hESC Registry Application Database

Detailed Listing for Request #: 2009-ACD-001

January 15, 2010

hESC Registry Application Search Results

Request #: 2009-ACD-001
Status: Pending

Review: ACD

Assurance: Yes (Section II(B))
Certification: Yes
Authority: Yes

Cell Lines: 1
Available: 1

Previous #:
2009-DRAFT-005

Email
Edit
Delete
Switch to ADM

Organization: WiCell Research Institute
Org Address: P.O. Box 7365 Madison, WI 53707-7365
DUNS: 122477024 **Grant Number(s):**
Signing Official (SO): Erik J. Forsberg / 608-334-7876 / eforsberg@wicell.org
Submitter of Request: / /

Line #1: WA01 (H1)
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes **Embryo Donated in Year:** 1998
Provider Name: WiCell Research Institute
Provider Phone: 888-204-1782
Provider Email: info@wicell.org
Provider URL: http://www.wicell.org
Provider Restrictions: 1) Intermixing of human embryonic cells with an intact embryo, either human or non-human, and

2) Attempting to make genetically identical whole embryos by any methods

NIH Restrictions:

Supporting Documents:
Document 1: (PDF - 09/23/2009) Summary of support documents
Document 2: (PDF - 09/23/2009) IRB approval
Document 3: (PDF - 09/23/2009) Consent form
Document 4: (PDF - 09/23/2009) Letter of assurance
Document 5: (PDF - 09/23/2009) SCRO approval letter
Document 6: (PDF - 09/23/2009) Thomson et al. 1998 hES cell derivation Science manuscript
Document 7: (PDF - 09/23/2009) Scott et al. 2009 use of hESC lines Nature manuscript
Document 8: (PDF - 09/29/2009) Letter of Assurance/Certification/Authority

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Administrative Comments: (10/05/2009--Ellen Gadbois)-Uploaded NIH Staff Analysis (prepared by NIH staff)

(09/29/2009 - Tom Turley) - Uploaded Letter of Assurance/Certification/Authority as provided by WiCell.

(11/12/2009--Ellen Gadbois)--Uploaded request from WG for more information

(11/25/2009 - B.Dean) - Uploaded additional supporting documents for WiCell.

Administrative Attachments:
Document 1: (XLS - 10/06/2009) NIH Staff Analysis
Document 2: (PDF - 11/12/2009) request from WG for more info
Document 3: (PDF - 11/24/2009) Email response to ACD Work Group
Document 4: (PDF - 11/24/2009) 1995 Protocol
Document 5: (PDF - 11/24/2009) 95E Complete Consents
Document 6: (PDF - 11/24/2009) 95G Complete Consents
Document 7: (PDF - 11/24/2009) HSC and Protocol Meeting Minutes
Document 8: (PDF - 11/24/2009) Summary of Additional Information

Status History
Draft: 09/23/2009
Pending: 09/23/2009

Emails Sent: 09/23/2009-New_Applicaton_Email

Added By: Commons\erikforsberg **On:** 09/23/2009 | **Last Updated By:** NIH\deanb **On:** 11/24/2009 | **Record ID:** 5

Total Record Count = 1

Administration Page

Logout of NIH Form 2890 Admin Site

Summary of Supporting Information Provided for WA01 (H1)

Document 1: Summary of Supporting Information – WA01 (H1)

This, the current document, is intended to provide the Working Group of the Advisory Committee to the [NIH] Director (ACD) with an explanation of how the supporting information provided address the materials that the Working Group will consider during its review of the use of the WA01 (H1) human Embryonic Stem Cell line with NIH funding.

Document 2: 1997 Thomson IRB Approval Notice

The derivation of the WA01 (H1) human Embryonic Stem Cell (hESC) line was conducted with the approval of the University of Wisconsin-Madison Health Sciences Institutional Review Board (IRB). This research protocol (95-623-239) was originally approved on July 24, 1995 and reapproved on an annual basis until the study was completed in 2000. The "1997 Thomson IRB Approval Notice" is a copy of the approval notice which was in affect during the time that the embryos used for the derivation of the WA01 (H1) hESC line were donated (January, 1998). Therefore the ACD should consider this document as demonstration that the derivation of the WA01 (H1) hESC line was conducted under IRB review and therefore meets the Health and Human Services regulations for the Protection of Human Research Subjects (45 C.F R. 46, Subpart A).

Document 3: 1997 Thomson Consent Form

University of Wisconsin-Madison Health Sciences Institutional Review Board reviewed and approved the consent form (Protocol # 95-623-239) that was signed by the donors of the embryos used for the derivation of the WA01 (H1) hESC line. The "1997 Thomson Consent Form" is a blank copy of this consent form which was signed in January of 1998 by both the male and female gamete donors, for whom the embryos were originally created for reproductive purposes.

While this consent form does not contain all of the elements listed in Section IIA of the July 7, 2009 NIH Guidelines on Human Stem Cell Research, it does contain the following elements:

Element 1. hESCs were derived from human embryos that were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose.

"Preimplantation embryos that are not chosen to be placed in the uterus will be cultured in various conditions in the laboratory for 10 days or less."

Element 4. No payments, cash or in kind, were offered for the donated embryos.

"You will not be financially compensated for participation."

Element 5. Policies and/or procedures were in place at the health care facility where the embryos were donated that neither consenting nor refusing to donate embryos for research would affect the quality of care provided to potential donor(s).

"If you choose not to take part in this study, your medical care will not be affected in any way."

Element 8. Donor(s) should have been informed that they retained the right to withdraw consent until the embryos were actually used to derive embryonic stem cells or until information that could identify the donor(s) was no longer retained by the researchers, if applicable.

"You are free at any time to refuse permission for the use of any of your excess embryos, up until the moment when the inner cells of the embryos are isolated for culture."

During the consent process, the donor(s) were informed of the following:

Element 9. The embryos would be used to derive hESCs for research.

While the term "human Embryonic Stem Cell Research" is not used in the consent, the research is described in lay terms as follows:

"Because these embryonic cells will be from the preimplantation embryo before the development of any specific tissue type they are called "undifferentiated". These cells can be cultured in this undifferentiated state in the laboratory, potentially indefinitely. However, by changing how they are cultured, they will sometimes randomly develop into "differentiated" cells (for example, cells that look and behave like the cells of the placenta, bone, skin, or blood). By studying how these embryonic cells differentiate, in the future it may be possible to direct their differentiation to specific cell types in culture. Because many diseases (such as diabetes mellitus or Parkinson's disease) result from the death or dysfunction of specific cell types, it might one day be possible to treat many diseases by the transplantation of differentiated cells derived in tissue culture from embryonic cell lines."

Element 10. What would happen to the embryos in the derivation of hESCs for research.

"Preimplantation embryos that are not chosen to be placed in the uterus will be cultured in various conditions in the laboratory for 10 days or less. This incubation time is not long enough for the development of any fetal structures. During this incubation the embryo will be photographed. After this period of time, the outer cells of the embryo will be separated from the inner cells of the embryo and discarded. The inner cells will then be cultured for an indefinite time ("cell lines") and studied. Note that the inner cells are not a complete embryo; as such they would not develop into a fetus if transferred to a uterus."

Element 11. hESCs derived from the embryos might be kept for many years.

"The inner cells will then be cultured for an indefinite time ("cell lines") and studied."

"These cell lines will be permanent, that is they will continue to divide in culture indefinitely."

Element 13. The research was not intended to provide direct medical benefit to the donor(s).

"You will not directly benefit from participation, but future patients may benefit from this study."

Element 14. The results of research using the hESCs may have commercial potential, and that the donor(s) would not receive financial or any other benefits from any such commercial development.

> *"You will not be financially compensated for participation. If embryonic cell lines are successfully isolated, the cell lines would become the property of the University of Wisconsin Alumni Research Foundation (WARF). Because of the possibility that differentiated cells derived from embryonic cell lines might one day be used to treat human disease, embryonic cell lines might have significant commercial value, and WARF may apply for patent protection for the isolation technique of the cell lines and on the properties of the cell lines. If a patent is granted, WARF would own the patent."*

Element 15. Whether information that could identify the donor(s) would be available to researchers.

> *"Nothing discovered about the donors from this research will be conveyed to the donor's physician in a way that could be personally identified."*

> *"Neither the men nor women who provide the gametes for the embryos used in this study will be identified in any communication or publication that may result from this study."*

Document 4: Letter of Assurance – WA01 – Sept 2009

A letter of assurance was obtained from the attending physician responsible for obtaining consent from the embryo donors whose embryos resulted in the derivation of the WA01 (H1) hESC line.

In addition to other information, this document provides supporting information demonstrating that the WA01 (H1) hESC line was derived from human embryos:

1. that were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose; and
2. that were donated by donor(s) who gave voluntary written consent for the human embryos to be used for research purposes.

It also provides the Working Group with written assurances that the principles articulated in Section IIA of the July 7, 2009 NIH Guidelines on Human Stem Cell Research as well as the HHS regulations for the Protection of Human Research Subjects (45 C.F R. 46, Subpart A) were followed.

Additionally this document provides written assurances that during the informed consent process (written and oral) that the donor(s):

1. were informed of other available options pertaining to the use of the embryos;
2. would not be offered any inducements for the donation of the embryos; and
3. were informed about what would happen to the embryos after the donation for research.

Therefore the ACD should consider this document as demonstration that the derivation of the WA01 (H1) hESC line was conducted in accordance with all of the eligibility requirements specified in Section IIB of the July 7, 2009 NIH Guidelines on Human Stem Cell Research.

Document 5: 2009 SCRO Approval Notice

The University of Wisconsin-Madison Stem Cell Research Oversight (SCRO) committee had not yet been established at the time (1998) when the WA01 (H1) hESC line was derived, however this committee has reviewed and approved (SC-2008-0014) the derivation of new hESC lines as well as the use of the WA01 (H1) hESC line by researchers at the University of Wisconsin-Madison.

Document 6: Thomson – Science - 1998

The derivation of the WA01 (H1) hESC line, along with four additional hESC lines was published in 1998:

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz, MA, Swiergiel JJ, Marshall VS, and Jones JM. "Embryonic stem cell lines derived from human blastocysts". Science 282:1145-1147, 1998.

Document 7: Use of hESC Lines – Nat Biotech Aug 2009

As reported in the August 2009 issue of Nature Biotechnology, 60.9% of all hESC publications reported using the WA01 (H1) hESC line. Additionally, of the 1,217 unique requests for National Stem Cell Bank lines, fully 77% asked for just two lines (H1, H9).

Scott CT, McCormick JB, and Owen-Smith J. "And then there were two: use of hESC lines". Nature Biotechnology 27(8):696-697, 2009.

Therefore the ACD should consider this information as demonstration that the WA01 (H1) hESC line has been and will likely remain an important cell line for hESC research and that both the WA01 (H1) and the WA09 (H9) hESC lines will continue to be important reference standards for newly derived hESC as well as iPS cell lines.

Thomson 95-623-239

Notice of Approval

Meeting Date: September 8, 1997

Protocol Number: 95-623-239
(Refer to this number when making inquiries)

To: James Thomson, VMD, Ph.D.
Assistant Professor, Dept. of Primate Research
1223 Capitol Ct.

From: Jane C. Fitchen / Shelley Logally
IRB Administrator

RE: **Protocol entitled, "Human Embryonic Stem Cells"**

The Human Subjects Committee has reviewed and approved the above research protocol. Approval is effective for one year. Please note the following additional information and requirements:

Institutional Endorsement: If a granting agency requires notification of HSC approval, submit the name and address of an individual at that agency. Our DHHS Multiple Project Assurance ID is M1285-0l.

Adverse Reactions: If any serious, unexpected adverse reaction occurs as a result of this study, you must notify the IRB administrator immediately.

Amendments: If you wish to change any aspect of the study (design, procedures, consent forms, or subject population, etc.), please submit your changes with a progress report on a Change of Protocol form. *The change may not be initiated until HSC approval has been given.*

Renewal: You are required to renew approval annually for as long as the study is active. Contact the HSC office for renewal forms.

Termination of Research: Please promptly notify the HSC in writing of the termination of this project.

Consent Forms: All subjects should be given a copy of the consent form(s).

VA Patients: All research involving VA Patients must be reviewed by the VA Research and Development Committee. Call the VA Research Office, 125-7863.

Cancer Patients Approval of all research involving cancer patients is conditional upon review and approval of the protocol by the WCCC Clinical Affairs Committee.

Please keep this notice with your copy of the approved protocol.

Change of Protocol - Increase collection of embryos to 50/year
Mailed September 10, 1997

Health Sciences Human Subjects Committee
H6/275 Clinical Science Center • 600 Highland Avenue • Madison, Wisconsin 53792-2457 • 608/263-2362

CP 9/8/97

Principal Investigator: James Thomson
(608) 263-3585

Consent to Use Excess Pre-implantation Embryos for Cell Line Isolation

YOU ARE INVITED TO TAKE PART IN A RESEARCH STUDY OF HUMAN CELL DIFFERENTIATION.

Purpose

All human tissues and organs are composed of small building blocks called cells. By growing cells from embryos which have not yet implanted in the uterus (preimplantation embryos) we hope to better understand normal embryo development, and ultimately find important clues for the treatment of infertility, miscarriage, birth defects, and other conditions.

What Will Be Done With The Embryos?

Preimplantation embryos that are not chosen to be placed in the uterus will be cultured in various conditions in the laboratory for 10 days or less. This incubation time is not long enough for the development of any fetal structures. During this incubation the embryos will be photographed. After this period of culture, the outer cells of the embryo will be separated from the inner cells of the embryo and discarded. The inner cells will then be cultured for an indefinite time ("cell lines") and studied. Note that the inner cells are not a complete embryo; as such they would not develop into a fetus if transferred to a uterus.

Why Are These Embryonic Cells Important?

Because these embryonic cells will be from the preimplantation embryo before the development of any specific tissue type they are called "undifferentiated". These cells can be cultured in this undifferentiated state in the laboratory, potentially indefinitely. However, by changing how they are cultured, they will sometimes randomly develop into "differentiated" cells (for example, cells that look and behave like the cells of placenta, bone, skin, or blood). By studying how these embryonic cells differentiate, in the future it may be possible to direct their differentiation to specific cell types in culture. Because many diseases (such as diabetes mellitus or Parkinson's disease) result from the death or dysfunction of specific cell types, it might one day be possible to treat many diseases by the transplantation of differentiated cells derived in tissue culture from embryonic cell lines. Although the potential for treating human diseases is great, significant biomedical advances will be needed before such treatments are possible.

Why Might These Embryonic Cell Lines Be Controversial?

These cell lines will be permanent, that is they will continue to divide in culture indefinitely. Although they are not whole embryos (i.e. they would not develop into a fetus if returned to the uterus) they will maintain many embryonic properties. Because of these embryonic properties, certain experiments which would be controversial for whole human embryos would be controversial for these cell lines. In particular, two experiments that will not be performed with embryonic cell lines derived from this study are: (i) Intermixing of human embryonic cells with an intact embryo, either human or nonhuman, and (ii) Attempting to make genetically identical whole embryos by any method.

Is There Any Medical Benefit For Participation?

You will not directly benefit from participation, but future patients may benefit from the study. The embryonic cells will provide information about normal human development that could lead to improved therapies for infertility or other conditions. Nothing discovered about the donors from this research will be conveyed to the donor's physicians in a way that could be personally identified.

Is There Any Financial Incentive For Participation?

You will not be financially compensated for participation. If embryonic cell lines are successfully isolated, the cell lines would become the property of the University of Wisconsin or the Wisconsin Alumni Research Foundation (WARF). Because of the possibility that differentiated cells derived from embryonic cell lines might one day be used to treat human diseases, embryonic cell lines might have significant commercial value, and WARF may apply for patent protection for the isolation technique of the cell lines and on the properties of the cell lines. If a patent is granted, WARF would own the patent. WARF is a Wisconsin corporation, organized in 1925 by University of Wisconsin-Madison alumni as an entity separate from the University. WARF is dedicated to supporting research at the UW-Madison. As part of its mission, WARF patents and licenses University inventions. Earnings are combined with WARF investment income to fund WARF's annual gift back to the University. The University then is responsible for deciding how the money will be applied. Inventors receive a share of earnings from their inventions, and by University policy, a part of what the University receives from WARF goes to a department or departments associated with the invention.

