

Licence Committee - minutes

Thursday 14 January 2016

HFEA, Finsbury Tower, 103-105 Bunhill Row, London, EC1Y 8HF

Centre 0246 (The Francis Crick Institute at Mill Hill) – application for research licence renewal for research project R0162

Committee members	Andy Greenfield (Chair) Bishop Lee Rayfield (Deputy chair) Kate Brian Anita Bharucha	
Members of the Executive	Trent Fisher Juliet Tizzard	Secretary Director of Strategy and Corporate Affairs (observer)
Legal Adviser	Sarah Ellson	Fieldfisher
Observers	None	

Declarations of interest:

- members of the committee declared that they had no conflicts of interest in relation to this item.

The committee had before it:

- 8th edition of the HFEA Code of Practice
- standard licensing and approvals pack for committee members

The following papers were considered by the committee:

- Renewal inspection report
- first peer review
- PR's response to the first peer review
- first peer review amended following PR's response (Section 6 amended only)
- PR's response to the amended first peer review

The following papers were considered by the committee (continued):

- second peer review
- PR's response to the second peer review
- cover letter from the PR
- application form
- supporting publications
- licensing minutes from the past three years

The following papers were tabled and considered by the committee:

- second peer reviewer's comments (following PR's response to the second peer review)
- clarification (wider background) email chain from PR dated 13 January 2016
- second clarification (wider background) email chain from PR dated 13 January 2016
- comments from second peer reviewer to committee (following PR's response to second peer review)

1. Consideration of application

- 1.1. The committee was presented with four additional papers regarding this application. On review of the papers the committee agreed for the papers to be tabled.
- 1.2. The committee noted that research project R0162 'Derivation of stem cells from human surplus embryos: the development of human embryonic stem cell (hES) cultures, characterisation of factors necessary for maintaining pluripotency and specific differentiation towards transplantable tissues' was first licensed in 2005. The current licence is due to expire on 26 March 2016.
- 1.3. The committee noted that the application was for a renewal of licence R0162-7-c. The application included adding additional research activities and objectives to the licence, including the use of a gene editing technique, CRISPR/Cas9, in human embryos.
- 1.4. The committee noted that the application also included an amendment of the research project title from:
 - Derivation of stem cells from human **surplus** embryos: the development of human embryonic stem cell (hES) cultures, characterisation of factors necessary for maintaining pluripotency and specific differentiation towards transplantable tissues.to:
 - Derivation of stem cells from human embryos: the development of human embryonic stem cell (hES) cultures, characterisation of factors necessary for maintaining pluripotency and specific differentiation towards transplantable tissues.
- 1.5. The committee noted that two peer reviews were submitted with the application and that both peer reviewers undertook an extensive review of the application and requested further information from the PR. The committee carefully considered the points raised by each peer reviewer.
- 1.6. The committee noted the PR had provided further information as requested and had sought to address the concerns raised by the peer reviewers. In the committee's view the detailed responses from the PR greatly assisted in providing the committee with a comprehensive outline of the proposed research project.
- 1.7. The committee noted that both peer reviewers raised concerns about the availability of zygotes or early cleavage stage embryos, which they considered would be needed for the proposed CRISPR/Cas9 gene editing. The committee noted that this might raise questions about the feasibility of the research; however, this potential concern was not a matter that the committee considered should affect its decision as to whether to renew the research licence. The committee noted that in her responses to the peer reviewers the PR had set out a very clear step-by-step approach to the proposed research, including estimated numbers of embryos required at each stage, with progression to the next step being sequential and subject to results in the earlier stages.
- 1.8. One of the peer reviewers had suggested using alternative techniques for gene disruption, such as gene expression knock-down using RNA interference (shRNA), instead of CRISPR/Cas9. However the Committee was satisfied that CRISPR/Cas9 had, in other studies, produced results suggesting that it was a highly efficient and targeted method of gene disruption, potentially superior to other techniques that were available. On balance, the proposed use of CRISPR/Cas9 was considered by the Committee to offer better potential for success, and was a justified technical approach to obtaining research data about gene function from the embryos used. The committee noted that the Executive considered that the PR's comprehensive response to the second peer reviewer addressed the key concerns and that the Committee considered that, in raising the prospect of alternative gene disruption techniques in their response to the PR (tabled at the meeting), the peer reviewer was supportive of the idea of using techniques to study the function of particular genes in human embryos.

