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The Procurement of Cells for the Derivation of Human Embryonic Stem Cell Lines for Therapeutic Use: Recommendations for Good Practice

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Abstract The donation of human embryos for the derivation of embryonic stem cell lines that may be used in the development of therapeutic products raises more complex ethical, practical and regulatory problems than the donation of embryos for non-clinical research. This review considers these issues and offers recommendations for good practice.

Keywords Human embryonic stem cells · Embryo research · Embryo donation · Regulation, Stem cell banks · Cell therapy · Stem cell therapy · Donor screening · Donor testing

Introduction

This position statement has been prepared by clinicians and scientists in the UK who are deriving embryonic stem cells (hESCs) for therapeutic use. It is intended to be a reference guide to recommended good practice. The recommendations were drafted in consultation with regulatory experts and regulators. Although centred on UK legislation, regulatory structures and practice, these recommendations are evidence-based and thus have international relevance.

The UK Medical Research Council (MRC) funded a number of centres to derive hESC lines that would be appropriately accredited and available internationally for therapeutic use. This was in recognition of the exceptional difficulty and cost incumbent with this process and the need to share good practice. Particular challenges in the derivation of hESC lines were identified related to the complex and overlapping regulatory environment in the UK. The novelty of the procedures resulted in delay and uncertainty, and sometimes the practical aspects of procurement were lost in the discussion.

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Therefore we have prepared this summary of recommendations for good clinical practice in the procurement of embryos for hESC line derivation. In the following, we first go through the different steps in the procurement and establishment of a hESC line which covers different ethical, clinical, regulatory and scientific considerations. In a second section, we focus on the implications for safety testing.

Separate recommendations which relate to the characterisation, storage, growth and banking of hESC lines are in preparation elsewhere [1, 2].

Steps in hESC Line Procurement

The pathway that leads from the procurement of the embryos (usually created initially for fertility treatment) to the use of ES cell lines in therapy traverses independent clinical and laboratory processes with different priorities. Related legislation and regulation of each stage reflects these individual priorities and thus potential conflict arises.

From Gametes to Embryos

Regulation of ART

The UK has comparatively permissive legislation governing the creation and use of embryos for both clinical and research purposes, supported by a substantial regulatory framework including general regulations relating to clinical care and laboratory procedures. Clinics providing ART treatment must be licensed by the Human Fertilisation and Embryology Authority (HFEA), established under the Human Fertilisation and Embryology Act 1990 as amended in 2008. Under this Act, a license can also authorise "activities in connection with the derivation from embryos of stem cells that are intended for human application". However, such authorisation can only occur under a license for research (Schedule 2, paragraph 3). Thus the HFEA has dual oversight of both the clinical ART procedures and the use of embryos for hESC derivation under a project of research. The legislation provides no power for the HFEA to authorise the procurement of embryos for the derivation of hESCs for use in treatment outside a research project. Finally, the HFEA can only authorise activities in connection with the procurement of embryos for the derivation of stem cells from embryos but the Act is silent on the scope and definition of derivation activities.

Donors Perspective

Almost all embryos are created in the clinical treatment setting with the intention that they will be used to establish a pregnancy. The best quality embryos are transferred to the uterus. Some of the remaining embryos are either not suitable or not wanted for cryopreservation. If not used in research, these 'fresh' embryos would be discarded.

Some remaining embryos of good quality may be cryopreserved for the patients' future fertility treatment. Sometimes couples subsequently decide that these embryos are no longer required for their fertility treatment. This is usually because a pregnancy has resulted from the initial treatment and they do not want a further child. Although the option to donate to other couples is given, this is not always accepted. Couples may then consider having the embryos destroyed or donated for research purposes. Immediately we therefore have two scenarios in which there are practical differences. There may be several years between cryopreservation and donation, whereas the donation of fresh embryos is contemporaneous with their creation. Although both sources of embryos are legal and appropriate for donation for hESC derivation, the procurement processes need to be modified to take account of these practical differences. This is particularly relevant to the testing of donors and the taking of a medical history (discussed below).

The experiences of patients undergoing fertility treatment who donate embryos to research have been well studied [3–7]. Overall, most patients agree to donate but from their perspective, fertility treatment is paramount. Those procuring embryos for research must be aware of and sensitive to this and the implications for the consent to donation procedures. **(→ Recommendation I)**

Ethical Principles of Donation, Including Consent

The background of hESC derivation is of ethical and political sensitivity. From the viewpoint of those deriving ES cell lines, we recognise the ethical debate but note that most of the issues raised in this context are not unique in medical research [8]. New scientific developments must be translated into new therapies without undue delay but must also be within ethically acceptable practice. For those who have fundamental objections to any use of human embryos outside of the context of fertility treatment, the priority is to stop the research. The resulting high profile media interest has resulted in political anxiety. It is within this environment that complex regulatory decisions are being made. Despite the similarity of international debates, the variance in conclusions has resulted in major legislative and regulatory differences between countries.