Confidentiality of Participation Neither the men nor women who provide the gametes for the embryos used in this study will be identified in any communication or publication that may result from this study.

If You Change Your Mind You are free at any time to refuse permission for the use of any of your excess embryos, up until the moment when the inner cells of the embryos are isolated for culture. Once these cells are isolated for culture, they become the property of the University of Wisconsin.

YOU MAY TAKE AS MUCH TIME AS YOU WISH TO THINK THIS OVER. IF YOU CHOOSE NOT TO TAKE PART IN THIS STUDY, YOUR MEDICAL CARE WILL NOT BE AFFECTED IN ANY WAY. BEFORE YOU SIGN THIS FORM, PLEASE ASK ANY QUESTIONS ON THE ASPECTS OF THIS STUDY WHICH ARE NOT CLEAR TO YOU. WE WILL ATTEMPT TO FULLY ANSWER ANY QUESTIONS YOU MAY HAVE PRIOR TO, DURING, OR FOLLOWING THIS STUDY. If you have questions, they should be directed to: [ ] Professor, Department of Obstetrics and Gynecology, IVF Laboratories of the University of Wisconsin Hospital and Clinics, phone number (608)

AUTHORIZATION: I have read the information, have had a chance to ask questions, and have decided to give consent for my embryos to be cultured in the laboratory for the purpose of research, as described above. I have been told that I will receive a signed copy of this consent form.

Name of Woman : ______________________
(Oocyte Donor) (please print)

____
Signature of Woman (Oocyte Donor) (date)

Witnessed by: ______________________
(please print)

____
Signature of Witness (date)

Name of Partner: ______________________
(Sperm Donor) (please print)

____
Signature of Partner (Sperm Donor) (date)

Witnessed by: ______________________
(please print)

____
Signature of Witness (date)

Assurance of Conditions of Consent for human Embryonic Stem Cell line WA01 (H1)

As the attending physician at the University of Wisconsin Hospital and Clinics (UWHC) responsible for obtaining consent from the embryo donors whose embryos resulted in the derivation of the WA01 (H1) human Embryonic Stem Cell (hESC) line, I, [ ], hereby provide the following written assurances that the embryos used for the derivation of the WA01 (H1) cell line:

1. were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose; and
2. were donated by individuals who sought reproductive treatment (hereafter referred to as "donor(s)") and who gave voluntary written consent for the human embryos to be used for research purposes.

Furthermore, during the consent process:

a. All disposition options available at UWHC at the time treatment was sought pertaining to the embryos no longer needed for reproductive purposes were explained to the individual(s) who sought reproductive treatment.
b. No payments, cash or in kind, were offered for the donated embryos.
c. While no specific written policies or procedures were in place at UWHC at the time of donation, I hereby provide assurance that neither consenting nor refusing to donate embryos for research affected the quality of care provided to potential donor(s).
d. There was a clear separation between my discussions related to a prospective donor's decision to create human embryos for reproductive purposes and a prospective donor's decision to donate human embryos for research purposes. Specifically:
   i. Decisions related to the creation of human embryos for reproductive purposes were made free from the influence of researchers proposing to derive or utilize human Embryonic Stem Cells (hESCs) in research. As the attending physician of individuals who sought reproductive treatment, I was responsible for their reproductive clinical care and obtaining informed consent from the donor(s). I was not directly involved in the laboratory procedures that resulted in the derivation of the WA01 (H1) cell line and did not plan to utilize hESCs in my own research.
   ii. At the time of embryo donation, consent for that donation was obtained from the individual(s) who had sought reproductive treatment. That is, even if potential donor(s) had given prior indication of their intent to donate to research any embryos that remained after reproductive treatment, consent for the donation for research purposes was given at the time of the donation.
   iii. Potential donor(s) were informed that they retained the right to withdraw consent for the donation of the embryo until the embryos were actually used to derive hESCs.
e. During the consent process, each donor was informed of the following:
   i. that the embryos would be used to derive hESCs for research;
   ii. what would happen to the embryos in the derivation of hESCs for research;
   iii. that hESCs derived from the embryos could be kept for many years;
   iv. that the donation of the embryos was made without any restrictions or directions as to the individual(s) who may receive medical benefit from the use of the hESCs, such as who may be the recipients of cell transplants;

v. that the research using hESCs derived from the embryos was not intended to provide direct medical benefit to the donor(s);

vi. that the results of research using the hESCs may have commercial potential, and that the donor(s) would not receive financial or any other benefits from any such commercial development;

vii. that the identity of the donor(s) would not be provided to researchers.

To the best of my knowledge, I, [ ], hereby certify that the above represents a true, accurate and complete description of the consent process used for the donation of the embryos which resulted in the derivation of the WA01 (H1) human Embryonic Stem Cell line. A blank copy of the UW-Madison Health Sciences Human Subjects Committee consent form (Protocol # 95-623-239) that was used for the donation of the embryos which resulted in the derivation of the WA01 (H1) human Embryonic Stem Cell line is attached to this letter of assurance. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001).

[ ]

9/12/09
Date

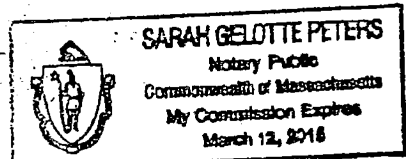
UWHC Attending Physician at the time of donation

See attached certificate
Susan [illegible]

Commonwealth of Massachusetts
County of Dukes County

On this 17th day of September 2009, before me, the undersigned notary public personally appeared [ ], proved to me through satisfactory evidence of identity, which was Massachusetts Drivers License, to be the person whose name was signed on page two of the preceding document, titled *Assurance of Conditions of Consent for human Embryonic Stem Cell Line WA01 (H1)*, in my presence.

Sarah Gelotte Peters
My commission expires March 12, 2015



As the IVF Laboratory Director at the University of Wisconsin Hospital and Clinics (UWHC) responsible for the creation of the embryos which resulted in the derivation of the WA01 (H1) human Embryonic Stem Cell (hESC) line, I, [ ], hereby certify that the embryos used for the derivation of the WA01 (H1) cell line:

1. were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose; and
2. were donated by individuals who sought reproductive treatment and who gave voluntary written consent for the human embryos to be used for research purposes.

I certify that these statements are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001).

[ ] 9/14/09
Date

HCLD(~~ABB~~)
UWHC IVF Laboratory Director at the time of donation

As the Principle Investigator of UW-Madison Health Sciences Human Subjects Committee Protocol # 95-623-239, I, James A. Thomson, V.M.D., PhD., hereby certify that the derivation of the WA01 (H1) cell line was conducted under IRB review and therefore meets the requirements detailed in 45 C.F. R. 46, Subpart A. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001).

James Thomson 9/14/09
Date

James A. Thomson, V.M.D., Ph.D., Diplomate A.C.V.P.
Principle Investigator
UW-Madison Health Sciences Human Subjects Committee Protocol # 95-623-239

My commission expires april 15, 2012
Dane County, State of Wisconsin
Notary Kathleen Mary Rand - 9/14/09

CP 9/8/97

Principal Investigator: James Thomson
(608) 263-3585

Consent to Use Excess Pre-implantation Embryos for Cell Line Isolation

YOU ARE INVITED TO TAKE PART IN A RESEARCH STUDY OF HUMAN CELL DIFFERENTIATION.

Purpose

All human tissues and organs are composed of small building blocks called cells. By growing cells from embryos which have not yet implanted in the uterus (preimplantation embryos) we hope to better understand normal embryo development, and ultimately find important clues for the treatment of infertility, miscarriage, birth defects, and other conditions.

What Will Be Done With The Embryos?

Preimplantation embryos that are not chosen to be placed in the uterus will be cultured in various conditions in the laboratory for 10 days or less. This incubation time is not long enough for the development of any fetal structures. During this incubation the embryos will be photographed. After this period of culture, the outer cells of the embryo will be separated from the inner cells of the embryo and discarded. The inner cells will then be cultured for an indefinite time ("cell lines") and studied. Note that the inner cells are not a complete embryo; as such they would not develop into a fetus if transferred to a uterus.

Why Are These Embryonic Cells Important?

Because these embryonic cells will be from the preimplantation embryo before the development of any specific tissue type they are called "undifferentiated". These cells can be cultured in this undifferentiated state in the laboratory, potentially indefinitely. However, by changing how they are cultured, they will sometimes randomly develop into "differentiated" cells (for example, cells that look and behave like the cells of placenta, bone, skin, or blood). By studying how these embryonic cells differentiate, in the future it may be possible to direct their differentiation to specific cell types in culture. Because many diseases (such as diabetes mellitus or Parkinson's disease) result from the death or dysfunction of specific cell types, it might one day be possible to treat many diseases by the transplantation of differentiated cells derived in tissue culture from embryonic cell lines. Although the potential for treating human diseases is great, significant biomedical advances will be needed before such treatments are possible.

Why Might These Embryonic Cell Lines Be Controversial?

These cell lines will be permanent, that is they will continue to divide in culture indefinitely. Although they are not whole embryos (i.e. they would not develop into a fetus if returned to the uterus) they will maintain many embryonic properties. Because of these embryonic properties, certain experiments which would be controversial for whole human embryos would be controversial for these cell lines. In particular, two experiments that will not be performed with embryonic cell lines derived from this study are: (i) Intermixing of human embryonic cells with an intact embryo, either human or nonhuman, and (ii) Attempting to make genetically identical whole embryos by any method.

Is There Any Medical Benefit For Participation?

You will not directly benefit from participation, but future patients may benefit from the study. The embryonic cells will provide information about normal human development that could lead to improved therapies for infertility or other conditions. Nothing discovered about the donors from this research will be conveyed to the donor's physicians in a way that could be personally identified.

Is There Any Financial Incentive For Participation?

You will not be financially compensated for participation. If embryonic cell lines are successfully isolated, the cell lines would become the property of the University of Wisconsin or the Wisconsin Alumni Research Foundation (WARF). Because of the possibility that differentiated cells derived from embryonic cell lines might one day be used to treat human diseases, embryonic cell lines might have significant commercial value, and WARF may apply for patent protection for the isolation technique of the cell lines and on the properties of the cell lines. If a patent is granted, WARF would own the patent. WARF is a Wisconsin corporation, organized in 1925 by University of Wisconsin-Madison alumni as an entity separate from the University. WARF is dedicated to supporting research at the UW-Madison. As part of its mission, WARF patents and licenses University inventions. Earnings are combined with WARF investment income to fund WARF's annual gift back to the University. The University then is responsible for deciding how the money will be applied. Inventors receive a share of earnings from their inventions, and by University policy, a part of what the University receives from WARF goes to a department or departments associated with the invention.

Confidentiality of Participation Neither the men nor women who provide the gametes for the embryos used in this study will be identified in any communication or publication that may result from this study.

If You Change Your Mind You are free at any time to refuse permission for the use of any of your excess embryos, up until the moment when the inner cells of the embryos are isolated for culture. Once these cells are isolated for culture, they become the property of the University of Wisconsin.

YOU MAY TAKE AS MUCH TIME AS YOU WISH TO THINK THIS OVER. IF YOU CHOOSE NOT TO TAKE PART IN THIS STUDY, YOUR MEDICAL CARE WILL NOT BE AFFECTED IN ANY WAY. BEFORE YOU SIGN THIS FORM, PLEASE ASK ANY QUESTIONS ON THE ASPECTS OF THIS STUDY WHICH ARE NOT CLEAR TO YOU. WE WILL ATTEMPT TO FULLY ANSWER ANY QUESTIONS YOU MAY HAVE PRIOR TO, DURING, OR FOLLOWING THIS STUDY. If you have questions, they should be directed to: [ ], Professor, Department of Obstetrics and Gynecology, IVF Laboratories of the University of Wisconsin Hospital and Clinics, phone number (608)

AUTHORIZATION: I have read the information, have had a chance to ask questions, and have decided to give consent for my embryos to be cultured in the laboratory for the purpose of research, as described above. I have been told that I will receive a signed copy of this consent form.

Name of Woman : ______________________ ______________________
(Oocyte Donor) (please print) Signature of Woman (Oocyte Donor) (date)

Witnessed by: ______________________ ______________________
(please print) Signature of Witness (date)

Name of Partner: ______________________ ______________________
(Sperm Donor) (please print) Signature of Partner (Sperm Donor) (date)

Witnessed by: ______________________ ______________________
(please print) Signature of Witness (date)

R. 3/6/09
[illegible]

Stem Cell Research Oversight (SCRO) Committee
NOTICE OF ACTIONs
Approval
Date of Correspondence: 2/26/2009

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Protocol:	SC-2008-0014 "Derivation of human Embryonic Stem (hES) Cell Lines"
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Your SCRO Committee Application "Derivation of human Embryonic Stem (hES) Cell Lines", including the supporting materials that you submitted with your application, was reviewed and approved by the SCRO Committee at its 2/25/2009 meeting. You may begin the research described in your application. The review period and expiration date of your approval are listed above.

We have received the information you sent regarding the above named protocol. This information complies with the modifications required by the Stem Cell Research Oversight (SCRO) Committee, and your protocol is now approved. You may now begin your research.

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Sincerely,

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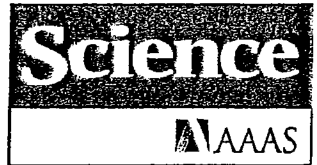

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson, *et al.*

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Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

Human blastocyst-derived, pluripotent cell lines are described that have normal karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize other early lineages. After undifferentiated proliferation in vitro for 4 to 5 months, these cells still maintained the developmental potential to form trophoblast and derivatives of all three embryonic germ layers, including gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm). These cell lines should be useful in human developmental biology, drug discovery, and transplantation medicine.

Embryonic stem (ES) cells are derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation in vitro (*1*, *2*). In chimeras with intact embryos, mouse ES cells contribute to a wide range of adult tissues, including germ cells, providing a powerful approach for introducing specific genetic changes into the mouse germ line (*3*). The term "ES cell" was introduced to distinguish these embryo-derived pluripotent cells from teratocarcinoma-derived pluripotent embryonal carcinoma (EC) cells (*2*). Given the historical introduction of the term "ES cell" and the properties of mouse ES cells, we proposed that the essential characteristics of primate ES cells should include (i) derivation from the preimplantation or periimplantation embryo, (ii) prolonged undifferentiated proliferation, and (iii) stable developmental potential to form derivatives of all three embryonic germ layers even after prolonged culture (*4*). For ethical and practical reasons, in many primate species, including humans, the ability of ES cells to contribute to the germ line in chimeras is not a testable property. Nonhuman primate ES cell lines provide an accurate in vitro model for understanding the differentiation of human tissues (*4*, *5*). We now describe human cell lines that fulfill our proposed criteria to define primate ES cells.

Fresh or frozen cleavage stage human embryos, produced by in vitro fertilization (IVF) for clinical purposes, were donated by individuals after informed consent and after institutional review board approval. Embryos were cultured to the blastocyst stage, 14 inner cell masses were isolated, and five ES cell lines originating from five separate embryos were derived, essentially as described for nonhuman primate ES cells (*5*, *6*). The resulting cells had a high ratio of nucleus to cytoplasm, prominent nucleoli, and a colony morphology similar to that of rhesus monkey ES cells (Fig. 1). Three cell lines (H1, H13, and H14) had a normal XY karyotype, and two cell lines (H7 and H9) had a normal XX karyotype. Each of the cell lines was successfully cryopreserved and thawed. Four of the cell lines were cryopreserved after 5 to 6 months of continuous undifferentiated proliferation. The other cell line, H9, retained a normal XX karyotype after 6 months of culture and has now been passaged continuously for more than 8 months (32 passages). A period of replicative crisis was not observed for any of the cell lines.

The human ES cell lines expressed high levels of telomerase activity (Fig. 2). Telomerase is a ribonucleoprotein that adds telomere repeats to chromosome ends and is involved in maintaining telomere length, which plays an important role in replicative life-span (*7*, *8*). Telomerase expression is highly correlated with immortality in human cell lines, and reintroduction of telomerase activity into some diploid human somatic cell lines extends replicative life-span (*9*). Diploid human somatic cells do not express telomerase, have shortened telomeres with age, and enter replicative senescence after a finite proliferative life-span in tissue culture (*10*–*13*). In contrast, telomerase is present at high levels in germ line and embryonic tissues (*14*). The high level of telomerase activity expressed by the human ES cell lines therefore suggests that their replicative life-span will exceed that of somatic cells.

