Materials Submitted to NIH
From Reproductive Genetics Institute (RGI)
Submissions #2009-ACD-006 & 2009-ACD-007

(Both submissions have the same supporting documentation)

I.	Submission Cover Page (2009-ACD-006)	p.l
II.	Submission Cover Page (2009-ACD-007)	p. 11
III.	Letter from RGI President	p. 19
IV.	Description of Supporting Information	p. 20
V.	Investigational Review Board Approval	p. 25
VI.	Research Consent	p. 26
VII.	Assurance Documents	p. 31
VIII.	Publications	p. 38
IX.	IIB Assurance	p. 56
X.	Additional Information:	

- Other Details via Email Correspondence p. 58
- IVF Clinical Consent (Couple A) p. 60
- Cryopreservation Consent (Couple A) p. 64
- Research Consent with Dates for Couple A; p. 69
  (Document text is identical to Item VI, p. 26)
- IVF Clinical Consent (Couple B) p. 74
- Research Consent with Dates for Couple B; p. 78
  (Document text is identical to Item VI, p. 26)
- Other Details via Email Correspondence p. 83
- Other Details via Email Correspondence p. 86

NOTE: Duplicative information in the submission is not included. Table of Clinical IVF and Research Consent Dates not included to protect patient privacy.

hESC Registry Application Database
Detailed Listing for Request #: 2009-ACD-006
April 21, 2010

hESC Registry Application Search Results

Request #: 2009-ACD-006 **Status: Pending** **Review: ACD** **Assurance:** Yes (Section **Certification:** Yes **Authority:** **Cell Lines:** 24 **Available:** 24 **Previous** #: 2009-DRAFT-022 Email	**Organization: Reproductive Genetics Institute** **Org Address:** 2825 North Halsted St., Chicago, IL 60657 USA **DUNS:** 790373369 **Grant Number(s):** n/a **Signing Official (SO):** Verlinsky Oleg / 773-472-4900 / verlin@rcn.com
Edit Delete	**Submitter of Request:** Strelchenko Nick / 773-472-4900 / nstrelch@wisc.edu:mail@reproductivegenetics.com
Switch to ADM	**Submitter Comments: (None)**
	Line #1: RG-148; DYSTROPHIA MYOTONICA 1 (DM1), affected, 46,XY **NIH Approval #:** **Available:** Yes **Embryo from U.S.:** Yes **Embryo Donated in Year(s):** 2003 **Provider Name:** Reproductive Genetics Institute **Provider Phone:** 773-472-4900 **Provider Email:** nstrelch@wisc.edu
	Provider URL: http://www.reproductivegenetics.com/
	Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production **NIH Restrictions:** **Additional Information:**
	Line #2: RG-153; DYSTROPHIA MYOTONICA 1 (DM1), affected, 46,XX **NIH Approval #:** **Available:** Yes **Embryo from U.S.:** Yes **Embryo Donated in Year(s):** 2003 **Provider Name:** Reproductive Genetics Institute **Provider Phone:** 773-472-4900 **Provider. Email:** nstrelch@wisc.edu
	Provider URL: http://www.reproductivegenetics.com/
	Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production **NIH Restrictions:** **Additional Information:**
i	**Line #3: RG-170; MUSCULAR DYSTROPHY, BECKER TYPE (BMD), affected, 46,XY** **NIH Approval #:** **Available: Yes** **Embryo from U.S.:** Yes **Embryo Donated in Year(s):** 2004

Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer

and chimera production **NIH**

Restrictions: Additional

Information:

Line #4: RG-186; HUNTINGTON DISEASE (HD), affected, 46,XX
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2004
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #5: RG-194; HUNTINGTON DISEASE (HD), affected, 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2004
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #6: RG-233; HEMOGLOBIN BETA LOCUS (HBB), affected (HbS/HbS - sickle cell anemia), 46,XX
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2005
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158

Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #7: RG-245; EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), carrier, 47,XXY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2005
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider-Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #8: RG-246; EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected, 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2005
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #9: RG-271; TORSION DYSTONIA 1 (DYT1), AUTOSOMAL DOMINANT, affected (N/GAG del), 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158

Provider Email: nstrelch@wisc.edu
Provider URL: http://www:reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer-Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #10: RG-283; MUSCULAR DYSTROPHY, DUCHENNE TYPE (DMD); affected, 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line#11: RG-288; CYSTIC FIBROSIS (CF), affected (deltaF508/deltaF508), 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #12: RG-289; CYSTIC FIBROSIS (CF), affected (deltaF508/de!taF508), 46,XX
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158

Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #13: RG-301; MUSCULAR DYSTROPHY, DUCHENNE TYPE (DMD) affected, 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #14: RG-302; MUSCULAR DYSTROPHY, DUCHENNE TYPE (DMD), carrier, 46,XX
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #15: RG-315; NEUROFIBROMATOSIS, TYPE I (NF1), affected (R19 47X/N), 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158

Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #16:RG-316; TUBEROUS SCLEROSIS, TYPE 1(TSC1), affected (N/IVS7+1 G-A)
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-.4900ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #17: RG-320; TUBEROUS SCLEROSIS, TYPE 1(TSC1), affected (N/IVS7+1 G-A)
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #18: RG-326; POPLITEAL PTERYGIUM SYNDROME (PPS),affected (R84H/N), 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158

7

Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #19: RG-328; FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHD), affected, 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #20: RG-330; FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHD), affected, 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #21: RG-333; FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHD), affected, 46,XX
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158

Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #22: RG-356; HEMOGLOBIN ALPHA LOCUS (HBA), affected (-alpha /--), 46,XX
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2007
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #23: RG-357; EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected, 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2007
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #24: RG-358; EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected, 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2007
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158

Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer

and chimera production

NIH Restrictions:

Additional Information:

Supporting Documents:

Document 1:	(PDF-10/07/2009) :	Cover Letter
Document 2:	(PDF-10/14/2009)	Summary of Supporting Information
Document 3	(PDF-10/14/2009) :	IRB Approval
Document 4	(PDF-10/14/2009)	Consent Form Blank
Document 5	(PDF-10/09/2009)	AssuranceLetter
Document 6	(PDF-10/14/2009)	Verlinsky RBM online2005
Document 7	(PDF-10/14/2009)	Verlinsky RBM online2006
Document 8	(PDF-10/14/2009)	Use of hESC lines RBM 2008
Document 9	(PDF-10/14/2009)	Certification and Assurance Letter

Administrative Comments: NIH staff analysis uploaded October 27, 2009 by E. Gadbois, minor revision October 30, 2009

Letter to RIG requesting addiitonal information from Working Group uploaded Nov 12,2009 by E. Gadbois

Certifications from SO reflected per updated by E. Gadbois November 12, 2009

Additional documentation requested from SCWG uploaded Nov 23, 2009 by B. Dean

Response from RGI uploaded Dec 17, 2009 by E. Gadbois

Response from RGI uploaded Jan 12, 2010 by E. Gadbois

Response from RGI uploaded Jan 26 2010 by E. Gadbois

Administrative Attachments:
Document 1: (DOC-10/30/2009) NIH staff analysis Document 2: (PDF -11/12/2009) request from WG for information Document 3: (PDF -11/23/2009) Additional Requested Documents Document 4: (PDF -12/17/2009) Dec 15 response from RGI Document 5: (PDF - 01/12/2010) Jan 11, 2010 response from RGI Document 6: (DOC - 01/26/2010) Jan 25 2010 response from RGI (table)

Status History
Draft: 10/07/2009
Pending: 10/14/2009

\

	Emails Sent: 10/14/2009-New_Applicaton_Email
	Added By: Commons\RGICELL1 On: 10/07/2009 | **Last Updated By:** NIH\gadboisel On: 01/26/2010 | **Record ID: 22**

Total Record Count = 1

Logout NIH Form *2890* Admin Site

\\

hESC Registry Application Database

Detailed Listing for Request #: 2009-ACD-007
April 21, 2010

hESC Registry Application Search Results

Request #: 2009-ACD-007 **Status: Pending** **Review: ACD** **1** **Assurance:** Yes (Section II(B)) **Certification:** Yes **Authority:** **Cell Lines: 23** **Available: 23** **Previous** #: 2009-DRAFT-023 Email	**Organization: Reproductive Genetics Institute** **Org Address:** 2825 North Halsted St., Chicago, IL 60077 **DUNS:** 790373369 **Grant Number(s): n/a** **Signing Official (SO):** Verlinskv Oleg / 773-472-4900 / verlin@rcn.com
	Submitter of Request: Strelchenko Nick / 773-472-4900 / nstrelch@wisc.edu;mail@reproductivegenetics.com
	Submitter Comments: (None)
Edit delete	**Line#1: RG-401; FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHD), affected, 46,XX** **NIH Approval #:** **Available:** Yes **Embryo from U.S.:** Yes **Embryo Donated in Year(s): 2007** **Provider Name:** Reproductive Genetics Institute **Provider Phone:** 773-472-4900 ext.2158 **Provider Email:** nstrelch@wisc.edu **Provider URL:** http://www.reproductivegenetics.com/
Switch to ADM	**Provider Restrictions:** cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production **NIH Restrictions:** **Additional Information:**
	Line #2: RG-402; FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHD), affected, 46,XX **NIH Approval #:** **Available:** Yes **Embryo from U.S.:** Yes **Embryo Donated in Year(s): 2007 .** **Provider Name:** Reproductive Genetics Institute **Provider Phone:** **Provider Email:** **Provider URL:** **Provider Restrictions:** cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production **NIH Restrictions:** **Additional Information:**
	Line #3: RG-403; FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHD), affected **NIH Approval #:** **Available:** Yes