The appropriate procedures for taking consent for medical research are fairly universal. The HFEA proscribes five 'special' pieces of information that must be imparted to prospective donors of hESC lines (no control over use, indefinite use, deposit in the UK Stem Cell Bank, potential commercial use, and potential patenting). The US National Institute for Health has also stipulated guidelines [9]. Thus, different countries and indeed different sponsors and recipients

of clinical treatments are likely to have different concepts of what constitutes acceptable ethical procurement. These questions are not linked in any way to the safety and efficacy of a hESC-based treatment and therefore cannot be made universal. **(→ Recommendation II)**

From Embryos to Stem Cells

Scientifically, the term embryo is non-specific; there are many words used to describe morphologically distinct stages of development both before and after fertilisation. In UK legislation "references to an embryo include an egg that is in the process of fertilisation or is undergoing any other process capable of resulting in an embryo" [10]. Thus an egg that undergoes parthenogenic activation or cloning is considered to be an embryo. In the USA, legal opinion related to whether federal funds could be used for hESC research defined an embryo as "an organism that when implanted in the uterus is capable of becoming a human being" [11]. Lay understanding is reflected in the Oxford English Dictionary which refers to an embryo as "The offspring of an animal before its birth" and "..restricted to the fetus in uterus before the fourth month of pregnancy". This is an ongoing, shifting debate reflecting the flexibility with which the terminology is used to meet stakeholder objectives.

Stem cells are typically derived from the inner call mass of early stage embryos by disaggregating the embryo [12] although other technologies are possible [13]. As the HFE Act generally applies to gametes and embryos, there is no indication that the Act was intended to apply to other cell types or that 'activities in connection with derivation' under this regime encompasses activities beyond the procurement and culture of embryos.

We therefore assume that 'activities in connection with derivation' are only those activities that precede the derivation event and that the remit of a license under the HFE Act does not extend to those cells that remain after the embryo is disaggregated and does not apply to cells removed from the embryo. This interpretation is supported by a joint statement published by the HFEA, HTA and MHRA in May 2007 which stated that "[d]uring the cell line derivation process the embryo is dissociated and it is at this processing stage that the HTA regulatory remit begins and the HFEA's regulatory remit ceases" [14].

Embryo Quality

Embryo quality is assessed firstly based on morphologic criteria: noted are the number of blastomeres at specific time points, cytoplasmic fragmentation, the size of blastomeres, presence of vacuoles, granularity, thickness of the outer layer (or zona pellucida), a 5-grade scale [15] is sometimes used for 3-day embryos; another scale for day 5 embryos correlates degree of expansion and hatching to inner cell mass and trophectoderm development [16]. Such methods are subject to observer variability [17] and efforts are being made to reduce this variability including the use of automation [18, 19]. Other methods are based on pre-implantation genetic screening mainly focussed at aneuploidy or going further using Comparative Genomic Hybridisation or the use of non-invasive metabolic assays. The value of these screens is not universally accepted.

Although good quality embryos are more likely to result in a pregnancy, poor quality embryos may be transferred without apparent detriment to the health of any resulting offspring [20]. In the UK, the HFEA does not specify the quality of embryo prior to transfer for fertility treatment. Consequently, we would not recommend that specific tests are used to exclude certain embryos from hESC line development, but we would suggest that abnormally fertilised embryos and embryos subject to an aneuploidy are not used for the derivation of a hESC line for therapeutic use. **(→ Recommendation III)**

Derivation Efficiency

The inherent ability of human embryo to develop in vitro, particularly those created from gametes of couples who already have a fertility problem, is relatively poor. The development rate to blastocyst from the donation of fresh surplus embryos is about ~30–50% [21] and from frozen embryos is ~60–70% [22]. From good quality blastocysts the derivation rate of hESC lines is <30%. However, under conditions necessary to derive hESC lines of therapeutic grade, the success rate has been reported to be much lower at 4.75% [23]. Thus, only a minority of donated embryos are likely to result in a hESC line in a derivation programme. It is therefore neither practical nor financially appropriate to undertake the full screening procedures on all donor couples since most donations will not result in a potential therapeutic product. In this respect, there is a clear difference between, for example, the donation of blood and blood products or organs for transplantation, and intended use of embryos for stem cells. This illustrates the need to implement regulations appropriately.

