SCRO Application Form

I. Investigator Information

Study Title:	Establishment of an integrated, peri-implantation human embryo model, including epiblast compartment, the trophectoderm and the hypoblast lineages	**SCRO record # *(office use only)*:**	
	Name	**Email**	**Phone**
Principal Investigator:	**P.M. Streak**	**streakp@university.edu**	**(xxx) abc-1234**
Faculty position:	**Professor**		
Department:	**Bioengineering**		
	Name	**Email:**	**Phone**
Contact Person if different from PI:			
Staff position:			
Department:			
***Mailing Address:**	**TBD**		

*This address is the location where you would like signed materials delivered.

Please note: Your proposal may require other institutional compliance reviews. The SCRO office will work with applicable Compliance Offices (e.g., EH&S, IACUC, IRB) to facilitate concurrent reviews and will notify these Offices promptly of SCRO approval. Please note that the Principal Investigator is responsible for obtaining the required compliance approvals.

II. Proposed Study

Abstract - Describe the following in lay terms (250-word limit):

- *goals of the research,*
- *brief description of the approach,*
- *rationale for using human embryos, hESC or hiPSCs, complex embryo models, or brain or gonadal organoids.*
- *significance of the research for human health.*

Note: If the research has ended, please indicate closure of the study and provide a brief description of study results.

We are interested in using human stem cells, including human pluripotent stem cells (hPSCs), with suitable 2D/3D in vitro culture systems, to model human embryonic development. Such in vitro human embryo models can provide powerful experimental platforms for studying human embryology, to uncover developmental principles and transcriptional complexity and gene regulation underlying cell fate patterning and tissue morphogenesis.

This research is important for providing a scientific foundation for prevention of pregnancy loss, birth defects and teratogenesis. This research has to involve hPSCs to mimic the epiblast compartment in the peri-implantation human embryo, given their molecular and developmental similarities.

We aim to establish an integrated, peri-implantation human embryo model, including epiblast compartment, the trophectoderm and the hypoblast lineages. To this end, we will develop in vitro co-culture systems that incorporate human trophoblast stem cell lines, human hypoblast stem cell lines, and hPSCs. We aim to culture this integrated, peri-implantation human embryo model for a prolonged period, till the onset of the gastrulation stage, when the primitive streak starts to emerge.

The emergence of the primitive streak will be determined through immunostaining for classic lineage markers. Other conventional biochemical assays such as in situ hybridization (ISH) will also be conducted to examine spatiotemporal expression of mRNA markers. Dynamic gene expression and lineage specification will be studied using reporter cell lines and single-cell RNA-sequencing tools. Comparative transcriptomic analysis will be conducted against human and monkey gastrula data to ensure the presence of the primitive streak-like cells in the model.

Empty field.

III. Intended Research

Check all that apply and provide additional information where requested.

☒ Studies involving *in vitro* passage or differentiation of hESC lines. **Complete Appendix A**

☐ Studies that involve the destruction of human embryos in your lab or in another lab that is sharing cells or embryos with your lab for analysis. **Complete Appendix B**

☐ Transplantation of hESCs, hiPSCs, or cells derived from either, into non-human research animals. **Complete Appendix C**

☒ Generation of embryos, complex embryo models (e.g., blastoids, gastruloids, assembloids), brain organoids, or gametes from either hESCs or hiPSCs. **Complete Appendix D**

**Check with SCRO if your intended research is not captured in the above categories.*

IV. Additional Oversight

Complete the information for all other Oversight that may apply to the research described in this application.

Other Committees/Offices	**Oversight Office** *if known*	**Approval date** *(office use only)*
Environmental Health & Safety (EH&S)	BUA # TBA	
Institutional Animal Care and Use Committee (IACUC)	# NA	
Institutional Review Board (IRB)	# NA	
Office of Technology Transfer	MTA # TBA	
Financial Conflict of Interest (FCOI) disclosure	# No COI	

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V. Conflict of Interest

Does the Principal Investigator, any co-investigator, or research coordinator involved with this study (or in aggregate with his/her spouse, dependents, or member of his/her household) have a financial relationship with the source of funding that requires filing out a Significant Financial Interest Disclosure (SFID) Form?

☐ ***Yes** ☒ **No**

** If **Yes**, ESCRO will not approve the protocol until the relevant conflict-of-interest approvals have occurred.*

VI. Investigator Certification

I, the Principal Investigator:

a. am responsible for assuring that **all** personnel (researchers and staff) involved with this proposal understand and comply with the University's policies.
b. will participate in an annual update describing any significant changes in specific aims for this research.
c. will adhere to all University Oversight Office requirements that are applicable to this proposal.
d. certify that I have answered all questions on this document and its attachments truthfully.