The human ES cell lines expressed cell surface markers that characterize undifferentiated nonhuman primate ES and human EC cells, including stage-specific embryonic antigen (SSEA)–3, SSEA-4, TRA-1-60, TRA-1-81, and alkaline phosphatase (Fig. 3) (*4*, *5*, *15*, *16*). The globo-series glycolipid GL7, which carries the SSEA-4 epitope, is formed by the addition of sialic acid to the globo-series glycolipid Gb5, which carries the SSEA-3 epitope (*17*, *18*). Thus, GL7 reacts with antibodies to both SSEA-3 and SSEA-4 (*17*, *18*). Staining intensity for SSEA-4 on the human ES cell lines was consistently strong, but staining intensity for SSEA-3 was weak and varied both within and among colonies (Fig. 3, D and C). Because GL7 carries both the SSEA-4 and SSEA-3 epitopes and because staining for SSEA-4 was consistently strong, the relatively weak staining for

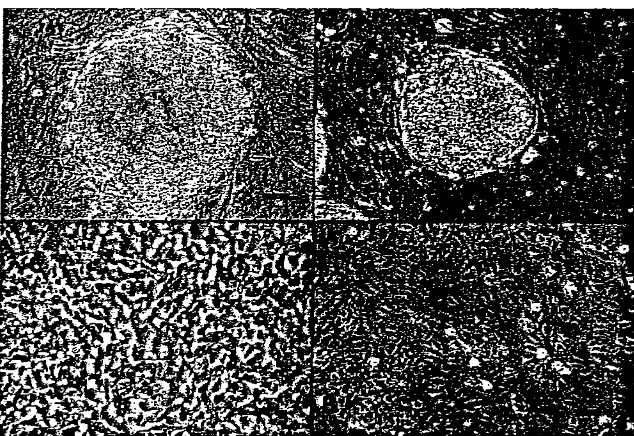

Fig. 1. Derivation of the H9 cell line. **(A)** Inner cell mass–derived cells attached to mouse embryonic fibroblast feeder layer after 8 days of culture, 24 hours before first dissociation. Scale bar, 100 μm. **(B)** H9 colony. Scale bar, 100 μm. **(C)** H9 cells. Scale bar, 50 μm. **(D)** Differentiated H9 cells, cultured for 5 days in the absence of mouse embryonic fibroblasts, but in the presence of human LIF (20 ng/ml; Sigma). Scale bar, 100 μm.

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SSEA-3 suggests a restricted access of the antibody to the SSEA-3 epitope. In common with human EC cells, the undifferentiated human ES cell lines did not stain for SSEA-1, but differentiated cells stained strongly for SSEA-1 (*15*) (Fig. 3). Mouse inner cell mass cells, ES cells, and EC cells express SSEA-1 but do not express SSEA-3 or SSEA-4 (*17*, *19*), suggesting basic species differences between early mouse and human development.

The human ES cell lines were derived by the selection and expansion of individual colonies of a uniform, undifferentiated morphology, but none of the ES cell lines was derived by the clonal expansion of a single cell. The uniform undifferentiated morphology that is shared by human ES and nonhuman primate ES cells and the consistent expression by the human ES cell lines of cell surface markers that uniquely characterize primate ES and human EC cells make it extremely unlikely that a mixed population of precursor cells was expanded. However, because the cell lines were not cloned from a single cell, we cannot rule out the possibility that there is some variation in developmental potential among the undifferentiated cells, in spite of their homogeneous appearance.

The human ES cell lines maintained the potential to form derivatives of all three embryonic germ layers. All five cell lines produced teratomas after injection into severe combined immunodeficient (SCID)–beige mice. Each injected mouse formed a teratoma, and all teratomas included gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm) (Fig. 4). In vitro, the ES cells differentiated when cultured in the absence of mouse embryonic fibroblast feeder layers, both in the presence and absence of human leukemia inhibitory factor (LIF) (Fig. 1). When grown to confluence and allowed to pile up in the culture dish, the ES cell lines differentiated spontaneously even in the presence of fibroblasts. After H9 cells were allowed to differentiate for 2 weeks, both α-fetoprotein (350.9 ± 14.2 IU/ml) and human chorionic gonadotropin (hCG, 46.7 ± 5.6 mIU/ml) were detected in conditioned culture medium, indicating endoderm and trophoblast differentiation (*20*).

Human ES cells should offer insights into developmental events that cannot be studied directly in the intact human embryo but that have important consequences in clinical areas, including birth defects, infertility, and pregnancy loss. Particularly in the early postimplantation period, knowledge of normal human development is largely restricted to the description of a limited number of sectioned embryos and to analogies drawn from the experimental embryology of other species (*21*). Although the mouse is the mainstay of experimental mammalian embryology, early structures including the placenta, extraembryonic membranes, and the egg cylinder all differ substantially from the corresponding structure of the human embryo. Human ES cells will be particularly valuable for the study of the development and function of tissues that differ between mice and humans. Screens based on the in vitro differentiation of human ES cells to specific lineages could identify gene targets for new drugs, genes that could be used for tissue regeneration therapies, and teratogenic or toxic compounds.

Elucidating the mechanisms that control differentiation will facilitate the efficient, directed differentiation of ES cells to specific cell types. The standardized production of large, purified populations of euploid human cells such as cardiomyocytes and neurons will provide a potentially limitless source of cells for drug discovery and

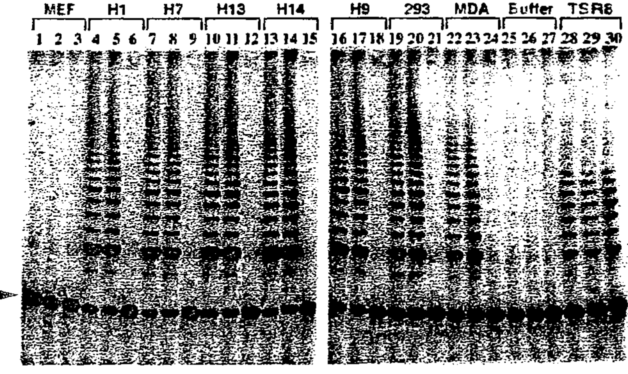

Fig. 2. Telomerase expression by human ES cell lines. MEF, irradiated mouse embryonic fibroblasts used as a feeder layer for the cells in lanes 4 to 18; 293, adenovirus-transformed kidney epithelial cell line 293; MDA, breast cancer cell line MDA; TSR8, quantitation control template. Telomerase activity was measured with the TRAPEZE Telomerase Detection Kit (Oncor, Gaithersburg, Maryland). The ES cell lines were analyzed at passages 10 to 13. About 2000 cells were assayed for each telomeric repeat amplification protocol assay, and 800 cell equivalents were loaded in each well of a 12.5% nondenaturing polyacrylamide gel. Reactions were done in triplicate with the third sample of each triplet heat inactivated for 10 to 15 min at 85°C before reaction to test for telomerase heat sensitivity (lanes 6, 9, 12, 15, 18, 21, 24, and 27). A 36–base pair internal control for amplification efficiency and quantitative analysis was run for each reaction as indicated by the arrowhead. Data were analyzed with the Storm 840 Scanner and ImageQuant package (Molecular Dynamics). Telomerase activity in the human ES cell lines ranged from 3.8 to 5.9 times that observed in the immortal human cell line MDA on a per cell basis.

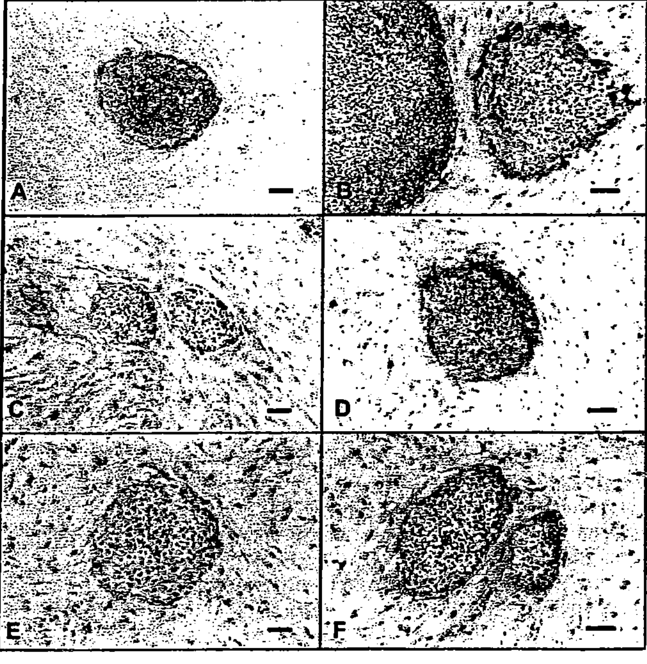

Fig. 3. Expression of cell surface markers by H9 cells. Scale bar, 100 μm. **(A)** Alkaline phosphatase. **(B)** SSEA-1. Undifferentiated cells failed to stain for SSEA-1 (large colony, left). Occasional colonies consisted of nonstained, central, undifferentiated cells surrounded by a margin of stained, differentiated, epithelial cells (small colony, right). **(C)** SSEA-3. Some small colonies stained uniformly for SSEA-3 (colony left of center), but most colonies contained a mixture of weakly stained cells and a majority of nonstained cells (colony right of center). **(D)** SSEA-4. **(E)** TRA-1-60. **(F)** TRA-1-81. Similar results were obtained for cell lines H1, H7, H13, and H14.

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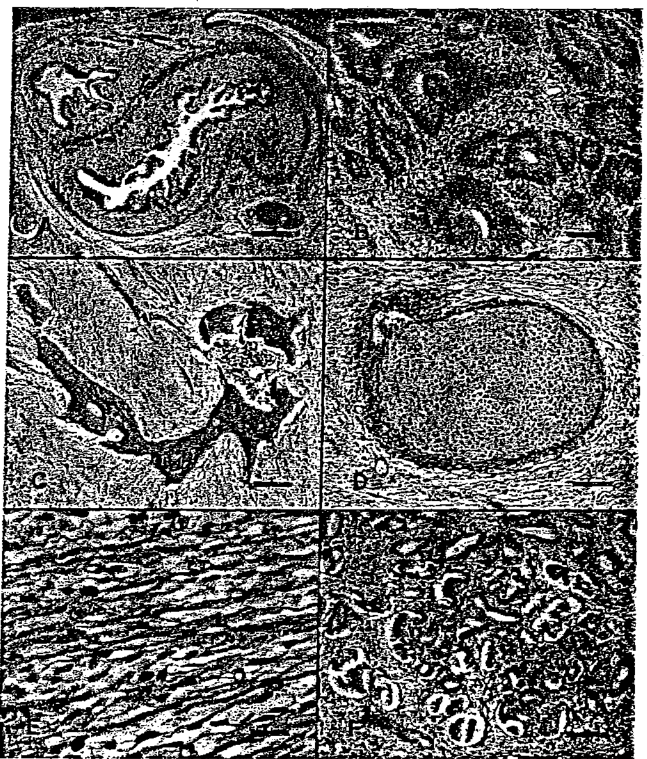

Fig. 4. Teratomas formed by the human ES cell lines in SCID-beige mice. Human ES cells after 4 to 5 months of culture (passages 14 to 16) from about 50% confluent six-well plates were injected into the rear leg muscles of 4-week-old male SCID-beige mice (two or more mice per cell line). Seven to eight weeks after injection, the resulting teratomas were examined histologically. **(A)** Gutlike structures. Cell line H9. Scale bar, 400 μm. **(B)** Rosettes of neural epithelium. Cell line H14. Scale bar, 200 μm. **(C)** Bone. Cell line H14. Scale bar, 100 μm. **(D)** Cartilage. Cell line H9. Scale bar, 100 μm. **(E)** Striated muscle. Cell line H13. Scale bar, 25 μm. **(F)** Tubules interspersed with structures resembling fetal glomeruli. Cell line H9. Scale bar, 100 μm.

transplantation therapies. Many diseases, such as Parkinson's disease and juvenile-onset diabetes mellitus, result from the death or dysfunction of just one or a few cell types. The replacement of those cells could offer lifelong treatment. Strategies to prevent immune rejection of the transplanted cells need to be developed but could include banking ES cells with defined major histocompatibility complex backgrounds or genetically manipulating ES cells to reduce or actively combat immune rejection. Because of the similarities to humans and human ES cells, rhesus monkeys and rhesus ES cells provide an accurate model for developing strategies to prevent immune rejection of transplanted cells and for demonstrating the safety and efficacy of ES cell–based therapies. Substantial advances in basic developmental biology are required to direct ES cells efficiently to lineages of human clinical importance. However, progress has already been made in the in vitro differentiation of mouse ES cells to neurons, hematopoietic cells, and cardiac muscle (*22–24*). Progress in basic developmental biology is now extremely rapid; human ES cells will link this progress even more closely to the prevention and treatment of human disease.

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6. Thirty-six fresh or frozen-thawed donated human embryos produced by IVF were cultured to the blastocyst stage in G1.2 and G2.2 medium (*25*). Fourteen of the 20 blastocysts that developed were selected for ES cell isolation, as described for rhesus monkey ES cells (*5*). The inner cell masses were isolated by immunosurgery (*26*), with a rabbit antiserum to BeWO cells, and plated on irradiated (35 greys gamma irradiation) mouse embryonic fibroblasts. Culture medium consisted of 80% Dulbecco's modified Eagle's medium (no pyruvate, high glucose formulation; Gibco-BRL) supplemented with 20% fetal bovine serum (Hyclone), 1 mM glutamine, 0.1 mM β-mercaptoethanol (Sigma), and 1% nonessential amino acid stock (Gibco-BRL). After 9 to 15 days, inner cell mass–derived outgrowths were dissociated into clumps either by exposure to Ca^{2+}/Mg^{2+}-free phosphate-buffered saline with 1 mM EDTA (cell line H1), by exposure to dispase (10 mg/ml; Sigma; cell line H7), or by mechanical dissociation with a micropipette (cell lines H9, H13, and H14) and replated on irradiated mouse embryonic fibroblasts in fresh medium. Individual colonies with a uniform undifferentiated morphology were individually selected by micropipette, mechanically dissociated into clumps, and replated. Once established and expanded, cultures were passaged by exposure to type IV collagenase (1 mg/ml; Gibco-BRL) or by selection of individual colonies by micropipette. Clump sizes of about 50 to 100 cells were optimal. Cell lines were initially karyotyped at passages 2 to 7.
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27. We thank the personnel of the IVF clinics at the University of Wisconsin School of Medicine and at the Rambam Medical Center for the initial culture and cryopreservation of the embryos used in this study; D. Gardner and M. Lane for the G1.2 and G2.2 media; P. Andrews for the NTERA2 cl.D1 cells and the antibodies used to examine cell surface markers; C. Harris for karyotype analysis; and Geron Corporation for the 293 and MDA cell pellets and for assistance with the telomerase TRAP assay. Supported by the University of Wisconsin (UIR grant 2060) and Geron Corporation (grant 133-BU18).

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And then there were two: use of hESC lines

To the Editor:

Last month, the US National Institutes of Health (NIH) issued its final guidelines defining the types of human embryonic cell (hESC) lines that would be eligible for public funding. The number of NIH-approved hESC lines available for federal funding was a bellwether issue for scientists during the Bush era prohibition. Originally, the agency announced 78 lines could be used. This was later revised downward to 64, then downward again to 21, the number most often cited in the literature[1,2].

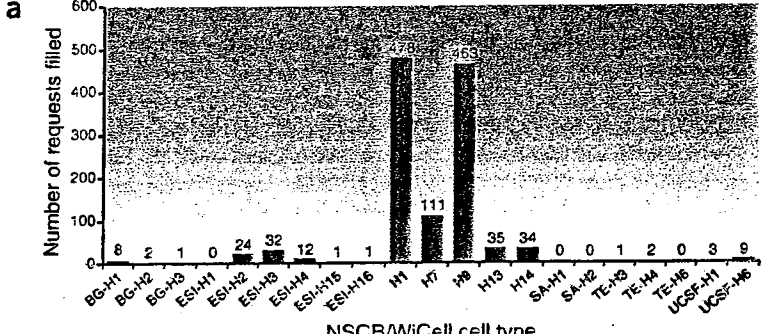
Among the criticisms of the remaining lines in the National Stem Cell Bank (NSCB) is that they have a limited range of genetic diversity[3]. Now that the NIH has announced its intention to set up a national registry of hESC lines, the biggest issue facing the agency will be how to rebuild the bank into a robust and valuable research resource.