From Stem Cells to Medicines

From Stem Cells to Cell Lines

It is currently a standard condition of a HFEA 'derivation' licence, that a sample of all hESC lines derived in the UK must be deposited in the UK Stem Cell Bank [24]. The Bank as a public sector curator also reviews and interprets regulatory compliance requirements [25].

However, there is an intervening stage during which cells are growing in the laboratory but are not yet fully characterised as hESC lines, and indeed may never become lines. It is therefore important, for compliance purposes, to distinguish between hESC and 'bankable' hESC 'lines'. A usual definition is a "defined unique population of cells obtained by culture from a primary source through numerous generations" [26]. It is generally assumed that a 'cell line' arises from a primary culture at the time of the first successful sub-culture. A 'successful' subculture in hESC line derivation, however, is not immediately obvious. Cell line cultures can be 'finite' or 'continuous'. A hESC cell line is generally assumed to be universally continuous [27]. If a hESC culture fails to proliferate after a certain growth period, the cells in culture are not assumed to be a fully formed 'cell line'. Here we suggest that the presence of a 'line' can be assumed if cells have multiplied from just a few hESCs to reach a mass of more than 3 million cells. This allows for sufficient testing for a minimum of 3 markers of pluripotency and 2 vials to be cryopreserved at 1 million cells per vial.

For the purpose of implementation of regulatory requirements, we therefore make the following recommendations:

1) During the period of development between the growth of the first cell colony and completion of the characterisation that may take at least 12 weeks [23], we recommend that these early colonies be called human embryonic stem cells (hESCs).
2) When hESCs are still able to proliferate, they should be called a hESC line after they have been proven to expand to 3 million cells. At this stage, banking can be commenced.

(→ Recommendation IV)

From Cell Lines to a Cell Bank

When the existence of a cell line has been ascertained, it should be banked in accordance with procedures and documentation according to internationally accepted criteria [28]. In addition the requirements of the UK Stem Cell Bank apply. We do not discuss those matters in this article, but from a procurement perspective we make the following recommendation:

The institution holding the master cell bank should request appropriate reassurances that sufficient information regarding the procurement process is held securely at the procuring institution. As long as that institution is operational and maintains a link with the organisation where the master cell bank is held, it is not necessary or desirable to transfer detailed procurement information to the Bank. This safeguards donor confidentiality and traceability.

(→ Recommendation V)

From Banked Cell Lines to Medicinal Products

A question arises whether the cells are also regulated according to formal legal criteria. In the UK, the procurement, storage and use of cells intended for therapeutic use are regulated by the Human Tissue Authority under the Human Tissue (Quality and Safety for Human Application) Regulations 2007. These Regulations represent the UK's implementation of those aspects of the European Directive 2004/23/EC on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells that apply to tissue and cells other than gametes and embryos. However, the regulations apply only to cells 'intended for human application'.

Freshly derived hESC are not themselves intended for immediate application, but require further expansion and potentially manipulation to achieve clinical utility. Such expansion and manipulation however, would render the hESC an 'advanced therapy medicinal product' (ATMP) according to Directive 2001/83/EC and Regulation EC No.1394/2007. Activities dealing with ATMP however, are under the remit of the Medicines and Healthcare Products Regulatory Authority (MHRA) and not the HTA. It is therefore imperative that the HTA or any similar organisation regulating the 'stop-gap' storage between hESC line creation and ATMP construction is liaising appropriately with the regulatory agencies 'downstream'.

Manufacturers of ATMP need to "to comply with the principles and guidelines of good manufacturing practice for medicinal products and to use as starting materials only active substances, which have been manufactured in accordance with the detailed guidelines on good manufacturing practice for starting materials" (2001/83/EC Art. 46 (f)).

According to Annex 1, PART I Module 3.2.1.1.(b) of that Directive starting materials shall mean all the materials from which the active substance is manufactured or extracted, expressly including any substance of biological origin. This would include hESC lines—for treatments based on them, but does it include pre-line hESC, embryos, and gametes?

According to Annex 1, PART IV 2.b.1.1. "Starting materials and each step of the manufacturing process shall be fully documented including viral safety aspects" and "characteristics of the human source such as age, sex, microbiological status, exclusion criteria and country of origin shall be documented"—it is unclear here who the 'human source' is—e.g. if one were to focus on the donors, 'sex' would not be a relevant criterion.

