et al. 2005).

The hESC are obtained in the ICM of the blastocysts. This ICM is separated and isolated from the trophectoderm using immunosurgery (Solter et al. 1975), with mechanical processes (Bongso et al. 1994), with direct culture of the blastocysts (Kim et al. 2005) or with laser techniques (Tanaka et al. 2006). These cells are seeded on an inactive fibroblastic feeder layer (Richards et al. 2003), over an extracellular matrix (MatrigelTM) (Xu et al. 2001) or over a matrix that includes protein components solely derived from recombinant sources or purified

from human material (Ludwig et al. 2006). In this process, the colonies, which are set up with undifferentiated morphology are selected and subcultured in order to obtain phenotypically and genotypically stable cell lines which are free of contamination.

In our opinion, the figure of the embryologist is necessary to derive hESC lines. Also, facilities or installations such as those described in this manuscript are required. The embryology laboratory must be in a dynamic organization structure based on the application of both quality and biosafety assurance programmes in order to obtain hESC lines for a future human transplantation.

This work should be carried out in a specific area by personnel qualified in thawing

Fig. 1 The derivation and banking of hESC lines (obtained from Cobo et al 2006b)

processes and embryo culture until blastocyst. This technique is very similar to an IVF laboratory technique, but there are differences as regards the final destination of the embryos. However, this manipulation should be done, in our opinion, in cleanrooms procuring aseptic manipulation techniques and working with a good environmental monitorization system to reduce the risk of contamination.

The fast development of regenerative medicine makes the interpretation and harmony of international guidelines necessary to assure that these new procedures and therapies are greatly accepted for clinical use in humans. This would also involve the procurement process of hESC lines which must be developed in a specific cell procurement programme from this origin; this, will be tough, dynamic and cost effective. The processing of hESC lines will be mainly developed based on the characteristics of each centre, but can take into account the general principles reviewed here. The design and the specific implementation programmes for the area of embryology within stem cell banks and other research centres with this area but which do not use IVF techniques is vital to develop embryonic cell lines in optimum conditions for future therapeutic applications.

Acknowledgement Ms. Angela Helen Barnie for the English correction of the manuscript.

References

- Association of Clinical Embryologists (ACE) (2003) Accreditation Standards and Guidelines for IVF Laboratories. <http://ace.ivf.net/ace/training.php>
- AGRBM (2005) Criteria for the recognition as an embryologist in human reproduction and continued professional education: the concept of the German Society for Human Reproductive Biology. *Alpha* 32:12–13
- American Association of Tissue Banks (2002) Standards for tissue banking, 10th edn. American Association of Tissue Banks, McLean, VA
- Andalucian Parliament Law 7/2003, October 20 (2003) about the regulation of the research with nonviable human embryos to “in vitro” fertilization. *BOJA* 210
- Antinori S, Selman HA, Caffa B et al (1996) Zona opening of human embryos using non-contact UV laser for assisted hatching in patients with poor prognosis of pregnancy. *Hum Reprod* 11:2488–2492
- American Society for Reproductive Medicine (ASRM) (2004) Reviewed guidelines for human embryology and andrology laboratories. www.asrm.org
- Bongso A, Fong CY, Ng SC, Ratnam S (1994) Isolation and culture of inner cell mass cells from human blastocysts. *Hum Reprod* 9:2110–2117
- College of American Pathologist (CAP) (2002) Laboratory Improvement. www.cap.org
- Castilla JA, Alcaide A, Bonada M, Expósito A, Luna C, Núñez R, de los Santos MJ (eds) (2006) I. Guidelines about Human and Physical Requirements in the Assisted Reproduction Laboratory. ASEBIR Clinical Embryology Notebooks, Madrid, Spain
- Cobo F, Stacey GN, Hunt C, Cabrera C, Nieto A, Montes R, Cortes JL, Catalina P, Barnie A, Concha A (2005) Microbiological control in stem cell banks: approaches to standardization. *Appl Microbiol Biotechnol* 68:456–466
- Cobo F, Cabrera C, Catalina P, Concha A (2006a) General safety guidances in stem cell bank installations. *Cytotherapy* 8:47–56
- Cobo F, Stacey GN, Cortes JL, Concha A (2006b) Environmental monitoring in stem cell banks. *Appl Microbiol Biotechnol* 70:651–662
- Cohen J, Gilligan A, Garrisi J (2004) Setting up an ART laboratory. In: Gardner DK, Weissman A, Howles CM et al (eds) Textbook of assisted reproduction techniques. Laboratory and clinical perspectives, 2nd edn. Taylor & Francis, London, UK, pp 17–24
- Commission Directive 2006/17/EC of 8 February (2006) implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells. *Official Journal of the European Union*, L 38/40
- Cowan CA, Klimanskaya I, McMahon J, Atienza J, Witmyer J, Zucker JP, Wang S, Morton CC, McMahon AP, Powers D, Melton DA (2004) Derivation of embryonic stem-cell lines from human blastocysts. *N Engl J Med* 350:1353–1356
- Department of Health (2001) A code of practice for tissue banks. Department of Health, London, UK
- EC Guide to good Manufacturing Practice (2003) Revision to Annex 1. Manufacture of Sterile Medicinal Products. European Commission. Enterprise Directorate-General
- European Society of Human Reproduction and Embryology (ESHRE) (2000) Guidelines for good practice in IVF laboratories. www.eshre.com
- European Union (2004) Directive 2004/23/EC of the European Parliament and of the Council of 31 March (2004) on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells. *Official Journal of the European Union*, L 102/48
- FSA. The Fertility Society of Australia Reproductive Technology Accreditation Committee (RTAC) (2002) Code of practice for centres using assisted reproductive technology. www.fsa.au.com/rtac/