Getting many different stem cell lines—the fundamental tool of regenerative medicine—into numerous laboratories with varied scientific and clinical foci is a worthy goal. We caution, however, that social and institutional factors such as intellectual property rights, access fees, use restrictions and competition shape the choices scientists make about research materials[4,5]. Federal policies and institutional guidelines, established and well-understood protocols, assays, reagents and the prominence of some lines in the published literature also have an impact. State-level policies can be even more restrictive than federal rules, and the possibility of legal challenges makes transfer of materials to some locales more difficult than others.

Indeed, initial social differences (in accessibility, ease of use or availability of technical complements such as known reagents and laboratory procedures) can lead to increasing returns for the selection of particular materials and eventually to lock-in on a dominant but often sub-par technological standard for a field[6]. In short, the diversity of lines actually in use at the bench has as much to do with past policies and the field's history as it does with the variety of lines available in the banks.

We enter this discussion with systematic data on the patterns of use of cell lines from the largest US repositories, the NSCB and the Harvard Stem Cell Institute (HSCI; Cambridge, MA, USA). In prior work, we examined the geographical patterns of distribution of hESC lines from these two sources. We showed that despite federal funding restrictions, hESC research is vigorous and growing in the United States. We see that states with high levels of research activity can blunt local laws and policies that would seek to restrict or chill hESC research[7].

Using data on cell line shipments obtained from HSCI and WiCell—which curates the NSCB—we see that lines have been neither uniformly available nor uniformly used, indicating far less diversity of materials than most believe[8]. Our most recent data set tracks WiCell's distribution of five of its own lines (derived by James Thomson) beginning in 1999, those available from the NSCB after its inception in 2005 and those distributed by HSCI since April 2004. No more than 18 approved cell lines have ever been available through the NSCB and that number is only accurate for the first months of 2009. Importantly, we find that just two NSCB lines—H1 and H9—are commonly used. The story is slightly better for HSCI, where all 28



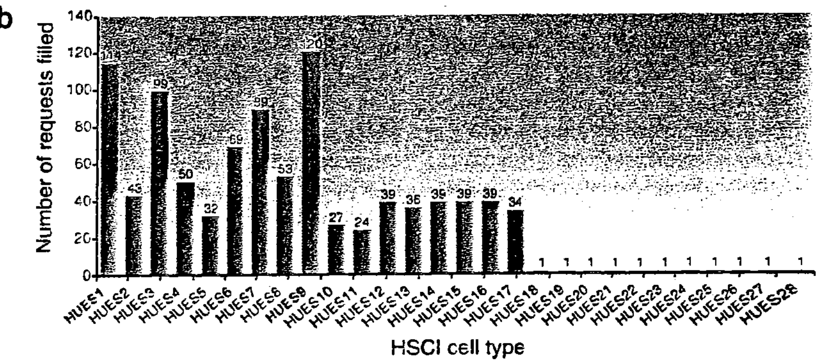


Figure 1 Requests for hESC lines. **(a)** Twenty-one approved cell lines with number of unique shipments from WiCell and NSCB from March 1999 to December 2008. Lines that are unavailable are listed as zeros. Highlighted bars denote the most requested cell lines. **(b)** Twenty-eight cell lines available from HSCI with number of unique shipments from April 2004 to December 2008. HUES18 through HUES28 have shipped only once and appeared in the published literature in 2009. The most requested lines are highlighted.

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of the lines derived by Harvard's Doug Melton were shipped at least once within 16 months of the announcement that they would be made broadly available[9]. HSCI has distributed nearly the same number of lines as WiCell/NSCB in half the time, but a large proportion of those shipments have gone to investigators outside the United States.

This pattern of diversity in banks is reflected somewhat differently at the bench. Figure 1 reports the number of unique requests that were filled, which we dub 'vials', for each available line. The patterns of use of materials are startling. Notice first that of the 1,217 unique requests for NSCB cell lines, fully 77% (941) asked for just two lines (H1, H9). Only one other line in the NSCB (H7) has been requested more than 100 times since 1999 and nine of the lines that have been available have been requested less than 10 times each. On a widely used measure of market concentration, 1-1/Herfindahl, a value of 1 represents a case of pure monopoly. For NSCB cell shipments, this measure is 0.968. For all intents and purposes, then, just two approved cell lines are widely used at the bench.

Shipments of HSCI lines also manifest high levels of concentration. We see that eleven of the 28 available have shipped just one time each. We note, however, that although those lines were shipped in 2005, they appear in peer-review literature only very recently[10]. We thus focus our attention on the 17 cell lines available from HSCI starting in 2004. Here we see less concentration. We observe 946 unique shipments of these 17 HSCI cell lines, but only 24.7% (234) of those are for the two most commonly requested lines. Nevertheless, the more nuanced concentration measure we mobilize is 0.868, reflecting a moderately high level of reliance on a relatively small number of available HSCI cell lines. HSCI has dramatically broadened the diversity of cell lines available for use, but it is notable that WiCell's H1 and H9 lines have been requested nearly as many times as all HSCI lines combined. We suspect one reason the 'diversity' bottleneck between the banks and the bench is stronger for NSCB lines is because federal funding restrictions introduce constraints that might cause investigators to select materials conservatively with an eye toward those with known procedures for access from the banks. It is possible other factors could have led to greater concentration of the WiCell lines. These include the quality of the cells, their early characterization and proven record of productivity. There is also a first-mover advantage: laboratories requested the lines to repeat Thomson's methods and to establish local culture conditions. Understanding the scientific and social reasons behind investigator choice is an area for further study.

How does shipment data translate into on-the-ground research results? Do hESC lines used outside our data sets—including those approved lines before establishment of NSCB—find their way into the stem cell research community? We address these questions with a systematic search of 534 peer-reviewed publications using hESC lines during the period 1999–2008. Preliminary analysis shows that the high demand for three NSCB lines is reflected in the literature: 83.3% of publications used H9, 60.9% used H1 and 24.2% used H7. Approximately 68% of publications used one or more in combination with other NSCB lines. Fewer than 36% of the publications used NSCB lines other than H1, H7 and H9. By contrast, the HSCI lines, although nearly five years old, are unlikely to supplant the Thomson lines in the published literature any time soon. Our analysis suggests that the two most commonly requested Harvard lines each appear in just under 3% of stem cell publications to date. Even where federal restrictions on material use do not hold, scientists select an artificially limited range of lines and the early pattern of such selections has led a small number of lines to dominate research in the field[11].

It seems unlikely that the role the four most used cell lines—H1, H9, HUES1 and HUES9—have played in the field's development will be quickly diminished by the creation of new materials. New policy prescriptions concerned with increasing the diversity of research materials must attend to both lines that can be used and those that actually are used.

The lasting legacy of Bush era policies is an hESC science that relies very heavily on a small number of well-used but less than ideal cell lines derived under fragmented and inconsistent regulatory regimes[12]. This complicated tangle of rules and restrictions may lead researchers to focus on well-used and documented materials regardless of their technical and scientific characteristics. A consequence of restrictive policy may indeed have produced a benefit: a reproducible yet small number of well-characterized lines are now used as references for the community of stem cell researchers. Yet the startling near monopoly of just two cell lines among those that have been approved for federal funding suggests that future policies must take care to preserve the continued use of these materials while developing incentives to create, benchmark and utilize new, more appropriate materials in pursuit of scientific and clinical goals.

In sum, attending to both the scientific and the social pressures on materials selection by focusing attention on the diversity of lines in the banks and at the bench is necessary to create an efficacious policy. The first step is that federal funding be extended to all lines under reasonable ethical standards of derivation. The second step is to determine which lines are best suited to help researchers reach their goals, thereby creating the greatest public benefit. We suggest that new NIH policies focus equally on rationalizing rules and standards for derivation and use of new lines and creating incentives to develop and disperse diverse materials that are necessary to realize this field's promise.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Andrew Cohn and Susan Langbehn of Wisconsin Alumni Research Foundation and WiCell, Douglas Melton, Claire Fitzgerald and Brock Reeve of HSCI, and Mariana Craciun of the University of Michigan. C.T.S. was supported by funds from the Stanford Institute for Neuro-Innovation and Translational Neurosciences. J.O.-S. was supported by National Science Foundation grant no. 0545634. J.B.M.'s work on this publication was made possible by Grant Number 1 UL1 RR024150-01* from the National Center for Research Resources (NCRR), a component of NIH, and the NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

Christopher Thomas Scott[1], Jennifer B. McCormick[2] & Jason Owen-Smith[3]

[1]Program on Stem Cells in Society, Center for Biomedical Ethics, Stanford University, Stanford, California, USA. [2]Departments of Medicine and Health Sciences Research, Bioethics Research Program, Mayo Clinic and College of Medicine, Rochester, Minnesota, USA. [3]Department of Sociology and Organizational Studies Program, University of Michigan, Ann Arbor, Michigan, USA.

e-mail: cscott@stanford.edu or jdos@umich.edu

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Date: September 24, 2009

NIH Stem Cell Registry
Re: Request # 2009-ACD-001
Submitted by WiCell Research Institute on September 23, 2009

I hereby certify that the statements in the Request for Human Embryonic Stem Cell Line to be Approved for Use in NIH Funded Research (NIH Form 2890), submitted by Erik J. Forsberg, and below, are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties (U.S. Code, Title 18, Section1001).

I further confirm that that I have the legal authority and/or legal rights pertaining to the human embryonic stem cell line(s) identified in item 6 of the form to make this request for NIH review and determination of eligibility for use in NIH funded research (e.g., I am the owner, deriver or licensee or have written permission of the same to submit). Any and all restrictions on the use of the stem cell line are clearly and completely identified in item 8 of the form.

Assurance Statements (mark the appropriate statement with an "X"; you may only check one Assurance statement.):

_____ Assurance in accord with Section II(A) of the NIH Guidelines:

I hereby assure that the donation of the embryo from which the cell line(s) identified in item 6 was derived was in accordance with the elements of Section II(A) of the NIH Guidelines on Human Stem Cell Research.

OR

_X__ Assurance in accord with Section II(B) of the NIH Guidelines:

I hereby assure that the embryo from which the cell line(s) identified in item 6 of the form was derived was donated prior to July 7, 2009, and the embryo: 1) was created using in vitro fertilization for reproductive purposes and was no longer needed for this purpose; and 2) was donated by donor(s) who gave voluntary written consent for the human embryo to be used for research purposes. The applicant is advised that the Working Group of the Advisory Committee to the NIH Director will consider submitted materials taking into account the principles articulated in Section II(A) of the NIH Guidelines for Human for Human Stem Cell Research, 45 CFR 46 Subpart A, and the following points to consider: during the informed consent process, including written and oral communications, whether the donor(s) were: (1) informed of other available options pertaining to the use of the embryo ; (2) offered any inducements for the donation of the embryo ; and (3) informed about what would happen to the embryo after the donation for research.

OR

_____ Assurance in accord with Section II(C) of the NIH Guidelines:

P.O. Box 7365 • Madison, WI 53707-7365 • Phone: 888.204.1782 • Fax: 608.441-8011
www.wicell.org

I hereby assure that the embryo from which the cell line(s) identified in item 6 of the form was derived was donated outside the United States on or after July 7, 2009, and the alternative procedural standards of the foreign country where the embryo was donated provide protections at least equivalent to those provided by Section II(A) of the NIH Guidelines on Human Stem Cell Research.

I acknowledge that I have read, understood, and agreed to the information provided on the form, including the Instructions for completing the form, and the Certification, Authority and Assurance provided above.

[signature]

Erik J. Forsberg, Ph.D.
Executive Director
WiCell Research Institute
Phone: 608-334-7876
Email: eforsberg@wicell.org

September 24, 2009
Date

Summary of Additional Supporting Information Provided to the ACD Working Group for WA01 (H1)

Document 1: Summary of Additional Supporting Information Provided to the Working Group – WA01 (H1)

This, the current document, is intended to provide the Working Group of the Advisory Committee to the [NIH] Director (ACD) with an explanation of how the additional supporting information provided meets the October 19, 2009 request from the ACD Working Group.

Document 2: October 2009 E-mail Request from the ACD Working Group

This is document is a copy of the October 19, 2009 request from the ACD Working Group for additional information required for review of the WA01 (H1) cell line. Specific documents requested were:

1. The research protocol 95-623-239 (as approved by the University of Wisconsin Madison Human Subjects Committee),
2. The record of the Human Subjects Committee discussion of protocol 95-623-239, and
3. For a given embryo donor during the time period relevant to WA01 (or a pair of donors, if it was a couple seeking reproductive treatment):
   a) A signed, dated consent form(s) for clinical care
   b) A signed, dated consent form for embryo cryopreservation
   c) Documentation of the date of oocyte retrieval
   d) A signed, dated consent form for donation of the embryos for research

Please redact any signatures of patients or other patient identifiers from these documents.

Document 3: 1995 Thomson Protocol 95-623-239

This document includes a copy of:

1. The July 24, 1995 Approval Letter from the University of Wisconsin Madison Human Subjects Committee,
2. The research protocol (95-623-239) as approved by the University of Wisconsin Madison Human Subjects Committee on July 24, 1995, and
3. The approved consent form (August 1995 version) for research protocol 95-623-239.

Document 4: July 24 1995 HSC Minutes – Thomson 95-623-239

This document is a copy of the July 24, 1995 University of Wisconsin Madison Human Subjects Committee meeting minutes during which Dr. Thomson's research protocol (95-623-239) was approved.

Please note: *While the University of Wisconsin-Madison Human Subjects Committee conducts IRB meetings in closed session and generally does not consider meeting minutes to be public documents under Wisconsin state laws; the Committee believes that in this case, policy dictates that these minutes may be released to the public.*

Document 5: 95-E Complete Consents Redacted

This document includes the following documents for Donor 95-E:

1. A signed, dated (August 15, 1990) consent form(s) for clinical care
2. Physician procedure (Oocyte recovery by transvaginal ultrasound directed aspiration) note with documentation of date of oocyte retrieval (September 16, 1990)
3. A signed, dated consent form for embryo cryopreservation (September 16, 1990)
4. A signed, dated consent form for donation of the embryos for research (November 9, 1996)

The signatures of patients and other patient identifiers have been redacted from these documents

It should be noted that both fresh (non-frozen) and frozen embryos were donated under research protocol 95-623-239. These documents are from a couple (Donor-95E) whose embryos were frozen and who then determined that their embryos were no longer needed for their reproductive care. Since patient identifiers were removed prior to the derivation process, it would be difficult to determine if the embryos from this couple resulted the derivation of the WA01 (H1) cell line.

Document 6: 95-G Complete Consents Redacted

This document includes the following documents for Donor 95-G:

1. A signed, dated (January 7, 1998) consent form(s) for clinical care
2. Physician procedure (Transvaginal Ultrasound-directed Egg Retrieval) note with documentation of date of oocyte retrieval (January 15, 1998)
3. A signed, dated consent form for embryo cryopreservation (January 7, 1998)
4. A signed, dated consent form for donation of the embryos for research (January 22, 1998)

The signatures of patients and other patient identifiers have been redacted from these documents

It should be noted that both fresh (non-frozen) and frozen embryos were donated under research protocol 95-623-239. These documents are from a couple (Donor-95G) who donated fresh (non-frozen) embryos which were deemed to be of poor quality and likely would not have survived the freeze/ thaw process. Therefore, rather than discarding the embryos, the couple decided to donate them to research protocol 95-623-239. Since patient identifiers were removed prior to the derivation process, it would be difficult to determine if the embryos from this couple resulted the derivation of the WA01 (H1) cell line.