- 1.9.** The committee noted that at the time of inspection, which took place on 20 October 2015, there was one area of practice requiring improvement: the centre had not yet received ethics approval covering the additional activities described in the licence renewal application. It noted that ethics approval from the Research Ethics Committee (REC) is already in place for the research activities in the existing licence.
- 1.10.** The committee noted that the centre had initially received advice from the REC that it should wait for the HFEA to grant a licence including the additional research activities before applying for ethics approval. After several discussions between the PR and the REC, the REC had now allowed the centre to submit an application for ethics approval. The centre is currently awaiting the application to be considered. The PR for the centre has confirmed that no research activity for which research approval had not been provided will take place until the research project has received REC approval.
- 1.11.** The committee noted the advice from the legal adviser who stated that the committee could either consider adjourning its determination of the application or, if the committee was inclined to approve the application, consider placing an additional condition on the renewed licence. Such a condition could limit research activities until approval for them had been obtained from an appropriately constituted research ethics committee and evidence of this had been provided to the HFEA Executive.
- 1.12.** The committee had regard to its decision tree. It noted that this was a renewal application but that it should be satisfied as to the same matters as would be considered on an initial grant of a research licence. It accepted the advice of its legal adviser that it was not bound in any way to renew/grant the licence but, that to the extent it reached conclusions that might appear inconsistent with previous decisions of the Licence Committee, there was a heightened obligation to provide reasons for any such apparent difference. The committee was satisfied that the application was submitted in the form required and contained the supporting information required by General Directions 0008 except for evidence of ethics approval from the REC. It was satisfied that the appropriate fee had been paid. The committee noted that the application was made by the Person Responsible (PR) for a single research project with a number of objectives.
- 1.13.** The committee noted that patient information and consent forms had previously been submitted to the HFEA and were considered satisfactory by the Executive. The committee noted that the Research Ethics Committee would also have to carefully consider the patient information and consent forms and in particular whether these adequately covered the additional research activities including gene editing.
- 1.14.** The committee was satisfied that the PR possesses the required qualifications and experience and that the character of the PR is such as is required for supervision of the licensed activities. It was further satisfied that the PR will discharge their duties under section 17 of the Act.
- 1.15.** The committee was satisfied by the inspectorate's statement that the premises are suitable for the conduct of licensed activities.
- 1.16.** The committee was satisfied that the renewed research licence would not apply to more than one research project and that the activities applied for, permitted under the Act, are storage of embryos, keeping embryos and use of embryos. It noted that this research application did not include any application for the creation of embryos and that this activity did not, and would not, form part of the licensed activities for this project if renewed.
- 1.17.** The committee found that the use of human embryos is necessary because the proposed research aims to study the role of gene products in human embryos that are not present in mouse embryos at the same stage; moreover, human embryonic development is significantly different to that of animal model species in a number of respects and, additionally, not all functions required for preimplantation development can be modelled in embryonic stem cells. The Committee was also satisfied that the number of embryos which might be used in the research was justified by the

PR, and it was reassured by the detailed information from the PR about how embryos would be used in a limited way at each stage of the intended research.

1.18. The committee was satisfied that the activities to be licensed are necessary or desirable for the following purposes, specified in paragraphs 3A(1) and 3A(2) of Schedule 2 to the Act, for the following reasons:

- developing treatments for serious diseases or other serious medical conditions:

This study may facilitate, in the long term, the development of treatments for serious diseases or other serious medical conditions. The human embryonic stem cells (hESC) derived may also be used as a drug discovery platform for the testing of drugs and for the future development of GMP hESC which might be used directly in cell replacement therapies.

- increasing knowledge about the development of embryos:

The genes and proteins the team studies, eg pluripotency genes, and their role in human preimplantation development, may provide insight into the mechanisms of human embryo development.

- promoting advances in the treatment of infertility:

The committee noted that this was an additional purpose to be added to the research licence. It was satisfied that the genes or proteins the team will be studying may, in the long term, be important in understanding human embryo development and in developing biomarkers of embryonic health which might be used in clinical IVF treatment.

1.19. The committee was satisfied that the proposed research project does not involve any prohibited activities and specifically would never involve:

- placing in a woman any non-permitted embryos (including embryos which may have been subject to gene editing techniques) or non-permitted eggs or sperm; or,
- keeping or using an embryo after 14 days from creation or the appearance of the primitive streak if earlier than the 14 day period.

1.20. The committee noted that the proposed research does involve the derivation of human embryonic stem cells but that these are not intended for human application.

1.21. The committee noted that the recommendation from the inspectorate was that the centre's research licence be renewed for a period of three years with the following activities:

- keeping embryos
- use of embryos
- storage of embryos

1.22. The committee noted that most of the research project had been running since 2005 and that the PR could show progress was being made in the work. The research project had previously been licensed for three-year periods. The Committee did not, in these circumstances, consider it would be necessary or appropriate to grant a shorter period for the licence renewal and, in particular, noted the staged approach to gene editing planned by the PR.

1.23. The committee noted the Executive also recommended the committee approve the amendment to the project title.

2. Decision

2.1. The committee agreed to renew the research licence for project R0162 at centre 0246 for a period of three years.

- 2.2.** The committee agreed that, because part of application has not yet had research ethics approval, the following condition will be placed on the licence:
- None of the additional research activities are to be undertaken until approval for them has been obtained from an appropriately constituted research ethics committee and evidence of this has been provided to and acknowledged by the HFEA Executive.
- 2.3.** The licensed activities are:
- keeping embryos
 - use of embryos
 - storage of embryos
- 2.4.** The committee approved the amendment of the project title to:
- Derivation of stem cells from human embryos: the development of human embryonic stem cell (hES) cultures, characterisation of factors necessary for maintaining pluripotency and specific differentiation towards transplantable tissues.
- 2.5.** The committee requests that the Research Information Sheet and Data Sheet which the PR is required to submit annually to the Authority set out in paragraph 4 of General Directions 0002 be submitted to the Licence Committee for consideration.
- 2.6.** The committee request that any further applications or renewals submitted for R0162 are to come before the Licence Committee.
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3. Chair's signature

- 3.1.** I confirm this is a true and accurate record of the meeting.

Signature



Name

Andy Greenfield

Date

28/01/2016

Research renewal inspection report



Purpose of this inspection report

The HFEA licenses and monitors establishments undertaking human embryo research. This is a report of an inspection, carried out to assess whether this centre complies with essential requirements when carrying out such research. Licences for individual research projects can be granted for up to three years and this report provides information on the centre's application for a renewal of its existing licence. Our Licence Committee uses the application and this report to decide whether to renew the licence and, if so, whether any additional conditions should be applied to the licence.

Date of inspection: 20 October 2015

Purpose of inspection: Renewal of a research licence.

Inspection details:

The report covers the performance of the centre since the last inspection, findings from the inspection, and communications received from the centre.