Embryo from U.S.: Yes
Embryo Donated in Year(s): 2007
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL:
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer

and chimera production

NIH Restrictions:

Additional Information:

Line #4: RG-404; SPINAL MUSCULAR ATROPHY, TYPE I (SMA1), affected, 46,XY
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2007
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL:
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #5: RG-406; TORSION DYSTONIA 1, AUTOSOMAL DOMINANT (DYT1), affected (N/GAG del)
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2007
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL:
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer

and chimera production

NIH Restrictions:

Additional Information:

Line #6: RG-413; BREAST CANCER, FAMILIAL (BRCA2),affected (N/IVS7 GT del) & MULTIPLE ENDOCRINE NEOPLASIA, TYPE I (MEN1), affected (N/3036 4bp del) NIH Approval #:

Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2008
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL:
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #7: RG-414; MULTIPLE ENDOCRINE NEOPLASIA, TYPE I (MEN1), affected (N/3036 4bp del)
NIH Approval #:
Available: Yes
Embryo, from U.S.: Yes
Embryo Donated in Year(s): 2008
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL:
Provider Restrictions: ceil line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #8: RG-415; HUNTINGTON DISEASE (HD), affected
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2008
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL:
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #9: RG-416; CYSTIC FIBROSIS (CF), affected (deltaF508/1717-1 G-A)
NIH Approval #:
Available: Yes

Embryo from U.S.: Yes
Embryo Donated in Year(s): 2008
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer

and chimera production

NIH Restrictions:

Additional Information:

Line #10: RG-417; CYSTIC FIBROSIS (CF), affected (deltaF508/1717-1 G-A)
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2008
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #11: RG-418; HEMOGLOBIN BETA LOCUS (HBB), affected (cd8+G /619del)
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2008
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material TransferAgreement and cannot be used for experiment nuclear transfer

and chimera production

NIH Restrictions:

Additional Information:

Line #12: RG-420; HEMOGLOBIN BETA LOCUS (HBB), affected (cd8+G/619del) NIH Approval #: Available: Yes

15Embryo from U.S.: Yes
Embryo Donated in Year(s): 2008
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.21**58**
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #13: RG-422; CYSTIC FIBROSIS (CF), affected (N1303K/deltaF508)
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2008
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #14: RG-423; CYSTIC FIBROSIS (CF), carrier (N/deltaF508)
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2008
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email: nstrelch@.wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #15: RG-424; MULTIPLE ENDOCRINE NEOPLASIA, TYPE 2 (MEN2B), affected (M918T/N) NIH Approval #: Available: Yes
Embryo from U.S.: Yes

16

Embryo Donated in Year(s): 2008 Provider
Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL: http://www.reproductivegenetics.com/ **Provider Restrictions:** cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #16: RG-426; PELIZAEUS-MERZBACHER DISEASE (PMLD), affected
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2009
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used if or experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #17: RG-428; TUBEROUS SCLEROSIS, TYPE 1 (TSC1), affected (N/IVS7+1 G-A)
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2009
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #18:iRG-222
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2005

Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email:
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line#19: RG-230
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2005
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #20: RG-249
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2005
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email: nstrelch@wisc.edu
Provider URL: http://www.reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line#21:RG-308
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2006
Provider Name: Reproductive Genetics Institute
Provider Phone:
Provider Email: nstrelch@wisc.edu

Provider URL: http://www.reproductivegenetics.com/ **Provider Restrictions:** cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #22: RG-313 NIH Approval #: Available: Yes **Embryo from U.S.:** Yes **Embryo Donated in Year(s):** 2006 **Provider Name:** Reproductive Genetics Institute **Provider Phone: Provider Email:** ristrelch@wisc.edu **Provider URL:** http://www.reproductivegenetics.com/ **Provider Restrictions:** cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Line #23: RG-399; FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHD), affected, 46,XX
NIH Approval #:
Available: Yes
Embryo from U.S.: Yes
Embryo Donated in Year(s): 2007
Provider Name: Reproductive Genetics Institute
Provider Phone: 773-472-4900 ext.2158
Provider Email: hstreich@wisc.edu
Provider URL: http://www;reproductivegenetics.com/
Provider Restrictions: cell line limited for research purposes by Material Transfer Agreement and cannot be used for experiment nuclear transfer and chimera production