From:	Dean, Betsy (NIH/OD) [E]
To:	Dean, Betsy (NIH/OD) [E]
Subject:	FW: request from the ACD Working Group
Date:	Tuesday, November 24, 2009 12:42:27 PM

-----Original Message-----
From: Jones, Jeff [mailto:JJones@wicell.org]
Sent: Thursday, November 19, 2009 4:20 PM
To: HESCREGISTRY (NIH/NIDCD); Forsberg, Erik
Cc: Gadbois, Ellen (NIH/OD) [E]; Marino, Susan (NIH/NINDS) [E]; Dean, Betsy (NIH/OD) [E]
Subject: RE: request from the ACD Working Group

HESC Registry,

As requested, I have attached the following supplemental information for review by the ACD Working Group for the WA01 (H1) cell line:

1. Summary of Additional Supporting Information 2. October 2009 e-mail request from the ACD Working Group 3. 1995 Thomson Protocol 95-623-239 4. July 24 1995 HSC Minutes - Thomson 95-623-239 5. 95-E Complete Consents Redacted 6. 95-G Complete Consents Redacted

Please note that the first document (Summary of Additional Supporting Information) summaries all of the other documents and describes how we believe these documents fulfill the ACD Working Group's request for additional supplemental information.

Hopefully the ACD Working Group will be able to review these documents during their next scheduled meeting.

If you have any additional questions, please feel free to contact me directly.

Best Regards,

-Jeff

Jeffrey M. Jones, PhD. HCLD (ABB)
Director of Derivation
WiCell Research Institute
Associate Professor
University of Wisconsin-Madison
Department of Ob/Gyn
Phone: (608) 441-8016
Fax: (608) 441-8011

Disclaimer: This message and any attachments are for the designated recipient only and may contain privileged, proprietary or otherwise private information. If you have received this in error, please notify the sender and delete the original. Thank you.

-----Original Message-----
From: HESCREGISTRY (NIH/NIDCD) [mailto:hescregistry@mail.nih.gov]
Sent: Monday, October 19, 2009 1:17 PM
To: Forsberg, Erik
Cc: Gadbois, Ellen (NIH/OD) [E]; HESCREGISTRY (NIH/NIDCD); Jones, Jeff
Subject: request from the ACD Working Group

Dear Dr. Forsberg,

Thank you for your recent submission of information about human embryonic stem cell line WA01 (H1). The ACD Working Group for Human Embryonic Stem Cell Eligibility Review has conducted an initial review of your submission.

The Working Group requests that you provide NIH with additional documents that will assist in its review. The Working Group is interested in the consent process and documents for the clinical services provided to the donors and the sequence of events for a given donor(s) during the time of donation of the embryos used for the derivation of WA01. The Working Group specifically requests the following documents, to the extent they are available:

* The research protocol 95-623-239 (as approved by the University of Wisconsin Madison Human Subjects Committee).
* The record of the Human Subjects Committee discussion of protocol 95-623-239.
* For a given embryo donor during the time period relevant to WA01 (or a pair of donors, if it was a couple seeking reproductive treatment):
    - o A signed, dated consent form(s) for clinical care
    - o A signed, dated consent form for embryo cryopreservation
    - o Documentation of the date of oocyte retrieval
    - o A signed, dated consent form for donation of the embryos for research

Please redact any signatures of patients or other patient identifiers from these documents.

Documents should be submitted to the NIH hESC Registry mailbox (hescregistry@mail.nih.gov). If you can let me know when you anticipate submitting the documents, that will help for planning further review by the Working Group.

Please let me know if you have any questions about this request.

Sincerely,
Ellen L. Gadbois

Ellen L. Gadbois, Ph.D.
Office of Science Policy Analysis
Bldg 1 Room 218D
National Institutes of Health
voice: 301-594-2567
fax: 301-402-0280

THOMSON 95-623-239

Notice of Approval

Meeting Date: July 24, 1995

Protocol Number: 95-623-239
(Refer to this number when making inquiries)

To: James Thomson, V.M.D., Ph.D.
Assistant Scientist, Primate Research Center
1223 Capitol Court

From: Jane C. Fitchen / Shelley Logally
IRB Administrator

RE: Protocol entitled, "Human Embryonic Stem Cells"

The Human Subjects Committee has reviewed and approved the above research protocol. Approval is effective for one year. Please note the following additional information and requirements:

Institutional Endorsement: If a granting agency requires notification of HSC approval, submit the name and address of an individual at that agency. Our DHHS Multiple Project Assurance ID is M1285-01.

Adverse Reactions: If any serious, unexpected adverse reaction occurs as a result of this study, you must notify the IRB administrator immediately.

Amendments: If you wish to change any aspect of the study (design, procedures, consent forms, or subject population, etc.), please submit your changes with a progress report on a Change of Protocol form. *The change may not be initiated until HSC approval has been given.*

Renewal: You are required to renew approval annually for as long as the study is active. Contact the HSC office for renewal forms.

Termination of Research: Please promptly notify the HSC in writing of the termination of this project.

Consent Forms: All subjects should be given a copy of the consent form(s).

VA Patients: All research involving VA Patients must be reviewed by the VA Research and Development Committee. Call the VA Research Office, 125-7863.

Cancer Patients Approval of all research involving cancer patients is conditional upon review of the protocol by the WCCC Clinical Affairs Committee.

Please keep this notice with your copy of the approved protocol.

Previously Deferred
Mailed August 23, 1995

Human Subjects Committee • Center for Health Sciences

H6/275 Clinical Science Center 600 Highland Avenue Madison, Wisconsin 53792-2457 608/263-2362

WISCONSIN REGIONAL PRIMATE RESEARCH CENTER
University of Wisconsin / 1223 Capitol Court / Madison, Wisconsin 53715-1299
FAX (608) 263-3524

James Thomson, V.M.D., Ph.D.
Associate Research Animal Veterinarian
(608) 263-3585

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August 18, 1995

To: Jane C. Fitchen
IRB Administrator
H6/275 Clinical Science Center

From: James Thomson
Wisconsin Regional Primate Research Center

Please find attached a revised version of the protocol, "Human Embryonic Stem Cells" (Protocol number: 95-623-239). All of the modifications required in your letter of 8/4/95 are included.

UNIVERSITY OF WISCONSIN-MADISON
CENTER FOR HEALTH SCIENCES
HUMAN SUBJECTS COMMITTEE

REQUEST FOR EXPEDITED REVIEW: CATEGORY I, "NO APPARENT RISK"
(See Guidelines, "Procedure for Expedited Review")

HSC Protocol # 95-623-239

This application must be submitted to and approved by the Human Subjects Committee prior to the initiation of any research involving human subjects or human material. The original plus 2 (total 3 copies) should be sent to Jane Fitchen, IRB Administrator, H6/275 Clinical Sciences Center, 600 Highland Avenue, Madison, Wisconsin 53792, Telephone: 263-2362. Deadline for submission of material is 12:00 noon Monday, one week prior to the meeting.

Note to file: The PI did not have to include this face sheet, but I am retaining it as evidence that Dr. Shapiro is familiar with the status of the protocol. [initials] 8/23/95

Date: 8/17/95

Project Title: Human Embryonic Stem Cells

Principal Investigator: James Thomson, V.M.D., Ph.D. (Name) Assistant Scientist (Rank)

1223 Capitol Court (Campus Mailing Address) Primate Research Center (Department) 263-3585 (Telephone)

Supporting Agency: Pending University-Industry Relations TIF Grant (Name) (Grant Number)

Is project federally funded? YES ___ NO X

NOTE: If project is federally funded, please provide a name and address of an individual at NIH to whom official notification (HHS form 596) should be sent.

Type name of faculty sponsor if below rank of Instructor: Sander S. Shapiro, M.D.

SIGNATURES: Principal Investigator: [signature]

Department Chairperson: [signature]

Faculty Sponsor (if required): [signature]

(HSC USE ONLY)

Approval Date: 7/24/95

SIGNATURE: ____________________
(HSC Chairperson)

0656I

(OVER)

Complete the form below. If necessary, continue answers on separate sheets and attach.

1. What is the purpose of the study?

   See attached.

2. What will be done (e.g., questionnaire, interview, observation, etc.)?

   See attached.

3. Who will the subjects be (e.g., high school students, staff nurses, etc.)? Estimate sample size.

   See attached.

4. How will the subjects be recruited and by whom?

   See attached.

5. Where will the study be done?

   See attached.

6. Do you think there are any risks to the subjects? If yes, explain.

   See attached.

7. a. Will any of the following be eligible subjects?

	YES	NO
pregnant women	___	x
mentally infirm	___	x
mentally retarded	___	x
patients	x	___
minors	___	x
prisoners	___	x
fetuses or abortuses	___	x
VA Hospital patients	___	x

	YES	NO
b. Will information be recorded in a way that subjects could be identified?	___	x
c. If the information did become known, would it put the subject at risk for criminal or civil liability or affect employability or insurability?	___	x
d. Does the study deal with sensitive aspects of behavior, such as illegal conduct, drug use, alcohol use, or sexual behavior?	___	x
e. Do you plan to recontact subjects after you collect the data?	___	x

NOTE: If the answer to any part of question #7 is "yes," please explain in more detail on an attached sheet.

1. What is the purpose of the study?

Embryonic stem (ES) cells, derived from preimplantation embryos [1,2], are undifferentiated, immortal cells capable of differentiating into derivatives of all three embryonic germ layers. We have recently isolated ES cell lines from blastocysts from an Old World Primate species, the rhesus monkey [3], and from a New World primate species, the common marmoset (unpublished). In the present study, we will use our experience gained from the nonhuman primate ES cells lines to isolate similar ES cell lines from excess *In Vitro* Fertilization (IVF)-produced preimplantation human embryos.

Well-characterized ES cells had previously been derived only from rodents [1,2,4,5]. The mechanisms controlling differentiation of specific lineages can be studied with mouse ES cells grown *in vitro*; however, there are significant differences between early human and mouse development. For example, human and mouse embryos differ in the timing of embryonic genome expression [6], in the structure and function of the fetal membranes and placenta [7,8], and in formation of an embryonic disc instead of an egg cylinder. Nonhuman primate ES cells provide a more accurate model of the differentiation of human tissues, but the best *in vitro* model would be provided by human ES cells. Other research groups are currently attempting to derive human ES cell lines from excess IVF-produced embryos, but have not yet succeeded in deriving permanent cell lines [9].

Human ES cells would offer exciting new possibilities for transplantation medicine. Because ES cells have the developmental potential to give rise to all adult cell types, any disease resulting from the failure of specific cell types would be potentially treatable through the transplantation of differentiated cells derived from ES cells. ES could be genetically manipulated prior to differentiation to reduce immunogenicity, or banks of ES cell lines could be derived with different Major Histocompatibility Complex (MHC) backgrounds. Because of the range of diseases potentially treatable by this approach, isolating human ES cells and elucidating the basic mechanisms controlling the differentiation of specific cell types has potentially dramatic clinical significance.

2. What will be done? Excess human preimplantation embryos will be cultured for 10 days or less, until formation of a blastocyst, a developmental stage which occurs before implantation. After this period of culture, the outer cells of the embryo (trophectoderm) will be separated from the inner cells of the embryo (inner cell mass) and discarded. The inner cell mass cells will be cultured and studied. Note that the inner cell mass is no longer a complete embryo; as such it

would not develop into a fetus if transferred to a uterus. Inner cell mass-derived cells will be cultured, and used for *in vitro* studies of differentiation.

The National Institutes of Health is not currently funding any research on IVF-produced human embryos so there are no federal guidelines currently regulating such research. However, the Report of the Human Embryo Research Panel, Ad Hoc Group of Consultants to the Advisory Committee to the Director, NIH (September, 1994; executive summary attached) recommended research **acceptable** for federal funding should include:

" Research involving the development of embryonic stem cells, but only with embryos resulting from IVF for infertility treatment or clinical research that have been donated with the consent of the progenitors. [10]"

Types of research that the panel found **unacceptable** included:

1) "Studies designed to transplant embryonic or adult nuclei into an enucleated egg, including nuclear cloning, in order to duplicate a genome or to increase the number of embryos with the same genotype with transfer."
2) "Development of human-nonhuman or human-human chimeras with or without transfer."

Under no circumstances will nuclei from human cell lines derived from this study be used for nuclear transfer to enucleated oocytes. Under no circumstances will chimeras be formed between human cells derived from this study and an embryo, either human or nonhuman.

3. **Who will be the subjects?** Excess IVF-produced preimplantation embryos will be derived from patients undergoing standard *In Vitro* Fertilization procedures at the University of Wisconsin Hospital and Clinics, and who do not wish to have them transferred to either themselves or to other recipients. Since these embryos would otherwise be discarded, the use of these embryos for the proposed research will not have an impact on the treatment outcome of the patients. Once embryonic tissue is entered into this study, it will be identified by code and no reference will be made to the particular persons whose gametes produced these embryos. Results of experiments with cells from embryos of particular donors will not be reported to the clinician in a way that would allow the identification of the donors. Fewer than 25 embryos per year will be used for this study.

4. How will the subjects be recruited and by whom? will explain the proposed research to patients undergoing standard *In Vitro* Fertilization procedures at the University of Wisconsin Hospital and Clinics and will provide them with consent forms if they choose to donate their excess preimplantation embryos for this project.

5. Where will the study be done? Excess preimplantation embryos will be cultured to the blastocyst stage by in the IVF laboratories of the University of Wisconsin Hospital and Clinics, H4/635B Clinical Science Center. After removes the trophectoderm, the isolated inner cell mass cells will be grown by Dr. Thomson in his laboratory at the Wisconsin Regional Primate Research Center (Room 120). Only staff of the IVF laboratories of the University of Wisconsin Hospital and Clinics will handle intact human embryos; no intact embryos will be removed from that laboratory.

6. Do you think that there are any risks to the subjects? No

References.

1. Martin G. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA 1981; 78:7634-7638.

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4. Doetschman T, Williams P, Maeda N. Establishment of hamster blastocyst-derived embryonic stem (ES) cells. Dev Biol 1988; 127:224-227.

5. Iannaccone PM, Taborn GU, Garton RL, Caplice MD, Brenin DR. Pluripotent embryonic stem cells from the rat are capable of producing chimeras. Dev Biol 1994; 163:288-292.

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7. Benirschke K, Kaufmann P. Pathology of the Human Placenta. New York: Springer-Verlag, 1990:

8. Mossman H. Vertebrate Fetal Membranes. New Brunswick: Rutgers, 1987:

9. Bongso A, Fong CY, Ng SC, Ratnam S. Isolation and culture of inner cell mass cells from human blastocysts. Hum Reprod 1994; 9:2110-2117.

10. Ad Hoc Group of Consultants to the Advisory Committee to the Director NIH. Report of the Human Embryo Research Panel. Bethesda, Maryland: National Institutes of Health, 1994:

RECEIVED AUG 18 1995

Consent to Use Excess Pre-implantation Embryos for Cell Line Isolation

YOU ARE INVITED TO TAKE PART IN A RESEARCH STUDY OF HUMAN CELL DIFFERENTIATION.

Purpose

All human tissues and organs are composed of small building blocks called cells. By growing cells from embryos which have not yet implanted in the uterus (preimplantation embryos) we hope to better understand normal embryo development, and ultimately find important clues for the treatment of infertility, miscarriage, birth defects, and other conditions.

What Will Be Done With The Embryos?

Preimplantation embryos that are not chosen to be placed in the uterus will be cultured in the laboratory for 10 days or less. This incubation time is not long enough for the development of any fetal structures, and the embryo will not yet reach the stage when implantation in the uterus occurs. During this incubation the embryos will be photographed. After this period of culture, the outer cells of the embryo will be separated from the inner cells of the embryo and discarded. The inner cells will then be cultured for an indefinite time ("cell lines") and studied. Note that the inner cells are not a complete embryo; as such they would not develop into a fetus if transferred to a uterus.

Why Are These Embryonic Cells Important?

Because these embryonic cells will be from the preimplantation embryo before the development of any specific tissue type they are called "undifferentiated". These cells can be cultured in this undifferentiated state in the laboratory, potentially indefinitely. However, by changing how they are cultured, they will sometimes randomly develop into "differentiated" cells (for example, cells that look and behave like the cells of placenta, bone, skin, or blood). By studying how these embryonic cells differentiate, in the future it may be possible to direct their differentiation to specific cell types in culture. Because many diseases (such as diabetes mellitus or Parkinson's disease) result from the death or dysfunction of specific cell types, it might one day be possible to treat many diseases by the transplantation of differentiated cells derived in tissue culture from embryonic cell lines. Although the potential for treating human diseases is great, significant biomedical advances will be needed before such treatments are possible.