Inspectors: Douglas Gray, Louise Winstone

Date of Licence Committee: 14 January 2016

Centre details:

Project title	Derivation of stem cells from human surplus embryos: the development of human embryonic stem cell (hES) cultures, characterisation of factors necessary for maintaining pluripotency and specific differentiation towards transplantable tissues
Centre name	The Francis Crick Institute at Mill Hill
Centre number	0246
Research project number	R0162
Centre address	New Polio Building, The Ridgeway, London, NW7 1AA
Person responsible	Kathy Niakan
Licence holder	Joachim Payne
Treatment centres donating to this research project	Bourne Hall, 0100 Cambridge IVF, 0051
Date licence issued	27 March 2013
Licence expiry date	26 March 2016
Additional conditions applied to this licence	none

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Brief description of the centre and its licensing history:

The Francis Crick Institute at Mill Hill is a research only centre and holds a HFEA licence for research project R0162. This project was first licensed in 2005. The current licence was issued by a Licence Committee for three years and expires in March 2016. There are no additional conditions on the licence. The premises were last inspected in November 2014 as part of a routine announced interim inspection.

The research licence renewal application includes additional research activities and objectives compared to the research currently licensed, including the use of gene editing techniques (CRISPR/Cas9) in human embryos.

Summary for licensing decision:

Taking into account the essential requirements set out in the Human Fertilisation and Embryology (HF&E) Act 1990 (as amended), the HFE Act 2008 and our Code of Practice (CoP), the inspection team considers that it has sufficient information to conclude that:

Administrative requirements:

- the centre has submitted an appropriately completed application form
- the centre has submitted the supporting information required by General Direction 0008, including patient information and consent forms, although a recommendation has been made in relation to the ethics approval submitted.
- the application has designated an individual to act as the person responsible (PR)
- the proposed licence applies to one project of research
- the centre has submitted fees to us in accordance with requirements.

Research activities applied for:

An application has been made for the following activities for the purpose of research:

- keeping embryos
- use of embryos
- storage of embryos

The proposed research project does involve the derivation of human embryonic stem cells but as these are not intended for human application research licence conditions R41-89 do not apply to this research project.

Purposes for which research activities may be licensed:

The activities specified above are required for the following purposes, as defined in Schedule 2 3A (1) and (2) of the HFE Act 1990 (as amended):

- developing treatments for serious disease or other serious medical conditions
- increasing knowledge about the development of embryos
- promoting advances in the treatment of infertility

The Committee should note that the last of these purposes is a new addition to this licence.

Prohibited research activities:

The activities to be licensed are not prohibited by the HFE Act 1990 (as amended) and do not include those activities specifically prohibited by Sections 3, 3ZA, 4 or 4A, or by Schedule 2, paragraph 3 of this Act.

Use of embryos:

The PR considers the use of human embryos necessary; this is supported by a peer reviewer. The other peer reviewer required more information before accepting the use of embryos as being necessary. The PR has provided a comprehensive response to the peer reviewer which is discussed further in the 'Peer review' section of this report.

PR considerations:

The PR is suitable and has discharged her duty under Section 17 of the HFE Act 1990 (as amended).

Premises:

The premises are suitable.

Peer review:

The application was sent to two expert peer reviewers. The peer reviewers both requested further information from the PR, particularly relating to the proposed CRISPR/Cas9 gene modification objective. The PR has provided comprehensive responses to both peer reviewers; the peer reviews and PR's responses are annexed to this report. At the time of writing this report, the PR's responses had been sent to the peer reviewers. Any further responses from the peer reviewers will be presented to the Committee alongside this report.

The first peer reviewer raised concerns whether the researchers would use embryos of an appropriate stage (zygote), whether these would be normal, and whether they would be available in sufficient numbers. Based on these concerns, the peer reviewer was not able to support the research. The PR has responded to these concerns and it is the opinion of the Executive that these concerns have been broadly addressed. Whilst the peer reviewer raises some important questions about the feasibility of the research, it is the Executive's opinion that these concerns do not necessarily affect whether the research is necessary or desirable.

The second peer reviewer raised concerns about whether the experimental design minimises the use of embryos, the freeze/thaw success rates achieved by the researchers, the stage of embryos to be used, and the experience of the PR and her team in the proposed experimental techniques. The PR has again provided a comprehensive response to the peer reviewer that the Executive considers addresses the key concerns. The Executive can confirm the comments made by the applicant with respect of apparently poor freeze/thaw success rates were the subject of discussions with the centre donating embryos to this research programme during their recent inspection, and it is our opinion that the issue has been appropriately addressed with the treatment centre.

Recommendation:

The Licence Committee is asked to note that at the time of the inspection, a recommendation was made to address one major area of non compliance. The PR has committed to implementing this recommendation.

Major areas of non compliance

- The PR should obtain ethics approval with respect of the additional research objectives described in the licence renewal application. Licensable activity related to these additional objectives should not commence until satisfactory evidence of approval has been submitted to the Executive.

The Executive recommends that the Committee considers whether it has sufficient information available to renew the centre's licence for a period of up to three years for the following activities:

- keeping embryos
- use of embryos
- storage of embryos

For the following principal purposes as defined in Schedule 2 3A (1) and (2) of the HFE Act 1990 (as amended):

- developing treatments for serious disease or other serious medical conditions
- increasing knowledge about the development of embryos
- promoting advances in the treatment of infertility

The PR has also requested that reference to 'surplus' embryos is removed from their project title, from:

Derivation of stem cells from human **surplus** embryos: the development of human embryonic stem cell (hES) cultures, characterisation of factors necessary for maintaining pluripotency and specific differentiation towards transplantable tissues.

to

Derivation of stem cells from human embryos: the development of human embryonic stem cell (hES) cultures, characterisation of factors necessary for maintaining pluripotency and specific differentiation towards transplantable tissues.