VII. Principal Investigator Signature

Date:	Print Name: P.M. Streak
Signature:	P.M Streak **I am responsible for ensuring this research complies with the policies listed in the Investigator Certification and any other relevant guidelines from the University and Federal and State agencies.**

SCRO Office Use Only

SCRO record #: _____

SCRO review/approval type: New: ☒ Renewal # ☐ Closure: ☐
SCRO review/approval type: Full ☒ Administrative: ☐
SCRO agenda date

_____ _____
SCRO Committee Chair signature or designee SCRO Chair release date

SCRO approval from date: approval to date:

Copy sent to:
☐ IACUC ☒ EH&S ☐ IRB ☐ OTT ☒ FCOI ☐ School or College (write in name) _____

Date sent:
ESCRO staff initials:

Appendix A
Studies involving in vitro passage or differentiation of hESC lines

Please provide the name(s) of the cell line(s) and source information (name of investigator and institution from which you received the cell lines). If you have derived the cell line(s) directly from specimens donated by a human donor, please write "donor" and the IRB protocol number.*

Name of cell line(s)	**Source**	**NIH hESC Registry # (if applicable)**
H1	WiCell	
H9	WiCell	
hiPSC line 1	PI lab	
hiPSC line 2	PI lab	

***For hESC research that is not currently eligible for federal funding:**

a) Append the approved consent-of-origin form if using hESCs not listed on the NIH Human Embryonic Stem Cell Registry. If the consent form is not available, please explain.

b) Explain your funding source.

National Science Foundation of the United States

Appendix B
Studies involving destruction of human embryos

Please provide the following information:

1. Describe:

   - Source and number of embryos that will be used.
   - Significant aspects of the methods, including how cells will be derived (if applicable).
   - Process for disposal of human tissues.

2. Scientific justification, including:

   - Reason for destroying embryos.
   - Where applicable, rationale for using particular genotypes.

Applicants must:

Append the approved original donor consent-of-origin form and consent for use of embryos for research.

Please note: IRB approval is required.

Appendix C

Transplantation of hESCs, hiPSCs, or cells derived from either, into non-human research animals

Please provide the following information:

1. Name(s) of the cell line(s) and source information (name of investigator and institution from which you received the cell lines). If you have derived the cell line(s) directly from specimens donated by a human donor, please write "donor" and the IRB protocol number.*

Name of cell line(s)	**Source**	**NIH hESC Registry # (if applicable)**

2. Species of research animal(s) used as recipient. *

3. Specific aims of research, brief description of methods, and endpoints of transplant studies.*

4. Scientific justification for transplanting hESCs or hiPSCs into research animals.

5. Address any ethical issues that these experiments might raise.

FOR TRAINING PURPOSES ONLY

SCRO application

***For hESC research that is not currently eligible for federal funding and if applicable, for hIPSCs you are proposing to use:**

a) Append the approved consent-of-origin form if using hESCs not listed on the NIH Human Embryonic Stem Cell Registry as well as for hIPSCs you are proposing to use. If the consent form is not available, please explain.

b) Explain your funding source.

Please note: IACUC approval is required.

Appendix D

Generation of embryos, complex embryo models (e.g., blastoids, gastruloids, assembloids), brain organoids, or gametes from either hESCs or hiPSCs.

Please describe:

1. Overall research goals.

   We aim to generate an integrated human embryo model that can progressively develop passing the onset of the gastrulation stage, exhibiting cellular structures mimicking the primitive streak.

2. Source of hESCs or hiPSCs that will be used.*

   See the table above.

3. Specific aims and brief description of methods.

   We aim to use this model to study the role of extraembryonic lineages, including the trophectoderm and the hypoblast, in guiding human primitive streak formation. The role of these extraembryonic lineages in the progressive lineage diversification and cell fate specification of hPSCs will be evaluated using immunocytochemistry to stain for relevant lineage markers. Conventional biochemical assays such as in situ hybridization (ISH) will also be conducted to examine spatiotemporal expression of mRNA markers. Dynamic gene expression and lineage specification will be studied using reporter cell lines and single-cell RNA-sequencing tools. Standard gain/loss of function assays involving pharmacological treatments and siRNA will be conducted to examine the involvement of different developmental signaling pathways in the embryonic-extraembryonic interactions. Key genes governing the embryonic-extraembryonic interactions will be investigated using KO lines that will be generated using the *CRISPR/Cas9 gene editing tools.*

4. Scientific justification (for example, if generating an organoid model, briefly state what will be learned from an organoid model compared to a standard two-dimensional cell culture model). Explain any ways you will control or limit culture of embryos or gametes (i.e., prevention of allowing development to extend past legal or ethical limits; prevention of fertilization occurring between sperm and eggs).

   Human gastrulation remains mysterious and difficult to study. Human gastrulation remains a bottleneck for successful pregnancy. Improving understanding of human gastrulation will advance human reproductive biology and medicine.

5. Address any ethical issues that these experiments might raise.

   We will closely monitor the emergence of primitive streak-like structures in the model (using imaging tools). Once such structures start to form, we will promptly stop the culture. The samples will be used immediately for downstream analysis (immunostaining or scRNA-seq).

*For hESC research that is not currently eligible for federal funding and if applicable, for hIPSCs you are proposing to use:

a) Append the approved consent-of-origin form if using hESCs not listed on the NIH Human Embryonic Stem Cell Registry as well as for hIPSCs you are proposing to use. If the consent form is not available, please explain.

b) Explain your funding source.

Please note: Other Oversight Office approval, such as IRB, IACUC, may be required.