Why Might These Embryonic Cell Lines Be Controversial?

These cell lines will be permanent, that is they will continue to divide in culture indefinitely. Although they are not whole embryos (i.e. they would not develop into a fetus if returned to the uterus) they will maintain many embryonic properties. Because of these embryonic properties, certain experiments which would be controversial for whole human embryos would be controversial for these cell lines. In particular, two experiments that will not be performed with embryonic cell lines derived from this study are: (i) Intermixing of human embryonic cells with an intact embryo, either human or nonhuman, and (ii) Attempting to make genetically identical whole embryos by any method.

Is There Any Medical Benefit For Participation?

You will not directly benefit from participation, but future patients may benefit from the study. The embryonic cells will provide information about normal human development that could lead to improved therapies for infertility or other conditions. Nothing discovered about the donors from this research will be conveyed to the donor's physicians in a way that could be personally identified. } added

Is There Any Financial Incentive For Participation?

You will not be financially compensated for participation. If embryonic cell lines are successfully isolated, the cell lines would become the property of the University of Wisconsin or the Wisconsin Alumni Research Foundation (WARF). Because of the possibility that differentiated cells derived from embryonic cell lines might one day be used to treat human diseases, embryonic cell lines might have significant commercial value, and WARF may apply for patent protection for the isolation technique of the cell lines and on the properties of the cell lines. If a patent is granted, WARF would own the patent. WARF is a Wisconsin corporation, organized in 1925 by University of Wisconsin-Madison alumni as an entity separate from the University. WARF is dedicated to supporting research at the UW-Madison. As part of its mission, WARF patents and licenses University inventions. Earnings are combined with WARF investment income to fund WARF's annual gift back to the University. The University then is responsible for deciding how the money will be applied. Inventors receive a share of earnings from their inventions, and by University policy, a part of what the University receives from WARF goes to a department or departments associated with the invention.

Confidentiality of Participation Neither the men nor women who provide the gametes for the embryos used in this study will be identified in any communication or publication that may result from this study.

<u>If You Change Your Mind</u> You are free at any time to refuse permission for the use of any of your excess embryos, up until the moment when the inner cells of the embryos are isolated for culture. Once these cells are isolated for culture, they become the property of the University of Wisconsin.

YOU MAY TAKE AS MUCH TIME AS YOU WISH TO THINK THIS OVER. IF YOU CHOOSE NOT TO TAKE PART IN THIS STUDY, YOUR MEDICAL CARE WILL NOT BE AFFECTED IN ANY WAY. BEFORE YOU SIGN THIS FORM, PLEASE ASK ANY QUESTIONS ON THE ASPECTS OF THIS STUDY WHICH ARE NOT CLEAR TO YOU. WE WILL ATTEMPT TO FULLY ANSWER ANY QUESTIONS YOU MAY HAVE PRIOR TO, DURING, OR FOLLOWING THIS STUDY

Name of Woman : ______________________ (please print)
(Oocyte Donor)

____
Signature of Woman (Oocyte Donor) (date)

Witnessed by: ______________________ (please print)

____
Signature of Witness (date)

Name of Partner: ______________________ (please print)
(Sperm Donor)

____
Signature of Partner (Sperm Donor) (date)

Witnessed by: ______________________ (please print)

____
Signature of Witness (date)

CONFIDENTIAL

Previously Deferred

95-623-239 -- James Thomson, V.M.D., Ph.D., Assistant Scientist, Primate Research Center.
Human Embryonic Stem Cells (Fost/Madsen)

The protocol was initially reviewed on June 5, 1995, and action was deferred with the recommendation that the principal investigator revise the consent form to emphasize the potential of the technology, the fact that a successful technology could have great commercial value, and that the opportunities the research might reveal could be a source of ethical controversy. Although the study meets the criteria to be a Category I protocol ("No Apparent Risk"), the full Committee requested the opportunity to review the study due to its ethically sensitive nature.

The reviewers agreed that the principal investigator's revision had successfully addressed many of the issues raised in using gametes in research. The Committee agreed that consent obtained from female donors was sufficient, but debated whether sperm donors would be given adequate opportunity for informed consent regarding experimental use of their gametes in situations where the donor is anonymous, e.g., sperm purchased from a sperm bank. Because embryos will be gathered only from women undergoing standard *In Vitro* Fertilization procedures at the University of Wisconsin Hospital and Clinics, and because that site will not perform *In Vitro* fertilization on single women, the Committee agreed that the protocol should be approved only if both male and female donors are known to the clinician and can both be approached for consent to donate their gametes for research purposes, i.e., anonymous donation will be excluded.

The Committee voted unanimous approval for the project, but final approval is conditional upon the following conditions and modifications:

1) The donors of both male and female gametes must be able to give consent for use of their reproductive material for research purposes. The research is limited to donations from couples; embryos formed from the use of sperm from anonymous donors may not be used in this protocol. The Authorization section of the consent form should be modified to obtain consent from the sperm donor.

2) A statement that results of the research will not be reported to the clinician should be added to the protocol section, #3. Who will be the subjects? The Benefits section of the consent form should state that nothing discovered about the donors from this research will be conveyed to the donor's physicians in a way that could be personally identified.

3) The consent form section, What will be done with the embryos?, should explicitly state that cell lines will be developed, and briefly define what cell lines are.

4) Modifications as noted on the attached, edited copy of the consent form should be made.

CONFIDENTIAL

New Business

95-623-239 -- James Thomson, V.M.D., Ph.D., Assistant Scientist, Primate Research Center.
Human Embryonic Stem Cells (Cat. I) (Fost/Thomas)

Description: To use experience gained from the nonhuman primate embryonic stem (ES) cell lines to isolate similar ES cell lines from excess *In Vitro* Fertilization (IVF)-produced preimplantation human embryos.

Subjects: Excess preimplantation human embryos will be derived from patients undergoing standard *In Vitro* Fertilization procedures at UWHC, who do not wish to have them transferred to either themselves or to other recipients. These embryos would otherwise be discarded. Once embryonic tissue is entered into this study, it will be identified by code only.

Intervention: Excess human preimplantation embryos will be cultured for 10 days or less, until an early blastocyst forms, a developmental stage before implantation would normally occur. After this period of culture, the outer cells of the embryo (trophectoderm) will be separated from the inner cells of the embryo (inner cell mass) and discarded. Inner cell mass-derived cells will be cultured and used for *in vitro* studies of differentiation.

In discussion, the Committee expressed concern that in developing immortal lines, the cells were potentially clonable and could potentially have commercial value. One reviewer acknowledged that although the protocol is staying within the guidelines of the NIH Human Embryo Research Panel (9/94),there could be religious objections to the research. The Committee agreed that while the intervention was minimal risk, the controversial nature of the research required more explicit information in the consent form.

The Committee voted unanimously to defer action on the protocol pending an opportunity for review by all members of the Committee. The Committee suggested that the principal investigator revise the consent form to emphasize the potential of the technology, the fact that a successful technology could have great commercial value, and that the opportunities the research might reveal could be a source of ethical controversy.

The Committee requested that 25 copies of the protocol be submitted for review at a future meeting.

Donor 95-E

INFORMED CONSENT: IN VITRO FERTILIZATION

I have been unable to become pregnant due to blocked or absent Fallopian tubes which cannot be corrected surgically or I have some other reason for infertility which is not treatable with other currently available methods. I wish to attempt to become pregnant using in vitro fertilization and embryo transfer (test bue baby), carried out by the physicians at the Endocrinology-Infertility Unit within the Department of Obstetrics and Gynecology and the University of Wisconsin Center for Health Sciences and any assistants selected by them.

I understand and have discussed with my physicians at the Endocrinology-Infertility Unit that, while other alternative procedures to attain fertility may exist, in vitro fertilization and ovum transfer offer the best opportunity to attain this goal in my particular case.

I understand that the following is an outline of the steps required in this procedure:

1) Determination by standard infertility tests that I am a suitable candidate for in vitro fertilization.
2) Use of "fertility drugs" (usually clomiphene citrate, Pergonal, and human chorionic gonadotropin or Pergonal and human chorionic gonadotropin) to produce ovulation at a predictable time.
3) Ultrasound examinations to assist in predicting the time of expected ovulation.
4) Undergoing transvaginal aspiration with insertion of a needle into my ovary to obtain one or more eggs.
5) Obtaining sperm from my husband by masturbation and treating it to prepare for fertilization in the laboratory.
6) Mixing the egg and the sperm together to allow fertilization to occur.
7) After fertiliztion, transferring the egg into a fluid for growth and after several cell divisions, if the embryo (conceptus) is developing normally, transferring the embryo into my uterus by means of a small plastic tube inserted through my cervix.
8) Obtaining blood samples four to six times during the two weeks after attempted fertilization to determine if my hormone levels are normal and if pregnancy has occurred.
9) I understand that ova (eggs) may be recovered by either laparoscopy or transvaginal aspiration. I have discussed these two alternatives with the Reproductive-Endocrine staff and understand the reasons for their choice of procedure.

The process for transvaginal aspiration of follicles for ovum retrieval is performed in some IVF patients instead of laparoscopy. The procedure involves placing a needle, guided by ultrasound, into the ovary. The needle goes through the vagina and is placed under local anesthesia. This procedure is chosen in place of laparoscopy when it is known that the ovaries are hidden from view by adhesions. The process may result in a lower frequency of egg retrieval. The procedure eliminates the need for general anesthesia.

INFORMED CONSENT: IVF (page 2)

I understand that I may be hospitalized for the above procedure to be accomp..shed. I also understand that, despite the physicians and hospital employees proceeding with due care, any of the following may occur which would prevent the establishment of a pregnancy:

1) The time of ovulation may be misjudged, may be unable to be predicted, may occur prior to egg retrieval or ovulation may not occur in the monitored cycle, thus precluding any attempt at obtaining an egg.
2) Pelvic adhesions may prevent access to the ovary containing the ripe egg.
3) Ovulation may have occurred by the time transvaginal aspiration is performed.
4) Medical emergencies with other patients may make an operating room and anesthesia unavailable at the appropriate time for obtaining an egg.
5) The attempt at obtaining an egg may be unsuccessful.
6) The egg may not be normal.
7) My husband may be unable to obtain a semen specimen.
8) Fertilization may not occur.
9) Cleavage of cell division of the fertilized egg may not occur.
10) The embryo may not develop normally.
11) Implantation in the uterus may not occur.
12) A laboratory accident may result in loss or damage to the fertilized egg or embryo.

If pregnancy is established, miscarriage, ectopic pregnancy, stillbirth, or congenital abnormalities (birth defects) may occur.

I understand that insurance coverage for any or all of the above procedures may not be available and that I and my husband will be personally responsible for the expenses of this treatment and for all other expenses and costs to us or to our child(ren) for medical problems resulting from this procedure, such as miscarriage, ectopic pregnancy, stillbirth or birth defects. Expenses will consist of hospital, laboratory, and professional fees.

I understand that the success rate (chance of pregnancy) is about 20% in those institutions where _in vitro_ facilities are currently in use.

I know that the procedures for _in vitro_ fertilization and ovum transfer, as applied to humans, are relative new. I understand that careful records of my case will be kept and that samples of my blood or fluids obtained during my therapy will be preserved for subsequent evaluation to monitor and possibly improve the methodologies use for _in vitro_ fertilization. I know that up to five fertilized ova may be placed in my uterus and that twin or other multiple births may result from this procedure. I know that my _in vitro_ fertilization attempt is being carried out as therapy is not a research project.

If I have further questions concerning in vitro fertilization and its application in my case, I can contact or the other physicians in the Endocrine-Infertility unit at the University of Wisconsin Center for Health Sciences.

INFORMED CONSENT: IVF (Page 3)

Although the above statements refer mostly to my wife, I as the husband also understand all of the above, consent to the above and request the in vitro fertilization procedures.

We know that the practice of medicine and surgery is not an exact science and that therefore reputable practitioners cannot properly guarantee results. We acknowledge that no guarantee or assurance has been made by anyone regarding the in vitro fertilization procedures which we have herein requested and authorized.

We have read the above and understand it. We have received a full explanation of the above procedures. We request that the physicians at the Endocrinology-Infertility Unit and assistants selected by them perform the above procedures. Our signatures also indicate that we have received a copy of this consent form.

Wife — Date 8/15/90

Husband — Date 8/15/90

Witness — Date 8/15/90

University of Wisconsin Hospital and Clinics

NAME: [redacted] DATE: 09/16/90
NUMBER: [redacted]
PREOPERATIVE DIAGNOSIS: Tubal obstruction.

OPERATION: Oocyte recovery by transvaginal ultrasound directed aspiration.

POSTOPERATIVE DIAGNOSIS: Tubal obstruction.

ANESTHESIA:

PROCEDURE: The patient was placed in Trendelenburg position on an operating table and a speculum was placed in the vagina. The vagina was cleaned off with Betadine followed by sterile saline. It was then injected with about 10 cc of 1% Lidocaine. At this time, the patient received 150 mcg of fentanyl IV. The transducer was then introduced into the vagina and the single ovary was scanned. There were approximately 12 large follicles. A #17 needle was then introduced into the ovary and each of these follicles was serially aspirated, followed by irrigation. When all the follicles had been aspirated and the needle was removed and the vagina inspected. There was no bleeding from the vagina. Therefore, the patient had all instruments removed and was returned to the Recovery Room. The procedure went exceedingly well. There were no difficulties.

At the time of dictation, 12 eggs were found.

ATTENDING SURGEON:

OPERATIVE REPORT

AGREEMENT FOR CRYOPRESERVATION OF, AND REQUEST TO TRANSFER, EMBRYOS

We, [redacted] and [redacted], the undersigned Husband and Wife, are legally married and have already decided to participate in the In Vitro Fertilization and Embryo Placement clinical treatment program. We are requesting transfer of a number of embryos and granting permission for any remaining embryos to be cryopreserved (frozen) or to be left in culture. At this time, we acknowledge that granting this permission does not guarantee that there will be any embryos available for freezing.

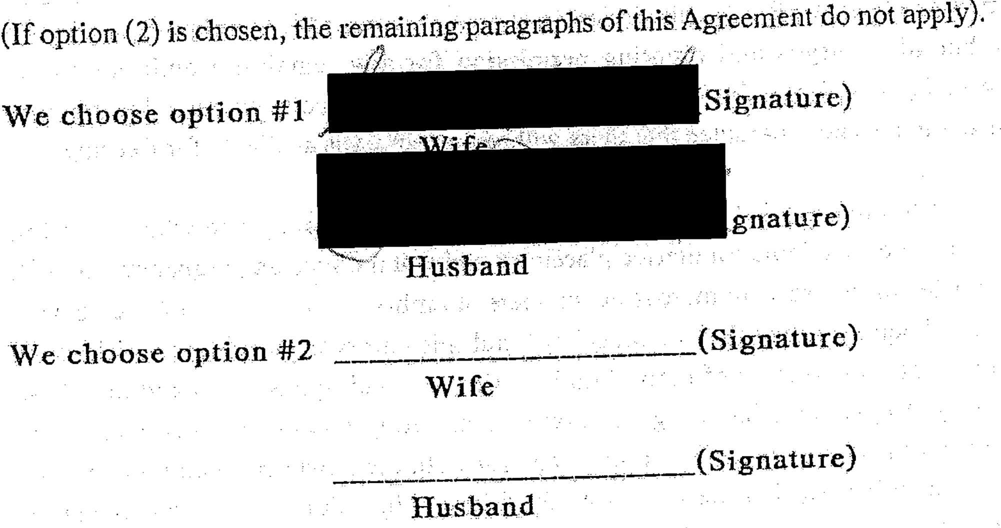
We have been informed that there is an increase in the pregnancy rate with increasing number of embryos available for uterine placement and that the success/pregnancy rate with embryo transfer increases with increasing numbers of embryos placed, up to 4-6 embryos. We have also been informed that the transfer of multiple embryos increases the risk of the establishment of a pregnancy of high plurality (triplets, quadruplets, etc.) and that these high plurality pregnancies have a great risk of terminating without the survival of the fetuses and may involve medical complications for both the newborn(s) and the mother. We have been informed that the risk of establishing a high plurality pregnancy can be reduced by placing a limited number of embryos in the uterus during the treatment cycle and freezing the rest of the embryos for uterine placement in a cycle subsequent to that treatment cycle.