The executive recommends the amendment of the project title.

This section summarises information submitted in the research licence application and from the peer reviewer.

Lay summary of the research project:

The aim of our research is to understand which factors human embryos require to develop successfully and in addition to establish new human stem cells for therapeutic applications. Our investigations will be important to assess whether IVF technologies could be improved to increase healthy pregnancy outcomes. Understanding human embryo development will also have important implications for developing more efficient methods to establish human stem cell lines, thus increasing the quality, number, and utility of human stem cells available for cell transplantation therapies. Human embryonic stem cells have the unique potential to become any cell within the human body and present an unlimited source of cells to serve as models for understanding diseases, to test the safety and efficacy of new medicines and to cure degenerative diseases. However, our current understanding of the requirements for the establishment of human stem cells from embryos is very limited. Our work aims to provide fundamental knowledge to establish clinically useful human embryonic stem cells from surplus IVF embryos for cell transplantation therapies. We also aim to establish stem cells that resemble early placental cells, which will have direct benefits for infertility and immunology. The proposed studies will lead to a deeper understanding of the events underlying early human development, thus promoting insights into the mechanisms of disease and infertility.

Objectives of the research:

Our specific aims are as follows:

- i) we aim to determine the relationship between the cellular and molecular properties of human preimplantation embryos and human embryonic stem cell lines.
- ii) we aim to establish defined, animal product-free conditions for the derivation of pluripotent human embryonic stem cell lines, ultimately leading to Good Manufacturing Practice (GMP) compatible approaches.
- iii) we aim to establish and characterise human extraembryonic stem cell lines.

Aim 1: Determine the relationship between the cellular and molecular properties of preimplantation human embryos and human embryonic stem cell (hESC) lines.

We seek to understand the molecular events underlying early human development and are specifically interested in how human pluripotent cells that give rise to the foetus diverge from trophoblast cells that will form foetal components of the placenta. As our current understanding of the requirements for successful derivation of hESCs from surplus IVF embryos is very limited we also seek to provide further insights about hESCs and their relationship to the pluripotent epiblast to ensure the clinical application of stem cells generated from donated embryos. To inform our research aims, we propose comparing the cellular and molecular properties of human embryos before and during hESC derivation with those of established hESC lines. Thus far, we have investigated global gene expression dynamics in embryos from the zygote to the blastocyst stage using RNA-sequencing analysis (Blakeley et al., 2015, Development, in press). While this has revealed both conserved and human-specific transcriptional programs, the number of embryos used in the analysis limits the statistical power to determine differential gene expression. Moreover, it will be an important complement to our existing dataset to examine the gene expression of earlier and later stage blastocysts to further study lineage specification in human preimplantation embryos compared to hESCs.

To further understand gene expression dynamics during early human development, we will use immunohistochemistry and in situ hybridisation analysis to define the expression pattern of genes identified from our RNA-sequencing analysis. For example, while we confirmed that the novel transcription factor KLF17 is uniquely expressed in the human epiblast, many other factors identified from our RNA-sequencing analysis have yet to be confirmed at the protein level. Importantly, these factors could prove to be novel human-specific reprogramming factors and markers of human pluripotent stem cells. To further understand how the gene expression changes we identify from our RNA-sequencing analysis relate to protein expression, we will measure the secretion of proteins using standard approaches (e.g. ELISA). Identifying key proteins that are expressed and/or secreted in human embryo culture would not only inform our Aims 2 and 3 but also may lead to novel insights facilitating infertility diagnosis or treatment.

We also seek to functionally test the requirement of human-specific genes during embryonic development, using new gene-editing techniques (CRISPR-Cas9). Our recently published RNA-sequencing data demonstrate several genes and signalling pathways that are specifically expressed during human embryo development, compared with mouse (Blakeley et al., 2015, Development, in press). Many of our candidate regulatory genes are also expressed in hESC lines. Therefore, gene-editing approaches will be optimised in hESC lines, prior to experiments using embryos. However, while we showed that hESC have a related gene expression state to the epiblast in the embryo, they are far from identical, which means that ultimately, we need to test the function of genes directly in the human embryo to determine if they are necessary for development. To do so, we will collaborate with researchers at The Francis Crick Institute who have successfully developed gene-editing methods in mouse embryos. Cas9 is an endonuclease that is guided to homologous DNA sequences by the guide RNA and causes DNA double strand breaks (DSBs) at target sites (Singh et al., Genetics: 199). The mechanisms used to repair the break can lead to mutations and loss of function of the targeted gene. A guide RNA (gRNA) DNA template for transcription will be generated in vitro. Microinjection or electroporation will be used to introduce the gRNA plus Cas9 mRNA or protein into zygotes or cleavage stage embryos, in addition to hESCs. CRISPR/Cas9-mediated gene targeting may lead to insertions or deletions that can be assayed using molecular biology techniques. If Cas9-derived mutations at target sites are detected, we would next assay off-target effects by next generation sequencing. If off-target activity occurs, then a modified Cas9 with nickase activity could be used, which leads to high-fidelity base excision repair or homology-directed repair. In parallel we will investigate gene function using alternative approaches for gene silencing or mis-expression, including the delivery of cDNA or shRNAs via lentiviral or retroviral transduction. We will also directly transfect mRNAs encoding factors of interest, or shRNA to achieve gene silencing via RNA interference. Zygote, cleavage or blastocyst stage embryos will be transfected or transduced using any one of the above modification options.