- Hoffman LM, Carpenter MK (2005) Characterization and culture of human embryonic stem cells. *Nat Biotechnol* 23:699–708
- ICMR-DBT. Indian Council of Medical Research (2006) Guidelines for stem cell research and therapy. www.icmr.nic.in
- ISO (1999) International Standard ISO 14644. Cleanrooms and associated controlled environments. Part 1: Classification of air cleanliness. International Organization for Standardization, Geneva, Switzerland
- ISO (2000) International Standard ISO 9001. Quality Management Systems-Requirements. International Organization for Standardization, Geneva, Switzerland
- ISO (2005) International Standard ISO 17025. General requirements for the competence of testing and calibration laboratories. International Organization for Standardization, Geneva, Switzerland
- Kim HS, Oh SK, Park YB, Ahn HJ, Sung KC, Kang MJ, Lee LA, Suh CS, Kim SH, Kim DW, Moon SY (2005) Methods for derivation of human embryonic stem cells. *Stem Cells* 23:1228–1233
- Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, Crandall LJ, Daigh CA, Conard KR, Piekarczyk MS, Llanas RA, Thomson JA (2006) Derivation of human embryonic stem cells in defined conditions. *Nat Biotechnol* 24:185–187
- Mortimer D (2005) A critical assessment of the impact of the European Union Tissues and Cells Directive (2004) on laboratory practices in assisted conception. *Reprod Biomed Online* 11:162–176
- National Academy of Medical Sciences of India (NAMSI) (2005) National Guidelines for accreditation, supervision and regulation of ART clinics in India. www.icmr.nic.in
- National Academies Press (NAP) (2005) Guidelines for Human Stem Cell Research. www.nap.edu/books
- National Institute of Health (NIH) (2002) Guidance for investigators and institutional review boards regarding research involving human embryonic stem cells, germ cells, and stem cell-derived test articles
- Noda Y, Goto Y, Umaoka Y et al (1994) Culture of human embryos in alpha modification of Eagle's medium under low oxygen tension and low illumination. *Fertil Steril* 62:1022–1027
- Pharmaceutical Inspection Co-operation Scheme (2002) Recommendation on sterility testing. Pharmaceutical Inspection Convention, PI 012-1, November 1, 2002
- Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A (2000) Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nat Biotechnol* 18:399–404
- Richards M, Tan S, Fong CY, Biswas A, Chan WK, Bongso A (2003) Comparative evaluation of various human feeders for prolonged undifferentiated growth of human embryonic stem cells. *Stem Cells* 21:546–556
- Sharp J (2005) GMP and quality assurance in sterile products manufacture. In: J Sharp (ed) *Good pharmaceutical manufacturing practice*. CRC Press, Florida, USA, pp 371–404
- Solter D, Knowles BB (1975) Immunosurgery of mouse blastocysts. *Proc Natl Acad Sci USA* 72:5099–5102
- Spanish Law 14/2006, May 26th, about Assisted Reproduction Techniques 2006. BOE 126
- Spanish Royal Decree 2132/2004, about the requirements and procedures to request the development of research projects with human embryonic stem cells. 2004. BOE 262
- Tanaka N, Takeuchi T, Neri QV, Sills ES, Palermo GD (2006) Laser-assisted blastocyst dissection and subsequent cultivation of embryonic stem cells in a serum/cell free culture system: applications and preliminary results in a murine model. *J Transl Med* 8(4):20 DOI 10.1186/1479-5876-4-20
- Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, Carpenter MK (2001) Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol* 19:971–974