NIH Restrictions:

Additional Information:

Supporting Documents:

Document 1 :	(PDF-10/08/2009)	Cover Letter
Document 2	(PDF -10/14/2009)	Summary of Supporting Information
Document 3	(PDF-10/14/2009)	IRB Approval Notice
Document 4	(PDF-10/14/2009)	Consent Form Blank
Document 5	(PDF -10/14/2009)	AssuranceLetter
Document 6;	(PDF -10/14/2009)	RBMonline2005
Document?:	(PDF -10/14/2009)	RBM online2006
Document 8	(PDF-10/14/2009)	Use of hESC lines RBM 2008
Document 9:	(PDF-10/14/2009)	Certification and Assurance Letter

www.reproductivegenetics.com
E-mail: rgiworld@gmail.com
FAX: 773-871-5221

Reproductive Genetics institute

Director: *Yury Verlinsky, Ph.D.* *2825 North Halsted St., Chicago, IL 60657* ***Tel.: 773-472-4900***

October 7, 2009

Raynard S. Kington, M.D., Ph.D
Acting Director of NIH National
Institute of Health Building l,
Room 144 9000 Rockville Pike
Bethesda, MD 20892

Dear Dr. Kington,

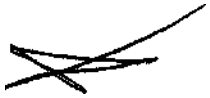
Please find attached the list of hESC lines derived by Reproductive Genetics Institute before July 7, 2009, for your consideration to include them in a new Registry listing hESCs eligible for use in NIH funded research.

On behalf of Reproductive Genetics Institute, I certify that informed consent was obtained for donation of the embryos, which is attached for your information. This consent form was approved by IRB on June 28 2002, and is in compliance with NIH HESC Guidelines:

(1) HESC lines were created from human embryos using IVF for reproductive purposes and were no longer needed for this purpose; (2) embryos were donated by individuals who sought reproductive treatment, and who gave voluntary consent for the human embryos to be used for research purposes; (3) all option available were explained to donors; (4) no payment was offered for the donated embryos; (5) policy and procedure were in place ensuring that neither consenting or refusing to donate embryos would affect the quality of care; (6) there was no influence on decision to donate embryos; consent for donation for research purposes was given at the time of donation; (7) donors informed that they retained the right to withdraw consent for the donation until the embryo was actually used to derive hESCs; (8) donors informed what would happen to the embryos in the derivation of hESC for research; (9) that HESC might be kept for many years; (10) that the donors will not receive direct medical, financial or any other benefit.

I also assure that Reproductive Genetics Institute has on file documents that support the above and agree that NIH has the right to review this documentation as needed. I'm aware that any false, fictitious, or fraudulent statement or claims may subject Reproductive Genetics Institute to criminal, civil or administrative penalties.

Oleg Verlinsky
President
Reproductive Genetics Institute



Brief Description of Supporting Information for RGI hESC lines.

Document 1: Cover Letter

Document 2: Summary Brief Description of Supporting Information for RGI hESC lines.
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 is provided, and address the materials that the Working Group will consider during its review of the use of the RGI hESC lines with NIH funding.

Document 3: Consent form IRB Approval Notice Establishing the RGI hESC lines was conducted with the approval of the RGI Institutional Review Board (IRB). This research protocol was originally approved on June 28, 2002 and reapproved on March 24, 2004. Therefore the ACD should consider this document as demonstration that the derivation of the RGI hESC lines 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 4: Consent Form for Establishing human Embryonic Stem Cell Lines
Reproductive Genetics Institute Institutional Review Board reviewed and approved the consent form on June 28, 2002 and revised on June 20, 2003. A blank copy of this consent form is enclosed, which was signed 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 exactly expressed all of the components listed in Section IIA of the July 7, 2009 NIH Guidelines on Human Stem Cell Research, it does contain the following components:

Component 1.. Bl All options pertaining to use of embryos no longer needed for reproductive purposes were explained to the potential donor(s).

Page 3, paragraph 1 - ALTERNATIVES

Component 2.. B2 No inducements were offered for the donation.

Page 2, paragraph 4 and 5 - RISKS AND BENEFITS

Component 3.. B3 A policy was 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).

Page 3, paragraph 4 and 5 - VOLUNTARY PARTICIPATION

Component 4. B4 There was a clear separation between the prospective donor(s)'s decision to create human embryos for reproductive purposes and the prospective donor(s)'s decision to donate human embryos for research purposes.