Embryos with altered gene expression (either by gene editing or transfection) will be investigated using the extensive cellular and molecular biology techniques established in the laboratory. This will include immunocytochemistry, qRT-PCR, western analysis and cell culture/derivation experiments. The effect of mutation, mRNA overexpression or RNAi knockdown will be monitored by quantitative RT-PCR in the first instance. We will then apply our recently published single cell RNA-sequencing approach to measure transcription genome-wide. Oct4-deficient mouse embryos lack a pluripotent inner cell mass and fail to generate embryonic stem cells (Nichols et al., 1998, Cold Spring Harb Perspect Biol: 4). Our analysis suggests temporal distinctions in the expression dynamics of OCT4/Oct4 between humans and mice (Blakeley et al., 2015, Development, in press; Niakan and Eggan 2013, Dev Biol: 375). It is therefore important to functionally test the requirement of factors such as OCT4 in human

embryogenesis, to directly test conserved versus specific roles compared to the mouse. As OCT4 is likely to play a role this gene will also serve as a first proof of concept. Following OCT4 we will focus on human-specific epiblast enriched genes, such as KLF17 that we recently identified. Importantly, we also identified several human-specific factors, whose expression is absent in any of the pluripotent stem cell lines established to date, such as ARGFX.

It will be important to functionally test if these genes, or others like them, are functionally required for human pluripotency in the embryo. Investigating gene function in early human embryogenesis would allow us to gain significant insights into the fate of cells during early human development. This in turn would enable us to develop more objective criteria for embryo selection and increase the efficiency of stem cell derivation. Moreover, insights gained from these studies would also be used in comparative studies of early mouse development. In all, these studies are expected to yield critical new knowledge about early human development and to contribute to the derivation of clinically applicable sources of specialised cells for regenerative medicine. Elucidating key molecular differences in early embryonic lineages will significantly inform our Aims 2 and 3 allowing us to strategically test methods to accurately and more efficiently propagate in vitro stem cells that reflect an in vivo pluripotent (epiblast) or extraembryonic (trophectoderm or endoderm) cell type. These analyses will also be important in aiding the development of optimal hESC derivation methods.

Aim 2: Derive and maintain human embryonic stem cells under defined and animal product-free culture conditions.

While attempts at improving the efficiency of hESC derivation have been noted (Chen et al., 2009, *Cell Stem Cell*: 4), an efficient method to derive and grow hESCs remains a challenge. Early steps in the derivation process are crucial since the majority of embryos either fail to generate a viable inner cell mass, or the outgrowth from the isolated inner cell mass differentiates upon initial passaging. Moreover, existing hESCs are thought to represent a later stage of development than their mouse embryonic stem cell (mESC) counterparts, despite both being derived from preimplantation blastocysts. Indeed, hESCs share several characteristics with postimplantation-derived mouse epiblast stem cells (EpiSCs), including morphological similarities, LIF-independent growth and a reliance on FGF and Activin/Nodal signalling (Brons et al., 2007, *Nature*: 448; Tesar et al., 2007, *Nature*: 448). Addition of Mek and Gsk3b inhibitors together with LIF (2i+LIF) allows mESCs to be propagated in defined medium thought to represent a “ground state” of pluripotency that is more similar to mouse preimplantation epiblast cells, as compared to classical serum and LIF mESCs (Boroviak et al., 2014, *Nat Cell Biol*: 16; Ying et al., 2008, *Nature*: 453).

Recent attempts to derive ground state hESCs have utilised combinations of ectopic transgene expression, growth factors and inhibitors to modulate signalling pathways (Chan et al., 2013, *Cell Stem Cell*: 13; Gafni et al., 2013, *Nature*: 504; Takashima et al., 2014, *Cell*: 158; Theunissen et al., 2014, *Cell Stem Cell*: 15). Mek and Gsk3b inhibitors are often included, although 2i+LIF alone is unable to support the self-renewal of hESCs (Hanna et al., 2010, *PNAS*: 107). However, the benchmark against which these cells are assessed relies heavily on conclusions drawn from mouse ground state pluripotency, which our analysis suggests are not equivalent to the human pluripotent epiblast (Blakeley et al., 2015, *Development*, in press).

While fibroblast growth factor 2 (FGF2) has been demonstrated to be required for hESC maintenance (Vallier et al., 2005, *J Cell Sci*: 118), it is unclear whether it acts as a mitogen or survival factor, and whether other factors may also function to maintain pluripotent hESCs. Our preliminary data suggests that FGF signalling activation leads to the loss of the pluripotent

epiblast in embryos (manuscript in preparation), consistent with findings that inhibition of this pathway has no effect on epiblast development (Roode et al., 2013, *Dev Biol*: 361). Altogether this suggests that FGF may not be required for the establishment of hESCs and that alternative signalling pathways may be required.