It is generally asserted that where cells are procured as 'starting materials' for an ATMP, their donation, procurement and testing must comply with Directive 2004/23/EC [29]. However, it is unclear what this compliance entails specifically, as we shall discuss below.

It needs to be borne in mind that these requirements are presented as an application for marketing authorisation pursuant to Articles 8 and 10 (1) of the Directive—in this context the ultimate decision on safety and efficacy rests with the MHRA but after clinical trials any hESC based ATMP would ultimately need to be submitted to yet another body—the European Medicines Agency (EMA) for marketing authorisation through the centralised procedure.

The fact that, there are at least five 'official' bodies part of the process (HFEA, HTA, UKSCB, MHRA, EMA) is peculiar to the UK, but represents practical processes and spheres of regulatory scrutiny that are relevant to international practice. A recent report from The Academy of Medical Sciences proposes that embryo research should be regulated under a new framework and regulator that would oversee all medical research [30]. The politically and administratively transient nature of regulatory particulars [31] argues in support of standards for good clinical and laboratory practice that will transcend the existence of regulatory organisations.

It is also evident that what particular regulatory requirements apply to hESC is subject to interpretation based on some uncertain scientific boundaries. Regulatory regimes need to be linked with appropriate and proportionate controls of identifiable risks at the different stages of the process. **(→ Recommendation VI)**

Testing

Opportunities for testing fall into five phases:

(a) testing the donor;
(b) testing the donated gametes
(c) testing the donated embryo;
(d) testing the hESC line
(e) testing the final hESC-based product prior to administration

Only (a) (b) and (c) are closely connected to the procurement process, which is the focus of this paper. Testing donated gametes (b) or the embryo (c) to exclude infection is technically very difficult. Consequently, and in line with legal expectations, we will focus on the donor testing considerations in the procurement phase.

Assessment of the Donor

As mentioned above, there is at least a presumption that the requirements of Directive 2004/23/EC on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells apply to hESC lines and this is confirmed by Recital (7) of that Directive.

The Directive has a 'daughter' implementing Directive 2006/17/EC regards certain technical requirements for the donation, procurement and testing of human tissues and cells.

"Reproductive cells" (apparently defined in the 'mother Directive as sperm and eggs, but not embryos) are defined in the 2006/17/EC as "all tissues and cells intended to be used for the purpose of assisted reproduction". This implies that that gametes and embryos donated for use in the derivation of hESC lines will not be considered 'reproductive cells' under this Directive. As we have discussed, this is at odds with the clinical reality, where almost all gametes are originally intended to be used in assisted reproduction. The question arises why this distinction should matter.

Although the result of gamete fusion leads to the creation of a new entity that does not conserve many of the characteristics and potential contagions from any one donor, we see no legal basis for considering the embryo itself as a 'donor'. Consequently, donors of gametes for eventual hESC line derivation could be considered 'Allogeneic living donors'. Such donors, according to Directive 2006/17/EC "must be selected on the basis of their health and medical history, provided on a questionnaire and through an interview performed by a qualified and trained healthcare professional with the donor".

The Directive confirms, however, that "Selection criteria for allogeneic living donors must be established and documented by the tissue establishment […] based on the specific tissue or cells to be donated, together with the donor's physical status and medical and behavioural history and the results of clinical investigations and laboratory tests establishing the donor's state of health". This stresses the importance of clinical judgement in the selection of donors.

One can distinguish between selection by consideration of medical history and laboratory-based screening.

Medical History/Selection Criteria

A medical history is taken to screen and select potentially suitable donors who have a low risk of transmission of adverse factors to a recipient. With the exception of a very small minority who are known to have viral infections, ART patients are low risk donors because they are attending for fertility treatment, generally with a long term partner [32]. Unlike healthy volunteer donors for other purposes (e.g. blood donation), patients attending for ART treatment will have documentation of a full and recent medical history. This will include many of the questions that are required for the selection of potential blood donors. Examples of the information that should be routinely available from ART medical records are given in Table 1. For this reason we do not recommend that there is a need for specific medical history to be taken from embryo donors additional to that recorded as part of their fertility treatment.

Table 1 Medical history from embryo donors

A routine medical history is taken at the time that the embryo is created by the clinician responsible for the patient. The following information should be available in the contemporaneous medical records relating to both partners.