Page 2 - METHODOLOGY, paragraph 1, and RISKS AND BENEFITS, paragraph 1.

Component 5. B5 At the time of donation, consent for that donation was obtained from the individual(s) who had sought reproductive services. 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 should have been given at the time of the donation. Donor(s) were informed that they retained the right to withdraw consent until the embryos were actually used for research.

Page 3, paragraph 4 and 5- VOLUNTARY PARTICIPATION

Component 6. . B6 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 in research. Whenever it was practicable, the attending physician responsible for reproductive clinical care and the researcher deriving and/or proposing to utilize human embryonic stem cells should not have been the same person.

Page 3, paragraph 4 and 5 - VOLUNTARY PARTICIPATION

Component 7. B7 Written informed consent was obtained from individual(s) who sought reproductive services and who elected to donate human embryos for research purposes. The following

information, which is pertinent to making the decision of whether or not to donate human embryos for research purposes, was in the written consent form for donation and discussed with potential donor(s) in the informed consent process

Consent form at RGI includes next statements required by NIH Guidelines:

A.A statement that donation of the embryos for research: was voluntary:

Page 3, paragraph 4 and 5 - VOLUNTARY PARTICIPATION

B.A statement that donor(s) understood alternative options pertaining to use of the embryos:

Page 3, paragraph 1 -ALTERNATIVES

C.A statement that the embryos would be used to derive human embryonic stem cells for research:

Stated in Headline of the Consent form.

D. Information about what would happen to the embryos in the derivation of human embryonic stem cells for research:

Page 2 - METHODOLOGY

E. A statement that human embryonic stem cells derived from the embryos might be maintained for many years:

Page 2 - METHODOLOGY, last paragraph

F. A statement that the donation was made without any restriction or direction regarding the individual(s) who may receive medical benefit from the use of the stem cells:

Page 4 - DISCOVERY AND PATENTS

G. A statement that the research was not intended to provide direct medical benefit to *the* donor(s):

Page 4 -DISCOVERY AND PATENTS

H. A statement as to whether or not information that could identify the donor(s) would be retained prior to the derivation or the use of the human embryonic stem cells:

Page 3 - CONFIDENTIALLY

I. A statement that the results of research using the human embryonic stem cells may have commercial potential, and a statement that the donor(s) would not receive financial or any other benefits from any such commercial development:

Page 4 - DISCOVERY AND PATENTS

Document 5; Letter of Assurance— October 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 listed hESC lines.

In addition to other information, this document provides supporting information demonstrating that the

hESC lines were 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) 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 hESC lines was conducted in accordance with all of the eligibility requirements specified in Section 118 of the July 7, 2009 NIH Guidelines on Human Stem Cell Research.

Document 6: RBM online - 2005

Establishment of hESC lines, from PGD cases was published in 2005:
Verlinsky Y, Strelchenko N, Kukharenko V, Rechitsky S, Verlinsky O, Galat V, Kuliev A. Human embryonic stem cell lines with genetic disorders. Reprod Biomed Online. 2005 Jan;10(1):105-10.

Document 7: RBM online - 2006

Further expanding repository of hESC lines, was published in 2006:
Verlinsky Y, Strelchenko N, Kukharenko V, Shkumatov A, Rechitsky S, Verlinsky O, Kuliev A. Repository of human embryonic stem cell lines and development of individual specific lines using stembrid technology. Reprod Biomed Online. 2006 Oct;13(4):547-50. Review.

Document 8: Use of hESC Lines — RBM online 2008

Application for research using Reproductive Genetics Institute hESC lines has been published in 2008:
Niclis JC, Trounson AO, Dottori M, Ellisdon AM, Bottomley SP, Verlinsky Y, Cram DS. Human embryonic stem cell models of Huntington disease. Reprod Biomed Online. 2009 Jul;19(1):106-13.
Therefore the ACD should consider this information as demonstration that the hESC lines have been and will likely remain an important cell lines for hESC research.

Document 9: Certification and Assurance Letter

Letter signed by the SO that provides the required certifications.

Reproductive Genetics Institute
Investigational Review Board

June 14,2008

Yury Verlinsky, Ph.D
Reproductive Genetics Institute
2825 N Halsted Chicago, IL
60657

Dear Dr. Verlinsky,

At your request, please be advised the "Consent to Donate Human Embryos for a Research Study to Establish Human Embryonic Stem Cell Lines" was approved by the IRB actions on May 21,2003 and March 24, 2004. (Both Minutes Attached)

Sincerely,

Harvey Morowitz
IRB, Secretary

Cc Edwin Feldman
Chairman, IRB

-FINAL-6/28/02
Revised 6/20/03

CONSENT TO DONATE HUMAN EMBRYOS FOR RESEARCH STUDY TO ESTABLISH HUMAN EMBRYONIC STEM CELL LINES

We, the undersigned husband and wife, understand that we are being invited to participate in a medical research study to establish human embryonic stem cell lines. This study is being conducted by the Reproductive Genetics Institute (RGI) and the study protocol has been approved by RGI's Institutional Review Board (IRB).