We observed robust expression of multiple components of the transforming growth factor (TGF- β) superfamily in human preimplantation epiblast cells (Blakeley et al., 2015, *Development*, in press). Components of the TGF- β signaling pathway are also expressed in hESCs (Besser, 2004, *J Biol Chem*: 279; James et al., 2005, *Development*: 132; Levine and Brivanlou, 2006, *Development*: 133; Sato et al., 2003, *Dev Biol*, 260; Vallier et al., 2009, *Development*: 2009). TGF- β signaling contributes to the maintenance of hESCs by regulating pluripotency gene expression (Bertero et al., 2015, *Genes Dev*: 29; Brown et al., 2011, *Stem Cells*: 29; James et al., 2005, *Development*: 132; Vallier et al., 2005, *J Cell Sci*: 118; Vallier et al., 2004, *Dev Biol*: 275; Xu et al., 2008, *Cell Stem Cell*: 3). We showed that TGF- β signaling is also required to maintain key pluripotency marker expression in human epiblast cells (Blakeley et al., 2015, *Development*, in press). Determining which elements of the TGF- β signalling pathway are required for pluripotency in the epiblast and hESCs will provide future options for enhancing pluripotency using chemical activators or inhibitors of specific steps in the pathway, thus leading to chemically defined and feeder-free conditions and increased rates of hESC derivation. We propose to functionally test the requirement for key components of the TGF- β signalling pathway, such as NODAL, in human embryos using gene editing and/or further signalling modulation to determine the effect on the developing epiblast cells in the embryo. Moreover, our RNA-seq analysis allowed us to identify several components of other signalling pathways that are expressed in the developing human embryo.

We seek to test the requirement of these pathways in early human development and stem cell derivation. We will use known inhibitors or activators of these pathways in human embryo culture to test their effect on early human development, in blastocyst outgrowths and in stem cell derivations to determine the effect on epiblast versus extraembryonic growth. The expected outcome of these studies is the improved derivation and culture of hESCs by optimising culture conditions to derive hESC lines, which will be conducive to future basic biological and clinical developments.

Aim 3: Derivation of human extraembryonic cell lines as a resource for modelling early development and therapeutic applications.

The generation of in vitro models of human extraembryonic cells will go a long way toward addressing questions about early developmental events that would not be possible by any other means. Mouse blastocysts have been used to generate self-renewing in vitro models of extraembryonic lineages, including both trophoderm stem (TS), and extraembryonic endoderm (XEN) stem cell lines (Tanaka et al., 1998, *Science*: 282; Kunath et al., 2005, *Development*: 132) that are tractable systems for molecular genetic studies. Unlike the mouse, no stable in vitro extraembryonic cell lines have been derived from human embryos or embryonic stem cells. In vitro models of early human extraembryonic cells that have the potential to self-renew and differentiate into other extraembryonic cells will be crucial in understanding aspects of early human placental, yolk sac and amnion development, which will have direct benefits for infertility, embryonic development, and immunology.

Currently the limitation in understanding these early events is due to the advanced differentiated state of placental cells derived from early terminations of pregnancies and by the lack of protocols to maintain in vitro models of these early extraembryonic populations. While it has

been suggested that BMP4 treatment results in the differentiation of hESCs to trophectoderm cells (Xu et al., 2002, Nat Biotech: 20), our investigations in collaboration with the laboratories at the University of Cambridge and the Babraham Institute, suggest that these cells are not bona fide trophectoderm stem cells (Bernardo et al., 2011, Cell Stem Cell: 9) and that alternative techniques need to be developed to establish cell lines that more closely model in vivo extraembryonic cells.

We will attempt to derive extraembryonic cell lines from human embryos using a variety of methods including specialized media and candidate growth factors. It has recently been suggested that the same conditions reported for the establishment of extraembryonic cell lines from mouse preimplantation embryos are not applicable to extraembryonic stem cell derivation from human preimplantation embryos (Kunath et al., 2014, Placenta: 35), which confirms our unpublished observations. Specifically, we found the addition of FGF4 and heparin, which have been used to derive mouse trophoblast stem (TS) cells, does not lead to human TS cell derivation. Instead the human outgrowths arrest under these conditions after two weeks in culture, which suggests that alternative approaches are required to establish human extraembryonic stem cell lines. Importantly, the information we generate from Aim 1 will expand our understanding of the cell surface receptors and candidate signalling pathways that are specific to extraembryonic lineages. This will be used to strategically inform our attempts to derive human extraembryonic cell lines from human embryos. Our approach will be to identify ligand-receptor pairs through transcriptional profiling with the aim of using these secreted proteins in successive derivation attempts. The most significant milestone of this aim will be evaluation of the cellular identity of the derived lines based on the expression of established genes. The information we glean from Aim 1 will also strategically inform the establishment of in vitro models of human extraembryonic endoderm (XEN) stem cells to model early yolk sac development. This will not only further provide new insight into early cell fate decisions but also have medical implications for the treatment of yolk sac related failures of pregnancy. To evaluate the identity of these XEN cell lines it will be important to investigate the expression of genes that we identified as uniquely expressed in the human primitive endoderm. Altogether, we seek to establish human embryonic and extraembryonic stem cells and, because of its clinical objective, the proposed studies can only be carried out with human stem cells and embryos. The outcome of our research will be an understanding of the requirements needed for human embryo development in order to generate reliable sources of continuous and expandable differentiated tissues to cure degenerative diseases, to test new cell-based medicines, to test the safety of new treatments and, as cellular models for understanding development and diseases.