- Have the donors had negative tests for HIV1/2, Hep B and Hep C in the 12 months preceding creation of the embryo?
- Have the donors had any signs of infection immediately preceding the creation of the embryo?
- Had either partner recently been in a country with an endemic risk of e.g. tuberculosis, malaria, West Nile fever, SARS, typhoid fever, toxoplasmosis, rabies, encephalitis, Lyme disease or brucellosis?
- Had the donor ever had a serious infection e.g. tuberculosis, malaria, West Nile fever, SARS, typhoid fever, toxoplasmosis, rabies, encephalitis, Lyme Disease or brucellosis?
- Did the donor have a neurosurgical operation for tumour or cyst of the spine/brain or implantation of dura mater before 1992?
- Had the donor ever received human pituitary extract?
- Were the donors taking any medication other than standard ART related treatment at the time preceding the creation of the embryo?

When frozen embryos are donated, the relevant history as recorded in medical records will have been taken at the time of the storage, which may have been several years before these embryos are donated. The history at the time of the subsequent donation may not be relevant and hence unnecessary. For instance, there have been suggestions that we ask donors questions about sexual history as is required from blood donors to identify those at risk of sexually transmitted disease [33]. We question the relevance of such questions to embryo donors since they are most likely to be with a long-standing partner at the time of embryo creation. Furthermore, it is inappropriate to take such history within the clinical setting, where generally the history is taken as a couple and the patient may withhold information about previous sexual experiences. Thus we do not recommend that a sexual history is necessary prior to embryo donation.

Screening Tests—Minimal Testing

The purpose of testing potential donors (identified from the medical history as being of low risk) is to ensure, as far as possible, that they do not carry transmissible infectious agents.

Directive 2006/17/EC sets out the biological tests which must be performed for all donors. The Directive draws several distinctions: firstly, a strong distinction between donors of reproductive cells and other donors. According to Annex III, donor selection criteria and laboratory testing do not need to be applied in the case of partner donation of reproductive cells for direct use. Reproductive cells that are processed and/or stored and reproductive cells that will result in the cryopreservation of embryos must be tested for HIV 1 and 2, hepatitis B and hepatitis C.

These provisions apply only to partner donations (between a man and a woman who declare that they have an intimate physical relationship). In the case of non-partner donations the donors must be negative for HIV 1 and 2, HCV, HBV and additionally syphilis on a serum or plasma sample. This set corresponds to the minimal testing requirements for donors of all other tissue as defined in Annex II.

Evidence suggests that the incidence within the ART population of seroconversion to HIV or hepatitis infection within 1 year of a negative test is exceptionally low, if it occurs at all [32]. Since this is only a screening test to identify suitable donors, we recommend that, within this donor population, negative screening within 1 year of the creation of the embryo is acceptable.

The question arises whether testing for syphilis (active infection with Treponema pallidum) should be a requirement for hESC line derivation as it is not required for partner donations of reproductive cells. Treponema pallidum grows on tissue cultures under anaerobic conditions (O_2 1.5%) much lower than the 5–20% routinely used for the growth of embryos and stem cells [34]. Moreover, the length of this motile spirochete (6–20 μm) is similar to a hESC so would be visible within the colony using dark field microscopy.

Thus we submit that, in light of the particular product development issues discussed below, testing for syphilis is not required. **(→ Recommendation VII)**

Screening Tests—Further Testing

Directive 2006/17/EC also sets out additional tests which may be appropriate in certain circumstances (e.g. RhD, HLA, malaria, CMV, toxoplasma, EBV, Trypanosoma cruzi).

The suitability of these and other tests for hESC lines is debatable. Certainly, it is prudent for laboratories only to work with cells from donors who have negative screening and thus we support the view that appropriate screening tests are carried out to identify unsuitable donors.

It is likely that other transmissible agents will be identified in the future, knowledge of which may be relevant for cellular-based products that have a long shelf life, such as hESC lines. We therefore suggest that the most useful time to engage in extensive testing is further downstream in the product development.

Donor Reference Sample

If the cells or tissue are intended for direct and immediate use (e.g. organ donation), surrogate/reference samples are tested, such as blood from the donor, and that sample is retained to enable traceability should a problem arise that could be related to transmission of infection, or the identification of (for example) a genetic abnormality that

may be relevant to the donor or to others who may have received the donation. This requirement is particularly relevant when the donated tissue is no longer available as it has all been passed to the recipient (e.g. whole organ donation), or where the donor is no longer be available as in *post mortem* donation.

For embryo donors, a sample of the original hESC line that was the starting material for the therapeutic product will have been retained, accessible and traceable. Being an expanded line, there will be sufficient material for pre-release testing.