PURPOSE

The purpose of this study is to create a supply of human embryonic stem cells for use in research related to human developmental biology, including research of the mechanisms of genetic expression at the cellular level. It is hoped that this research will provide information that may ultimately lead to new treatments for disease. The establishment of human embryonic stem cell lines is an experimental procedure.

BACKGROUND

Human embryonic stem cells are derived from the inner cells of preimplantation embryos. The embryo at this point in its development is microscopic in size and is made up of less than 300 cells. The scientific value of these cells is that they are undifferentiated; i.e., they have not yet developed into a cell that has a specific biological purpose. Other stem cell lines have been created by other researchers using animal embryos and human embryos. Many scientists are researching possible ways to direct the differentiation of stem cells to function as specific human body cells, such as heart tissue, brain tissue, and pancreatic cells etc. If the differentiation of stem cells can be directed, it may be possible to treat diseases using these new cells to replace defective cells in the human body. This treatment concept is known as cell transplantation therapy.

Human embryos are not the only potential source of stem cell lines. Other scientists, in studies unrelated to this research study, have explored, and continue to explore, establishment of stem cell lines using cells taken from umbilical cord samples and from adult marrow cells.

METHODOLOGY Embryos donated by couples undergoing in vitro fertilization (IVF) will be utilized in this research study to create lines of human embryonic stem cells for further research. Any nontransferred embryos will be used for this study.

Embryos donated to this study will be allowed to develop to the blastocyst stage, which is microscopic in size and is made up of less than 300 cells. Blastocysts are incapable of further development outside of the uterus. The stem cell colonies, once created from the blastocysts will be both frozen and stored for use in research at a later time, or will be directly utilized immediately.

It is anticipated that this study will involve approximately 200 donor couples as study subjects. The duration of the study is indefinite as its goal is to establish stem cell lines for current and future use in research. This study is expected to continue for a minimum of one year and may continue well beyond the initial one-year period. Our participation as a study subject is limited to donation of embryos from this current IVF cycle for which we have no desire for embryo transfer. Our participation in this study will conclude at the time of embryo donation.

RISKS AMD BENEFITS

We understand that our participation in this study is limited to donation of any untransferred embryos from this current IVF cycle. We also understand that mere are no medical risks to us as participants in this study, and acknowledge that risks and benefits related to the IVF process itself have been disclosed to us. Further, we have been informed and understand that embryos donated for this research will not be available to us for IVF transfer. We have also been informed and understand that embryos donated, as such, for this research will be used exclusively by RGI and will not be transferred to or sold to anyone else.

We also understand that we will receive no direct benefit of any kind as a result of our participation in this study. Potential benefits from this study are limited to benefits which may result from advances in science, including the possibility that research on human embryonic stem cells will lead to cell transplantation therapies or other, new ways to treat disease.

ALTERNATIVES

We have been informed and understand that the following alternatives are available to us for our nontransferred embryos,

1. We can instruct RGI to release our nontransferred embryos to us.
2. We can instruct RGI to allow our nontransferred embryos to degenerate in the laboratory.
3. We can donate our nontransfered embryos to this research study.
4. We can cryopreserve our nontransferred embryos for future transfer.

CONFIDENTIALITY

We understand that the information obtained about us during this study will be treated as confidential and that our identities will not intentionally be revealed without our prior written consent, except as otherwise required by state or federal law or other governmental regulatory authority, such as the FDA. In addition, we understand that any medical records related to this study may be inspected by RGI's Institutional Review Board.

CONTACT PERSONS

If we have any questions about this study, we may contact Dr. Yury Verlinsky at 773-472-4900. If we have any questions about our rights as participants in this research protocol, we may contact Dr. Ed Feldman, Chairperson of the Institutional Review Board at 773-472-4900.

VOLUNTARY PARTICIPATION

Our participation in this study is purely voluntary. We have received no direct or indirect payment or compensation as an incentive to participate, and we understand that we nave no right to payment or compensation for our participation in this study. We also understand that there are no costs to us for our participation in this study.