Summary of the research undertaken to date:

The aim of our research is to understand which factors human embryos require to develop successfully and in addition to establish new human stem cells for therapeutic applications. We have used cutting-edge techniques to investigate global gene expression in individual cells throughout human preimplantation development and compared this to mouse embryos at equivalent developmental stages, unraveling novel temporal-, lineage- and species-specific factors. We developed a computational pipeline to cluster single-cells into developmental stages based on their global gene expression profiles. Our analysis revealed that temporal expression dynamics of key developmental regulators and their co-expressed genes are largely distinct in human versus mouse. Significantly, we resolved lineage specific gene expression in humans including expression of a number of key components of the transforming growth factor (TGF- β) signaling pathway in the pluripotent epiblast. Treating human embryos with a potent TGF- β signaling inhibitor resulted in downregulation of the pluripotency marker NANOG, suggesting that this pathway is necessary to maintain the pluripotent epiblast. Intriguingly, our global gene

expression analysis allowed us to identify several components of other signalling pathways that are enriched in the developing human embryo, which may be functionally required to maintain pluripotency or placental progenitor cells. Importantly, although our analysis uncovered factors with conserved expression in human and mouse embryos, we identified several transcription factors that are exclusively expressed in the human epiblast, but not the mouse. Conversely, we also found that a number of factors functionally required for mouse pluripotent cells are absent from the human epiblast. Moreover, a number of key mouse trophectoderm (placental progenitor) factors were absent in the human trophectoderm, and vice versa. We found that although human embryonic stem cells expressed many epiblast-enriched genes, they also expressed genes that are absent in pluripotent epiblast cells of the embryo. Altogether, our comparison of human and mouse preimplantation development reveals previously unappreciated differences in gene expression and highlights the importance of further analyzing human preimplantation development rather than assuming equivalence to the mouse or existing human embryonic stem cells. Over the 3 year period of the licence, a total of 736 embryos were received: 367 embryos were thawed and 118 embryos were of sufficient quality to use in research.

Donation and use of embryos:

During 2014 316 embryos were donated to the project, 149 embryos were used, and 421 embryos remained in storage.

▶ **Principle:**

3. Have respect for the special status of the embryo when conducting licensed activities.

▶ **What we inspected against:**

Research Licence Conditions (RLC) R23, R24, R26, R27, R28, R29, CoP guidance note 22.

What the centre does well.

Observations during the inspection provided assurance that the project respects the special status of the human embryo:

- Processes, documented in standard operating procedures (SOPs), are in place to ensure that no embryo obtained for the purposes of the research project, is kept or used for any other research project or purpose (RLC R23). Staff training and their close supervision ensure procedures are adhered to, preventing the use of donated embryos in unlicensed activities.
- Recruitment practices ensure that no money or other benefit is given to those donating embryos to research unless authorised by directions (RLC R24).
- Each embryo used in the research project is uniquely labelled (RLC R26).
- Documented procedures have been established, implemented and complied with to ensure that clinical and research roles are separated (RLC R27).
- Procedures ensure that embryos do not develop after 14 days or the primitive streak has appeared (if earlier) (RLC R28). The culture and manipulation of each embryo is recorded in the laboratory records, which are regularly reviewed to prevent the culture of embryos beyond 14 days.
- A sample of all stem cell lines derived will be deposited in the UK Stem Cell Bank (RLC R30).

What they could do better.

The additional research objectives applied for involve the use of an incubator in a room that is shared with other research groups. This incubator does not yet have a lock to ensure that embryos are only used for purposes of the specified research project (RLC R23). This incubator is not yet in use and the PR has committed to inform the inspectorate when a lock has been fitted, and to not use the incubator until this time. The risk of use of embryos for purposes other than the specified research is further mitigated by the laboratory having controlled access and by robust procedures which ensure embryos are only used under the direct supervision of the PR. The inspection team therefore considers that a formal recommendation is not necessary.

▶ **Principle:**

5. Provide prospective and current patients and donors with sufficient, accessible and up-to-date information in order to allow them to make informed decisions.

6. Ensure that patients and donors have provided all relevant consents, before any licensed activity is undertaken.

▶ **What we inspected against:**

Information, counselling and consent; CoP guidance note 22, RLC R18, R19, R20, R21, R22. consent for storage; CoP guidance note 22, RLC R31, R32, R33, R34, R35, R36, R37, R38, R39.

What the centre does well.

Provision of information and counselling to those consenting to donate to research

Before giving consent, those donating to research should be provided with relevant information and given a suitable opportunity to receive counselling about the implications of their donation. Observations and discussion during the inspection provided assurance that:

- Before giving consent, those donating to research are given a suitable opportunity to receive proper counselling about the implications of their donation (RLC R18).
- Necessary information is provided to patients before giving their consent (RLC R19 and R20).
- Information is provided to patients by trained personnel in a manner and using terms that are easily understood (RLC R21). The competence of staff at the recruiting centres to provide information in this way, and to seek consent, has been assessed.
- A designated individual, who is not directly involved in the patient's treatment, is available to discuss with patients the project of research and the possibility of donating material to it (RLC R22). Contact details for this designated individual are provided in the patient information.

Consent for storage

Stored embryos are obtained only from centres to which a HFEA licence or third party agreement applies (RLC R32, R33).

No embryos are kept in storage for longer than the statutory storage period (RLC R36, R38 and R39), or the period of storage consented to by the gamete providers if less than the statutory storage period. This was assessed by reviewing the centre's record of stored embryos. A bring-forward system is maintained, ensuring embryos are stored only within the statutory storage period or the period consented to by the gamete providers, if less.

What they could do better.

Nothing noted.