We have been informed that there are two alternatives for excess embryos obtained through the In Vitro fertilization procedures:

1) The embryos may be cryopreserved (frozen) and stored for embryo transfer or use at a later date.
2) The embryos may continue their development in culture until their development ceases.

We have decided and instructed that the (1) remaining embryos of the 5 which are to be transferred during this cycle be cryopreserved (frozen) and stored for subsequent use or (2) the remaining embryos be left in culture until development ceases.

(If option (2) is chosen, the remaining paragraphs of this Agreement do not apply).



We choose option #1 (Signature)
Wife

(Signature)
Husband

We choose option #2 ______________________(Signature)
Wife

______________________(Signature)
Husband

We have been informed that, although there is limited data at the present time, available human data as well as animal data does not suggest that the embryo freezing process increases the risks of congenital anomalies (birth defects) in the resultant offspring; however, the long-term outcome in terms of pregnancy rate and consequences of freezing embryos remains to be demonstrated.

We have been informed that not all embryos survive the freezing process. We agree that, even though some embryos will not survive the freezing process, the overall advantages of the freezing as mentioned above outweigh the loss of embryos.

We acknowledge that neither our physicians nor University of Wisconsin Hospital and Clinics (UWHC) have made any warranties with respect to: 1) the viability of our frozen embryos, (2) the successful establishment of pregnancy following uterine placement of our previously frozen embryos, (3) the lack of risk of a birth defect, miscarriage, or pregnancy complication after embryo placement in the uterus or, (4) the infallibility of the Hospital's liquid-nitrogen-cooled regrigerators or any other of the Hospital's equipment.

We acknowledge that the freezing of embryos requires the use of mechanical support systems (liquid-nitrogen refrigerators), the failure of which can affect the ability of the embryo to grow. We agree that if an embryo does not grow after thawing for whatever reason, then that embryo will not be placed in the uterus.

We are aware that, for a variety of reasons (for example, our choice; death of one or both of us; or achieving our desired family size), one or more of our embryos may remain frozen and will not be wanted or needed by us. In such a situation, we agree and authorize our physicians at University of Wisconsin-Madison to maintain such unused embryos in a frozen state until the earliest in time of the following four events or dates:

1) three years from the date of execution of this agreement;
2) the death of both of us (it is understood that if one of us dies, the other can make decisions on use of the embryos);
3) our legal separation, dissolution, or divorce;
4) whenever we both consent.

With respect to Item 4, above, we understand that our physicians at UWHC reserve the right to prescribe the use of forms or other identification procedures in connection with such consent.

Prior to the end of three years from the date of execution of this Agreement, if in the medical judgement of our physicians, the frozen embryos can still be used, we shall be asked to reconsider the question of disposition of the remaining frozen embryos.

After the earliest of such events or dates as stated in Items 2, 3, and 4 above, or in the event we fail or decline to execute a new agreement regarding disposition of the remaining frozen embryos prior to the end of the three-year period, we authorize and request that any and all remaining frozen embryos be disposed of by our physicians at UWHC as follows:

> That one or more of our frozen embryos be made available for donation to a suitable anonymous recipient couple. The selection of such a couple shall be within the absolute discretion of our physicians at UWHC. In the event that no suitable recipient couple is available, decisions regarding the embryos will be made in accordance with professional ethical standards and any applicable legal requirements.

We have been informed that, other than the anticipated loss of some embryos during the freezing, preservation and thawing process, there are no known risks of discomfort. The development and application of human embryo freezing is anticipated to further increase the success rate, i.e. pregnancy establishment, following In Vitro Fertilization and Embryo Placement, while decreasing the risks and expenses associated with oocyte retrieval.

We agree that the full cost of the procedures, supplies and professional services involved in embryo freezing are our personal responsibility. Attached is a copy of the current fee schedule for embryo freezing and storage. In addition, we understand that there will be certain professional and laboratory costs involved in monitoring any subsequent cycle in which embryos are to be placed in the uterus as well as professional fees involved with the embryo placement procedure in that subsequent cycle.

Witnesses:

Signature of Physician/Date

Signature of Patient/Date

9-16-90

Additional Witness/Date

Signature of Spouse/Date

ESTIMATED COSTS FOR CRYOPRESERVATION AND TRANSFER OF CRYOPRESERVED EMBRYOS:* at the UNIVERSITY OF WISCONSIN MEDICAL CENTER

Ovulation monitoring (LH x 6 @ $51.00 each)	$ 306.00
Freezing and Thawing	$ 0.00
Medications	$ 50.00
Physician's Fee	$ 209.00
Transfer Charge	$ 251.00
OR Charges	$ 600.00
Pregnancy Test	$ 82.00

8/89

Principal Investigator: James Thomson
(608) 263-3585

Consent to Use Excess Pre-implantation Embryos for Cell Line Isolation

YOU ARE INVITED TO TAKE PART IN A RESEARCH STUDY OF HUMAN CELL DIFFERENTIATION.

Purpose

All human tissues and organs are composed of small building blocks called cells. By growing cells from embryos which have not yet implanted in the uterus (preimplantation embryos) we hope to better understand normal embryo development, and ultimately find important clues for the treatment of infertility, miscarriage, birth defects, and other conditions.

What Will Be Done With The Embryos?

Preimplantation embryos that are not chosen to be placed in the uterus will be cultured in various conditions in the laboratory for 7 days or less. This incubation time is not long enough for the development of any fetal structures, and the embryo will not yet reach the stage when implantation in the uterus occurs. During this incubation the embryos will be photographed. After this period of culture, the outer cells of the embryo will be separated from the inner cells of the embryo and discarded. The inner cells will then be cultured for an indefinite time ("cell lines") and studied. Note that the inner cells are not a complete embryo; as such they would not develop into a fetus if transferred to a uterus.

Why Are These Embryonic Cells Important?

Because these embryonic cells will be from the preimplantation embryo before the development of any specific tissue type they are called "undifferentiated". These cells can be cultured in this undifferentiated state in the laboratory, potentially indefinitely. However, by changing how they are cultured, they will sometimes randomly develop into "differentiated" cells (for example, cells that look and behave like the cells of placenta, bone, skin, or blood). By studying how these embryonic cells differentiate, in the future it may be possible to direct their differentiation to specific cell types in culture. Because many diseases (such as diabetes mellitus or Parkinson's disease) result from the death or dysfunction of specific cell types, it might one day be possible to treat many diseases by the transplantation of differentiated cells derived in tissue culture from embryonic cell lines. Although the potential for treating human diseases is great, significant biomedical advances will be needed before such treatments are possible.

Why Might These Embryonic Cell Lines Be Controversial?

These cell lines will be permanent, that is they will continue to divide in culture indefinitely. Although they are not whole embryos (i.e. they would not develop into a fetus if returned to the uterus) they will maintain many embryonic properties. Because of these embryonic properties, certain experiments which would be controversial for whole human embryos would be controversial for these cell lines. In particular, two experiments that will not be performed with embryonic cell lines derived from this study are: (i) Intermixing of human embryonic cells with an intact embryo, either human or nonhuman, and (ii) Attempting to make genetically identical whole embryos by any method.

Is There Any Medical Benefit For Participation?

You will not directly benefit from participation, but future patients may benefit from the study. The embryonic cells will provide information about normal human development that could lead to improved therapies for infertility or other conditions. Nothing discovered about the donors from this research will be conveyed to the donor's physicians in a way that could be personally identified.

Is There Any Financial Incentive For Participation?

You will not be financially compensated for participation. If embryonic cell lines are successfully isolated, the cell lines would become the property of the University of Wisconsin or the Wisconsin Alumni Research Foundation (WARF). Because of the possibility that differentiated cells derived from embryonic cell lines might one day be used to treat human diseases, embryonic cell lines might have significant commercial value, and WARF may apply for patent protection for the isolation technique of the cell lines and on the properties of the cell lines. If a patent is granted, WARF would own the patent. WARF is a Wisconsin corporation, organized in 1925 by University of Wisconsin-Madison alumni as an entity separate from the University. WARF is dedicated to supporting research at the UW-Madison. As part of its mission, WARF patents and licenses University inventions. Earnings are combined with WARF investment income to fund WARF's annual gift back to the University. The University then is responsible for deciding how the money will be applied. Inventors receive a share of earnings from their inventions, and by University policy, a part of what the University receives from WARF goes to a department or departments associated with the invention.

Confidentiality of Participation Neither the men nor women who provide the gametes for the embryos used in this study will be identified in any communication or publication that may result from this study.

If You Change Your Mind You are free at any time to refuse permission for the use of any of your excess embryos, up until the moment when the inner cells of the embryos are isolated for culture. Once these cells are isolated for culture, they become the property of the University of Wisconsin.

YOU MAY TAKE AS MUCH TIME AS YOU WISH TO THINK THIS OVER. IF YOU CHOOSE NOT TO TAKE PART IN THIS STUDY, YOUR MEDICAL CARE WILL NOT BE AFFECTED IN ANY WAY. BEFORE YOU SIGN THIS FORM, PLEASE ASK ANY QUESTIONS ON THE ASPECTS OF THIS STUDY WHICH ARE NOT CLEAR TO YOU. WE WILL ATTEMPT TO FULLY ANSWER ANY QUESTIONS YOU MAY HAVE PRIOR TO, DURING, OR FOLLOWING THIS STUDY. If you have questions, they should be directed to: , Professor, Department of Obstetrics and Gynecology, IVF Laboratories of the University of Wisconsin Hospital and Clinics, phone number

AUTHORIZATION: I have read the information, have had a chance to ask questions, and have decided to give consent for my embryos to be cultured in the laboratory for the purpose of research, as described above. I have been told that I will receive a signed copy of this consent form.

Name of Woman : ______________ (please print)
(Oocyte Donor)

______________ 11/9/96
Signature of Woman (Oocyte Donor) (date)

Witnessed by: ______________ (please print)

______________ 11/9/96
Signature of Witness (date)

Name of Partner: ______________ (please print)
(Sperm Donor)

____
Signature of Partner (Sperm Donor) (date)

Witnessed by: ______________ (please print)

______________ 11/9/96
Signature of Witness (date)

Donor 95-G

11/97

INFORMED CONSENT: IN VITRO FERTILIZATION

I have been unable to become pregnant due to blocked or absent Fallopian tubes which cannot be corrected surgically or I have some other reason for infertility which is not treatable with other currently available methods. I wish to attempt to become pregnant using in vitro fertilization and embryo transfer (test tube baby), carried out by the physicians at the Endocrinology-Infertility Unit within the Department of Obstetrics and Gynecology and the University of Wisconsin Center for Health Sciences and any assistants selected by them.

I understand and have discussed with my physicians at the Endocrinology-Infertility Unit that, while other alternative procedures to attain fertility may exist, in vitro fertilization and ovum transfer offer the best opportunity to attain this goal in my particular case.

I understand that the following is an outline of the steps required in this procedure:

1) Determination by standard infertility tests that I am a suitable candidate for in vitro fertilization.
2) Use of "fertility drugs" (usually Lupron, menotropins [Pergonal, Fertinex or Humegon], and human chorionic gonadotropin) to produce ovulation at a predictable time.
3) Ultrasound examinations to assist in predicting the time of expected ovulation.
4) Undergoing either transvaginal or laparoscopic aspiration with insertion of a needle into my ovary to obtain one or more eggs.
5) Obtaining sperm from my husband by masturbation and treating it to prepare for fertilization in the laboratory.
6) Mixing the egg and the sperm together to allow fertilization to occur.
7) After fertilization, transferring the egg into a fluid for growth and after several cell divisions, if the embryo (conceptus) is developing normally, transferring the embryo into my uterus by means of a small plastic tube inserted through my cervix.
8) Obtaining blood samples four to six times during the two weeks after attempted fertilization to determine if my hormone levels are normal and if pregnancy has occurred.
9) I have discussed the various alternative technical forms of IVF with the Reproductive-Endocrine Staff and understand the reasons for their choice of procedure.

The process of transvaginal aspiration of follicles for ovum retrieval is performed in most IVF patients. The procedure involves placing a needle, guided by ultrasound, into the ovary. The needle goes through the vagina and is placed after intravenous sedation. The procedure eliminates the need for general anesthesia. I understand that I may be hospitalized for the above procedure to be accomplished. I also understand that, despite the physicians and hospital employees proceeding with due care, any of the following may occur which would prevent the establishment of a pregnancy:

INFORMED CONSENT: IVF (PAGE 2)

1) The time of ovulation may be misjudged, may be unable to be predicted, may occur prior to egg retrieval or ovulation may not occur in the monitored cycle, thus precluding any attempt at obtaining an egg.
2) Pelvic adhesions may prevent access to the ovary containing the ripe egg.
3) Ovulation may have occurred by the time transvaginal aspiration is performed.
4) Medical emergencies with other patients may make an operating room and anesthesia unavailable at the appropriate time for obtaining an egg.
5) The attempt at obtaining an egg may be unsuccessful.
6) The egg may not be normal.
7) My husband may be unable to obtain a semen specimen.
8) Fertilization may not occur.
9) Cleavage of cell division of the fertilized egg may not occur.
10) The embryo may not develop normally.
11) Implantation in the uterus may not occur.
12) A laboratory accident may result in loss or damage to the fertilized egg or embryo.
13) The fluids used to maintain gametes (sperm and egg) and embryos contain biological materials that could carry infectious agents. I know that these products are screened for major infectious diseases, but that an absolute guarantee of sterility cannot be made.

If pregnancy is established, miscarriage, ectopic pregnancy, stillbirth, or congenital abnormalities (birth defects) may occur.

I understand that insurance coverage for any or all of the above procedures may not be available and that I and my husband will be personally responsible for the expenses of this treatment and for all other expenses and costs to us or to our child(ren) for medical problems resulting from this procedure, such as miscarriage, ectopic pregnancy, stillbirth or birth defects. Expenses will consist of hospital, laboratory, and professional fees.

I understand that the success rate (chance of pregnancy) is about 25% in those institutions where in vitro facilities are currently in use.

I know that the procedures for <u>in vitro</u> fertilization and ovum transfer, as applied to humans, are relatively new. I understand that careful records of my case will be kept and that samples of my blood or fluids obtained during my therapy will be preserved for subsequent evaluation to monitor and possibly improve the methodologies used for in vitro fertilization. I know that up to five fertilized ova may be placed in my uterus and that twin or other multiple births may result from this procedure. I know that my in vitro fertilization attempt is being carried out as therapy and is not a research project. However, I understand that embryos and ova may be photographed and that the information from these records will be used in an effort to better understand the IVF process, thereby, advancing scientific knowledge. Furthermore, I am aware that those ova that either fail to fertilize or fertilize abnormally will be discarded. However, before being discarded, they will be cultured and examined in an effort to learn more about the general process of ovum/zygote development. I have been informed that when there is failure of fertilization during the first 24 hours following ovum retrieval, the eggs become non-viable. That is, further efforts to fertilize the eggs will not produce embryos that can go on to normal pregnancy and fetal development. When this happens, the failed to fertilize eggs must be destroyed. I understand that the options are to simply discard them or

INFORMED CONSENT: IVF (PAGE 3)

to allow them to be used for practice manipulation. I know that practice manipulation means maintaining them in culture while attempting to place a single sperm into them by micromanipulation. I agree to allow the eggs to be used for micromanipulation, and understand that they will be discarded within 7 days. I also understand that the micromanipulation that will be done is being done so that the embryology staff at the University of Wisconsin can maintain their micromanipulation skills at a high level of competence. I know that this type of evaluation is a form of quality assessment and research and that information obtained in this way will not necessarily benefit me directly.

I understand that while my privacy in these medical matters will be strictly observed, the technical results of my IVF cycle (number of oocytes obtained, number of oocytes fertilized, number and quality of embryos, number of embryos transferred or cryopreserved, frequency of embryo implantation, number of births, etc.) will be made available to credentialling organizations and to other workers in the field of assisted reproductive technology. I know that when such information is provided to organizations outside of the University Health Care system, my name or identifying numbers will not be included unless required by Federal law.