Blood sample retention in hESC procurement is not only unnecessary to protect the ATMP recipient, it also puts a strain on donors and clinical personnel: as explained above, hESC lines are a rare by-product of fertility treatment. To procure a blood sample from each and every patient going through the ART process, and to store these samples for unspecified periods (potentially extensively in the case of sperm donors) is a burden that needs to be weighed against the utility of maintaining such a sample, which is, we find, very limited indeed.

Accordingly we find no reason to recommend that blood samples be retained from the embryo donors **(→ Recommendation VIII)**.

Product Testing

Products for clinical purposes need to be tested prior to release to provide, as far as possible, reassurance that the product will not harm the recipient.

Where an organ or tissue is processed before being given to the recipient, the processed product is tested before release. Comprehensive procedures are described elsewhere and include tests for the detection of human viral pathogens (HIV 1 and 2 provirus, HTLV 1 and 2 provirus, HAV, HBV, HCV, HHV6, HHV7, HHV8, hCMV, EBV, SV40 and B19) by Real Time Polymerase Chain Reaction (PCR). Such tests also relate to the identification of risks that could originate from critical materials used during the prolonged culture period as well as from the primary source [35]. Final product testing for the exclusion of viral and other transmissible infective agents should be the only decisive release criterion related to such factors.

ATMPs are novel products subject to extensive pre-clinical testing. Directive 2001/83 anticipates evidence of, *inter alia*, biodistribution, persistence and long-term engraftment, oncogenic transformation and cell/tissue lineage fidelity, and crucially potential immunogenic and immunotoxic effects. There are strict oversight requirements for the manufacturing of ATMPs to control process related impurities. In many cases, the hESC line-based intervention will not rely on the application of 'unadulterated' hESCs, but will modify and differentiate the cells to suit a particular therapeutic profile. Thus, the 'end product' will be tested extensively from different angles to ensure that it is safe before first-in-man trials on an initially small number of clinical trial subjects. This context is altogether different from the routine tissue transplantation context where the tests discussed above are the only controls used. We suggest that a compelling case can be made that it is in the best interests of regenerative medicine and its patients that testing requirements and regulatory controls are focussed on the 'applied' end of hESC clinical use, rather than on their procurement. **(→ Recommendation IX)**

Summary

Donation for hESCs is in many ways not comparable with donations for other tissues and organs. hESCs traverse different regulatory spheres which can lead to legal ambiguities. It is not possible or appropriate to resolve these complexities by applying every feasible regulatory provision, however remote its relevance. We suggest that for various reasons an over-reliance on testing at the procurement stage is inappropriate in the context of securing the safety of hESC based treatments. Instead, a risk-based focus needs to be placed on protecting the recipients of hESC-based treatments 'downstream'.

Consequently, we make the following recommendations for the derivation of hESC lines for clinical use.

I. The procurement of hESCs occurs in the context of infertility treatment. These cells are a very rare by-product of a different clinical process which cannot and should not be made subservient to hESC procurement. This context must be given appropriate consideration by clinicians, scientists and regulators.
II. Ethical discussions relevant to the procurement process should be conducted in accordance with the norms and laws at a local and individual level. However, these considerations should remain firmly distinct from the technical and safety assessment of best practice in hESC procurement for clinical use.
III. Embryos of different quality can be used for hESC line establishment, but abnormally fertilised and aneuploid embryos should be excluded.
IV. For regulatory purposes, hESCs are pluripotent cells removed from the embryo. If those cells continue to proliferate after expanding beyond 3 million cells, they can be defined to be cell lines.
V. If the procurement organisation and the organisation where the master cell bank is kept (the Bank) are two distinct entities, the Bank needs to retain sufficient records to allow traceability, but detailed information relating to procurement may remain at the procurement site.

VI. Any organisation regulating the interim storage between hESC line derivation (as defined above) and the manufacture of an (investigational) medicinal product needs to liaise appropriately with the regulatory agencies ultimately responsible for such products.

VII. The medical history and tests that are required for routine ART treatment are suitable and adequate screening tools to identify low-risk donors of embryos. There should not be additional obligatory requirements for donors of embryos for the derivation of therapeutic grade hESC lines.

VIII. There are no evidence-based reasons to retain blood samples from embryo donors. This can be replaced by appropriate samples from the cell line.

IX. Testing during procurement stages is only ever serviceable as background information. Regarding the quality and safety of an ATMP, the only ultimately important time-point is upon reseal of the product and any testing should be oriented as closely as possible towards that event.

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