<p>▶ Principle: 8. Ensure that all premises, equipment, processes and procedures used in the conduct of licensed activities are safe, secure and suitable for the purpose.</p> <p>▶ What we inspected against: Premises and facilities; RLC R10</p>
<p>What the centre does well.</p> <p>Premises and facilities The premises and facilities are secure, clean, well maintained and are suitable for carrying out the licensed activities (RLC R10).</p>
<p>What they could do better.</p> <p>Nothing noted.</p>

<p>▶ Principle: 10. Maintain proper and accurate records and information about all licensed activities</p> <p>▶ What we inspected against: Information and record keeping; RLC R13, R14, R15, R16, R17, General Direction 0002.</p>
<p>What the centre does well.</p> <p>A review of embryo storage and usage records indicate that proper records are maintained (RLC R13 and R15). These records are in a form that prevents the removal of data (RLC R16).</p> <p>Since the last renewal inspection, the centre has submitted the annual research information and data sheet to the HFEA within the required timeframe (RLC R14 and General Direction 0002).</p>
<p>What they could do better.</p> <p>Nothing noted.</p>

<p>▶ Principle: 11. Report all adverse incidents (including serious adverse events and reactions) to the us, investigate all complaints properly, and share lessons learned appropriately</p> <p>▶ What we inspected against: Incidents; RLC R40</p>
<p>What the centre does well.</p> <p>Processes are in place to detect, report and investigate adverse incidents (RLC R40).</p>
<p>What they could do better.</p> <p>Nothing noted.</p>

▶ **Principle:**

12. Ensure that all licensed research by the centre meets ethical standards, and is done only where there is both a clear scientific justification and no viable alternative to the use of embryos.

▶ **What we inspected against:**

HFE Act 1990 (as amended), Schedule 2 (3(5) and 3A).

What the centre does well.

The research project does not include any activities that have been prohibited by the HFE Act 1990 (as amended).

What they could do better.

The research described in the centre's current licence has been approved by the Cambridge Central Research Ethics Committee (REC). Evidence was provided by the PR that this approval remains active and covers the research activity currently undertaken. The existing ethics approval does not however cover additional aspects of research described in the licence renewal application (recommendation 1). The PR will need to submit a substantial amendment to the REC, but the REC has advised that they will not consider the amendment until a HFEA licence has been granted.

▶ **Principle:**

13. Conduct all licensed activities with regard for the regulatory framework governing treatment and research involving gametes or embryos within the UK, including:

- maintaining up-to-date awareness and understanding of legal obligations
- responding promptly to requests for information and documents
- co-operating fully with inspections and investigations by us or other agencies responsible for law enforcement or regulation of healthcare.

▶ **What we inspected against:**

Licensing; RLC R1, R2, R3, R5, R6. The person responsible; HFE Act 1990 (as amended) Section 16 & 17, RLC R8, R9.

What the centre does well.

Licensing

Inspection of the licensed premises indicated that all licensed research activities are performed only on the premises specified on the licence and under the supervision of the PR (RLC R1, R2).

The Person Responsible

The PR has a key role to play in implementing the requirements of the HFE Act 1990 (as amended) and is the person under whose supervision the licensed activities are authorised. The PR has the primary legal responsibility under Section 17 of the HFE Act 1990 (as amended) to secure:

- that suitable practices are used in undertaking the licensed activities

- that other persons working under the licence are suitable, and
- that the conditions of the licence are complied with.

The PR has academic qualifications in the field of biological sciences and has more than two years of practical experience which is directly relevant to the activity to be authorised by the licence (HFE Act 1990 (as amended), Section 16 (2) (c)). The PR has successfully completed our PR entry programme. The inspection team considered that the PR has fulfilled her responsibilities under Section 17 of the HFE Act 1990 (as amended).

What they could do better.

Nothing noted

Following an interim inspection in 2013, a recommendation for improvement was made in relation to one 'other' area of practice. The PR provided evidence that the recommendation was fully implemented within the agreed timescale.

The section sets out matters which the inspection team considers may constitute areas of non compliance. These have been classified into critical, major and others. Each area of non compliance is referenced to the relevant sections of the acts, regulations, standard licence conditions, directions or the Code of Practice, and the recommended improvement actions required are given, as well as the timescales in which these improvements should be carried out.

 **Critical areas of non compliance**

A critical area of non compliance is an area of practice which poses a significant direct risk of causing harm to a patient, donor or to an embryo. A critical area of non compliance requires immediate action to be taken by the person responsible.

Area of practice and reference	Action required and timescale for action	PR response	Executive review
None			

Major areas of non compliance

A major area of non compliance is a non-critical area of non compliance:

- which poses an indirect risk to the safety of a patient, donor or to an embryo through the procurement, use, storage or distribution of gametes and embryos, which do not comply with the centre’s licence
- which indicates a major shortcoming from the statutory requirements
- which indicates a failure of the person responsible to carry out his/her legal duties
- a combination of several ‘other’ areas of non compliance, none of which on their own may be major but which together may represent a major area of non compliance.

Area of practice and reference	Action required and timescale for action	PR response	Executive review
<p>1. The ethical approval for the project does not sufficiently cover all aspects of the research project proposed in the licence renewal application.</p> <p>General Direction 0008</p>	<p>The PR should seek to obtain ethics approval for the additional research activities and objectives described in their licence renewal application at the earliest opportunity. If provided, this should be communicated immediately to the HFEA.</p> <p>The PR should ensure that no research activity is undertaken for which clear approval has not been provided by a REC.</p>	<p>As discussed with the inspector, we were initially given advice by the REC to wait for the HFEA to decide on our licence application and then to submit an amendment. Following several discussions with the REC, they have subsequently allowed us to submit an amendment and we have an acknowledgment from the REC that our application will be considered within 35 days from December 11th 2015, when the amendment was received. We will notify the inspector once we have heard back from the REC. We confirm that no research activity will be undertaken until we have REC and HFEA approval.</p>	<p>We await the outcome of the REC. If necessary, the application will be referred back to the Licence Committee.</p>

 **'Other' areas of practice that require improvement**

'Other' areas of practice that require improvement are any areas of practice which cannot be classified as either a critical or major area of non compliance, but which indicates a departure from good practice.

Area of practice and reference	Action required and timescale	PR response	Executive review
None			

Additional information from the person responsible