Theoretic concern has also been voiced about the possibility that multiple ovulation may increase the long range potential for an increased incidence of ovarian cancer (1% in the general population). This has led to the suggestion that hyperstimulation patients who have completed their efforts at infertility therapy take oral contraceptive to inhibit further ovulation.

On the day of ovum recovery, a semen sample will be required. Usually this is produced by the husband six hours after ovum recovery. If you are concerned about your ability to produce a semen specimen at the time required, you should talk to the IVF physician about this matter. As an option semen can be frozen in advance of the stimulation cycle to be used as a "back up". Use of frozen samples may be less effective than use of fresh semen in attaining fertilization. This would require advanced notice to our laboratories of at least four weeks and would cost $250 per sample.

If I have further questions concerning in vitro fertilization and its application in my case, I can contact or the other physicians in the Endocrine-Infertility Unit at the University of Wisconsin Center for Health Sciences.

Although the above statements refer mostly to my wife, I as the husband also understand all of the above, consent to the above and request the in vitro fertilization procedures.

We know that the practice of medicine and surgery is not an exact science and that therefore reputable practitioners cannot properly guarantee results. We acknowledge that no guarantee or assurance has been made by anyone regarding the in vitro fertilization procedures which we have herein requested and authorized.

INFORMED CONSENT: IVF (PAGE 4)

We have read the above and understand it. We have received a full explanation of the above procedures. We request that the physicians at the Endocrinology-Infertility Unit and assistants selected by them perform the above procedures. Our signatures also indicate that we have received a copy of this consent form.

Wife ________________ Date 1/7/98

Husband ________________ Date 1/7/98

Witness ________________ Date 1-7-98

University of Wisconsin Hospital and Clinics

NAME: [redacted]
NUMBER: [redacted] ( ) DOB: [redacted]

SURGERY DATE: 01/15/98

PREOPERATIVE DIAGNOSIS:
Infertility.

OPERATION:
Transvaginal ultrasound-directed egg retrieval.

POSTOPERATIVE DIAGNOSIS:
Infertility.

ANESTHESIA:
IV sedation.

COMPLICATIONS:
None.

PROCEDURE:
The patient was taken to the operating room. She was prepped and draped in the usual fashion and the bladder was drained. Transvaginal ultrasound with a 17 gauge needle guide attached to a rocket aspirator was inserted into the vagina. Follicles in each ovary were aspirated and irrigated with human tubal fluid. A total of 11 eggs were retrieved without any difficulty.

Following the procedure the vagina was inspected for hemostasis. There were no complications. The patient tolerated the procedure well.

OPERATIVE REPORT

AGREEMENT FOR CRYOPRESERVATION OF, AND REQUEST TO TRANSFER, EMBRYOS

We, ________________ and ________________, the undersigned Husband and Wife, are legally married and have already decided to participate in the In Vitro Fertilization and Embryo Placement clinical treatment program. We are requesting transfer of a number of embryos and granting permission for any remaining embryos to be cryopreserved (frozen) or to be left in culture. At this time, we acknowledge that granting this permission does not guarantee that there will be any embryos available for freezing.

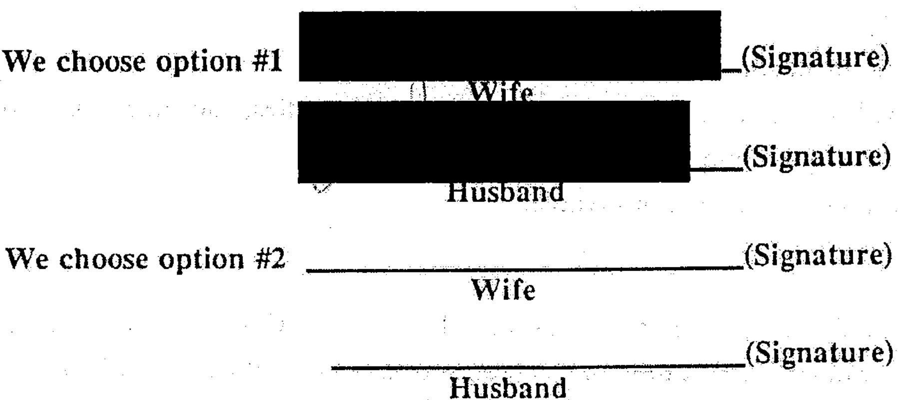
We have been informed that there is an increase in the pregnancy rate with increasing number of embryos available for uterine placement and that the success/pregnancy rate with embryo transfer increases with increasing numbers of embryos placed, up to 4-6 embryos. We have also been informed that the transfer of multiple embryos increases the risk of the establishment of a pregnancy of high plurality (triplets, quadruplets, etc.) and that these high plurality pregnancies have a great risk of terminating without the survival of the fetuses and may involve medical complications for both the newborn(s) and the mother. We have been informed that the risk of establishing a high plurality pregnancy can be reduced by placing a limited number of embryos in the uterus during the treatment cycle and freezing the rest of the embryos for uterine placement in a cycle subsequent to that treatment cycle.

We have been informed that there are two alternatives for excess embryos obtained through the In Vitro fertilization procedures:

1) The embryos may be cryopreserved (frozen) and stored for embryo transfer or use at a later date.
2) The embryos may continue their development in culture until their development ceases.

We have decided and instructed that the (1) remaining embryos of the 5 which are to be transferred during this cycle be cryopreserved (frozen) and stored for subsequent use or (2) the remaining embryos be left in culture until development ceases.

(If option (2) is chosen, the remaining paragraphs of this Agreement do not apply).



We have been informed that, although there is limited data at the present time, available human data as well as animal data does not suggest that the embryo freezing process increases the risks of congenital anomalies (birth defects) in the resultant offspring; however, the long-term outcome in terms of pregnancy rate and consequences of freezing embryos remains to be demonstrated.

We have been informed that not all embryos survive the freezing process. We agree that, even though some embryos will not survive the freezing process, the overall advantages of the freezing as mentioned above outweigh the loss of embryos.

We acknowledge that neither our physicians nor University of Wisconsin Hospital and Clinics (UWHC) have made any warranties with respect to: 1) the viability of our frozen embryos, (2) the successful establishment of pregnancy following uterine placement of our previously frozen embryos, (3) the lack of risk of a birth defect, miscarriage, or pregnancy complication after embryo placement in the uterus or, (4) the infallibility of the Hospital's liquid-nitrogen-cooled regrigerators or any other of the Hospital's equipment.

We acknowledge that the freezing of embryos requires the use of mechanical support systems (liquid-nitrogen refrigerators), the failure of which can affect the ability of the embryo to grow. We agree that if an embryo does not grow after thawing for whatever reason, then that embryo will not be placed in the uterus.

We are aware that, for a variety of reasons (for example, our choice; death of one or both of us; or achieving our desired family size), one or more of our embryos may remain frozen and will not be wanted or needed by us. In such a situation, we agree and authorize our physicians at University of Wisconsin-Madison to maintain such unused embryos in a frozen state until the earliest in time of the following four events or dates:

1) three years from the date of execution of this agreement;
2) the death of both of us (it is understood that if one of us dies, the other can make decisions on use of the embryos);
3) our legal separation, dissolution, or divorce;
4) whenever we both consent.

With respect to Item 4, above, we understand that our physicians at UWHC reserve the right to prescribe the use of forms or other identification procedures in connection with such consent.

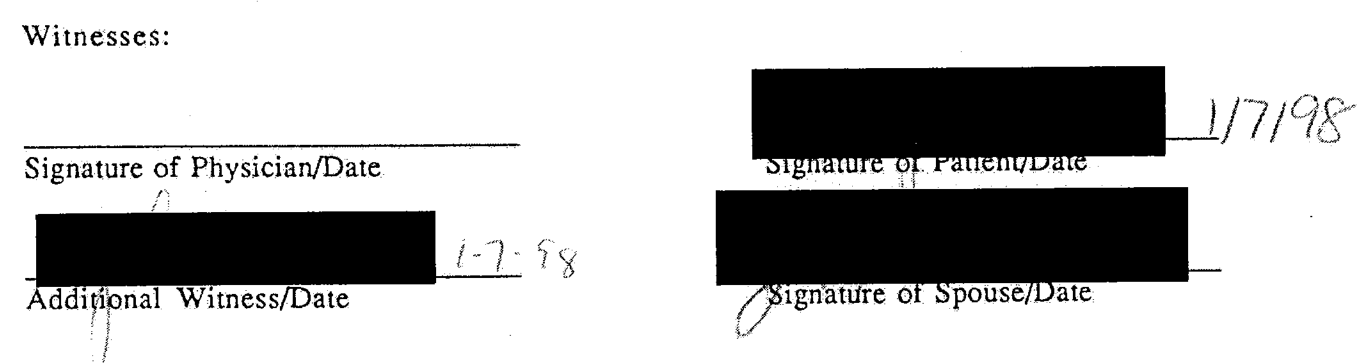
Prior to the end of three years from the date of execution of this Agreement, if in the medical judgement of our physicians, the frozen embryos can still be used, we shall be asked to reconsider the question of disposition of the remaining frozen embryos.

After the earliest of such events or dates as stated in Items 2, 3, and 4 above, or in the event we fail or decline to execute a new agreement regarding disposition of the remaining frozen embryos prior to the end of the three-year period, we authorize and request that any and all remaining frozen embryos be disposed of by our physicians at UWHC as follows:

> That one or more of our frozen embryos be made available for donation to a suitable anonymous recipient couple. The selection of such a couple shall be within the absolute discretion of our physicians at UWHC. In the event that no suitable recipient couple is available, decisions regarding the embryos will be made in accordance with professional ethical standards and any applicable legal requirements. We understand that, unless otherwise agreed to by us, destruction of the remaining embryos will occur by placing them in culture until they stop development and that during this incubation a photographic record will be made of their growth. This record will then be analyzed in an effort to learn more about and advance the science of embryo culture.

We have been informed that, other than the anticipated loss of some embryos during the freezing, preservation and thawing process, there are no known risks of discomfort. The development and application of human embryo freezing is anticipated to further increase the success rate, i.e. pregnancy establishment, following In Vitro Fertilization and Embryo Placement, while decreasing the risks and expenses associated with oocyte retrieval.

We agree that the full cost of the procedures, supplies and professional services involved in embryo freezing are our personal responsibility. Attached is a copy of the current fee schedule for embryo freezing and storage. In addition, we understand that there will be certain professional and laboratory costs involved in monitoring any subsequent cycle in which embryos are to be placed in the uterus as well as professional fees involved with the embryo placement procedure in that subsequent cycle.



ESTIMATED COSTS FOR CRYOPRESERVATION AND TRANSFER OF CRYOPRESERVED EMBRYOS:* at the UNIVERSITY OF WISCONSIN MEDICAL CENTER

Freezing and Thawing	$ 915.00
Medications	$ 600.00
Physician's Fee	$ 318.00
Transfer Charge	$ 305.00
OR Charges	$ 900.00
Pregnancy Test	$ 95.00

11/97

Principal Investigator: James Thomson
(608) 263-3585

Consent to Use Excess Pre-implantation Embryos for Cell Line Isolation

YOU ARE INVITED TO TAKE PART IN A RESEARCH STUDY OF HUMAN CELL DIFFERENTIATION.

Purpose

All human tissues and organs are composed of small building blocks called cells. By growing cells from embryos which have not yet implanted in the uterus (preimplantation embryos) we hope to better understand normal embryo development, and ultimately find important clues for the treatment of infertility, miscarriage, birth defects, and other conditions.

What Will Be Done With The Embryos?

Preimplantation embryos that are not chosen to be placed in the uterus will be cultured in various conditions in the laboratory for 7 days or less. This incubation time is not long enough for the development of any fetal structures, and the embryo will not yet reach the stage when implantation in the uterus occurs. During this incubation the embryos will be photographed. After this period of culture, the outer cells of the embryo will be separated from the inner cells of the embryo and discarded. The inner cells will then be cultured for an indefinite time ("cell lines") and studied. Note that the inner cells are not a complete embryo; as such they would not develop into a fetus if transferred to a uterus.

Why Are These Embryonic Cells Important?

Because these embryonic cells will be from the preimplantation embryo before the development of any specific tissue type they are called "undifferentiated". These cells can be cultured in this undifferentiated state in the laboratory, potentially indefinitely. However, by changing how they are cultured, they will sometimes randomly develop into "differentiated" cells (for example, cells that look and behave like the cells of placenta, bone, skin, or blood). By studying how these embryonic cells differentiate, in the future it may be possible to direct their differentiation to specific cell types in culture. Because many diseases (such as diabetes mellitus or Parkinson's disease) result from the death or dysfunction of specific cell types, it might one day be possible to treat many diseases by the transplantation of differentiated cells derived in tissue culture from embryonic cell lines. Although the potential for treating human diseases is great, significant biomedical advances will be needed before such treatments are possible.

Why Might These Embryonic Cell Lines Be Controversial?

These cell lines will be permanent, that is they will continue to divide in culture indefinitely. Although they are not whole embryos (i.e. they would not develop into a fetus if returned to the uterus) they will maintain many embryonic properties. Because of these embryonic properties, certain experiments which would be controversial for whole human embryos would be controversial for these cell lines. In particular, two experiments that will not be performed with embryonic cell lines derived from this study are: (i) Intermixing of human embryonic cells with an intact embryo, either human or nonhuman, and (ii) Attempting to make genetically identical whole embryos by any method.

Is There Any Medical Benefit For Participation?

You will not directly benefit from participation, but future patients may benefit from the study. The embryonic cells will provide information about normal human development that could lead to improved therapies for infertility or other conditions. Nothing discovered about the donors from this research will be conveyed to the donor's physicians in a way that could be personally identified.

Is There Any Financial Incentive For Participation?

You will not be financially compensated for participation. If embryonic cell lines are successfully isolated, the cell lines would become the property of the University of Wisconsin or the Wisconsin Alumni Research Foundation (WARF). Because of the possibility that differentiated cells derived from embryonic cell lines might one day be used to treat human diseases, embryonic cell lines might have significant commercial value, and WARF may apply for patent protection for the isolation technique of the cell lines and on the properties of the cell lines. If a patent is granted, WARF would own the patent. WARF is a Wisconsin corporation, organized in 1925 by University of Wisconsin-Madison alumni as an entity separate from the University. WARF is dedicated to supporting research at the UW-Madison. As part of its mission, WARF patents and licenses University inventions. Earnings are combined with WARF investment income to fund WARF's annual gift back to the University. The University then is responsible for deciding how the money will be applied. Inventors receive a share of earnings from their inventions, and by University policy, a part of what the University receives from WARF goes to a department or departments associated with the invention.

Confidentiality of Participation Neither the men nor women who provide the gametes for the embryos used in this study will be identified in any communication or publication that may result from this study.

If You Change Your Mind You are free at any time to refuse permission for the use of any of your excess embryos, up until the moment when the inner cells of the embryos are isolated for culture. Once these cells are isolated for culture, they become the property of the University of Wisconsin.

YOU MAY TAKE AS MUCH TIME AS YOU WISH TO THINK THIS OVER. IF YOU CHOOSE NOT TO TAKE PART IN THIS STUDY, YOUR MEDICAL CARE WILL NOT BE AFFECTED IN ANY WAY. BEFORE YOU SIGN THIS FORM, PLEASE ASK ANY QUESTIONS ON THE ASPECTS OF THIS STUDY WHICH ARE NOT CLEAR TO YOU. WE WILL ATTEMPT TO FULLY ANSWER ANY QUESTIONS YOU MAY HAVE PRIOR TO, DURING OR FOLLOWING THIS STUDY. If you have questions, they should be directed to:
Department of Obstetrics and Gynecology, IVF Laboratories of the University of Wisconsin Hospital and Clinics.

AUTHORIZATION: I have read the information, have had a chance to ask questions, and have decided to give consent for my embryos to be cultured in the laboratory for the purpose of research, as described above. I have been told that I will receive a signed copy of this consent form.

Name of Woman : ______ (please print) ______ 1/22/98
(Oocyte Donor) Signature of Woman (Oocyte Donor) (date)

Witnessed by: ______ (please print) ______ 1/22/98
Signature of Witness (date)

Name of Partner: ______ (please print) ______ 1/22/98
(Sperm Donor) Signature of Partner (Sperm Donor) (date)

Witnessed by: ______ (please print) ______ 1/22/98
Signature of Witness (date)