We understand that we may withdraw from this study by submitting a written request to withdraw to Dr. Yury Verlinsky, Reproductive Genetics Institute, 2825 North Halsted Street, Chicago, Illinois 60657. If at the time our written request to withdraw is received, our donated embryos can be identified and have not already

been cultured for stem cell production, our donated embryos will be withdrawn from this study in accordance with our written direction. We further understand, that as of the date of this consent, our embryos that were donated to this research study from this IVF cycle will no longer be suitable for transfer.

We have been informed and understand that we may discontinue participation in this study without penalty or loss of benefits to which we are otherwise entitled. We have also been informed and understand that discontinuing our participation in this study will not affect ongoing treatment, access to services offered by RGI, or our relationship with RGI.

DISCOVERIES AND PATENTS

By choosing to enroll in this research study, we acknowledge and agree that we will receive no payment of any kind for donation of our embryos and/or for participation in this study. We further acknowledge that RGI may realize monetary or other benefits and awards from its research utilizing our donated embryos and that RGI will be the sole and exclusive owner of any such monetary or other benefits and awards. We further agree that we, our heirs, successors, relatives, representatives and/or agents have no interest in, and will make no claim to, any monetary or other benefits and awards which RGI may derive, in whole or in part, from use of our donated embryos. We further agree that we, our heirs, successors, relatives, representatives and/or agents will not bring any action in law or in equity, or in any administrative setting, related to our participation in this study.

AGREEMENT TO PARTICIPATE

We have received a copy of this consent form and have carefully read and considered its contents. We have had an opportunity to ask questions about donation of our embryos and about the procedures, risks, potential outcomes and any additional considerations associated with this research that all of our questions have been answered to our satisfaction. We further acknowledge that we have had the opportunity to discuss any ethical concerns related to this study and that any ethical concerns have been answered to our satisfaction.

We have been informed and understand that our refusal to participate in this study will involve no penalty or loss of benefits to which we are otherwise entitled.

We have also been informed and understand that our refusal to participate in this study will not affect any ongoing treatment, access to services offered by RGI, or: our relationship with RGI.

By affixing our signatures, we acknowledge that we have had adequate time to reach our decision; that we voluntarily consent to donate all of our nontransferred embryos, from this current IVF cycle, Month Day Year, and we agree to participate in the above-described research study. We acknowledge that the procedures in this study are experimental and that there is no certainty that the results of this research will lead to medical benefits for ourselves or others.

Female Partner Date

Male Partner Date

Printed Names

Identification Confirmed by : _____(or signatures notarized)

WITNESS:

Date:

CERTIFICATION I certify that I have consulted with the above-named male and female partner and, to the best of my knowledge, have answered their questions and explained the procedures, benefits, risks, alternatives and costs, if any, involved in this study to their satisfaction.

Signature _____

Title _____

Date

Assurance of Conditions of Consent
For human Embryonic Stem Cell lines

As the attending physician of the patients that underwent IVF and PGD with the Reproductive Genetics Institute (RGI), who consented to embryo donation and whose embryos resulted in the derivation of the human Embryonic Stem Cell (hESC) lines, I, Ilan Tur-Kaspa, M. D., hereby provide the following written assurances that the embryos used for the derivation of the cell lines listed below in table:

ID	Name of Disorder	Karyotype
RG-148	DYSTROPHIA MYOTONICA 1 (DM1), affected	46,XY
RG-153	DYSTROPHIA MYOTONICA 1 (DM1), affected	46,XX
RG-170	MUSCULAR DYSTROPHY, BECKER TYPE (BMD), affected male	46,XY
RG-186	HUNTINGTON DISEASE (HD), affected	46,XX
RG-194	HUNTINGTON DISEASE (HD), affected	46,XY
RG-233	HEMOGLOBIN-BETA LOCUS (HBB), affected (HbS/HbS - sickle cell anemia)	46,XX
RG-245	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), carrier (XXY)	47,XXY
RG-246	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected (XY)	46,XY
RG-271	TORSION DYSTONIA 1, AUTOSOMAL DOMINANT(DYT1), affected (N/GAGdel)	46,XY
RG-283	MUSCULAR DYSTROPHY, DUCHENNE TYPE (DMD), affected male	46,XY
RG-288	CYSTIC FIBROSIS(CF) ,affected (AF508/AF508)	46,XY
RG-289	CYSTIC FIBROSIS(CF), affected (AF508/AF508)	46,XX
RG-301	MUSCULAR DYSTROPHY, DUCHENNE TYPE(DMD) affected male	46,XY
RG-302	MUSCULAR DYSTROPHY, DUCHENNE TYPE(DMD), carrier	46,XX
RG-315	NEUROFIBROMATOSIS, TYPE 1 (NFl),affected (R19 47X/ N)	46,XY
RG-316	TUBEROUS SCLEROSIS TYPE 1(TSC1), affected (N / IVS7+1 G->A)	
RG-320	TUBEROUS SCLEROSIS TYPE l(TSCl),affected (N / IVS7+1 G->A)	
RG-326	POPLITEAL PTERYGIUM SYNDROME (PPS),affected (R84H / N)	46,XY
RG-328	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46,XY
RG-330	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHMD1A), affected	46,XY
RG-333	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHMD1A), affected	46,XX
RG-356	HEMOGLOBIN ALPHA LOCUS(HBA), affected (- - / - -)	46,XX
RG-357	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected (XY)	46,XY
RG-358	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected (XY)	46,XY
RG-399	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46,XX
RG-401	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46,XX
RG-402	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46,XX
RG-403	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY lA(FSHMDlA), affected	
RG-404	SPINAL MUSCULAR ATROPHY, TYPE 1 (SMA1), affected	46,XY
RG-406	TORSION DYSTONIA 1, AUTOSOMAL DOMINANT (DYT1), affected (N/ GAG del)	
RG-413	BREAST CANCER, FAMILIAL (BRCA2),affected (N/IVS7 GT del) & MULTIPLE ENDOCRINE NEOPLASIA, TYPE l(MENl),affected(N/3036 4bp del)	
RG-414	MULTIPLE ENDOCRINE NEOPLASIA, TYPE l(MENl),affected (N/3036 4bp del)	
RG-415	HUNTINGTON DISEASE(HD), affected	
RG-416	CYSTIC FIBROSIS(CF), affected (AF508 / 1717-1 G>A)	
RG-417	CYSTIC FIBROSIS(CF), affected (AF508 /1717-1 G>A)	
RG-418	HEMOGLOBIN-BETA LOCUS (HBB), affected (cd8+G /619del)	
RG-420	HEMOGLOBIN-BETA LOCUS(HBB) affected (cd8+G/619del)	
RG-422	CYSTIC FIBROSIS(CF),affected (N1303K / AF508)	
RG-423	CYSTIC FIBROSIS(CF), carrier (AF508)	

RG-424	MULTIPLE ENDOCRINE NEOPLASIA TYPE 2 (MEN2B), affected (M918T/N)	
RG-426	PELIZAEUS-MERZBACHER DISEASE (PMLD), affected	
RG-428	TUBEROUS SCLEROSiS TYPE 1 (TSC1), affected (N/IVS7+1G^A)	
RG-222	Normal embryo (wish not to freeze, donated to hESC-research)	
RG-230	Normal embryo, family with Anemia Fanconi (non-match) (donated to hESC-research)	46,XX
RG-249	Normal embryo, family with NEMO GENE and flanking STRs (non-match) (donated to hESC-research)	
RG-308	Normal female (wish not to freeze, donated to hESC-research)	46,XX
RG-313	Normal female (wish not to freeze, donated to hESC-research)	46,XX

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.
3. the hESC lines that were created from the normal embryos were donated by couples who decided not to cryopreserve the embryos and elected to donate them for hESC-research and not to discard them.

Furthermore, during the consent process:

a. All disposition options available at RGI at the time of 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 RGI 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 only responsible for reproductive clinical care and to assure that informed consent was obtained from the donor(s). I was not directly involved in the derivation of the mentioned above cell lines nor did I 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. 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 indiyidual(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) or their children; 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;

To the best of my knowledge, I. Ilan Tur-Kaspa. M.D.. hereby certify that the above represents an accurate description of the consent process used for the donation of the embryos which resulted in the derivation of the listed above human Embryonic Stem Cell lines. A blank copy of the Reproductive Genetics Institute consent form that was used for the donation of the embryos which resulted in the derivation of the human Embryonic Stem Cell lines is attached to this letter of assurance.

10/6/09

Date

Ilan Tur-Kaspa. M. D.
Attending Physician of the patients at time of donation Medical Director, Institute for Human Reproduction Director, Clinical IVF-PGD Program, Reproductive Genetics Institute

As the IVF Laboratory Director at the Reproductive Genetics Institute (RGI) responsible for the creation of the embryos which resulted in the derivation of the human Embryonic Stem Cell (hESC) lines, I, Svetlana Rechitsky, PhD., hereby certify that the embryos used for the derivation of the cell lines listed in table below:

ID	Name of Disorder	Karyotype
RG-148	DYSTROPHIA MYOTONICA1 (DM1), affected	46,XY
RG-153	DYSTROPHIA MYOTONICA1 (DM1), affected	46,XX
RG-170	MUSCULAR DYSTROPHY, BECKER TYPE (BMD), affected male	46,XY
RG-186	HUNTINGTON DISEASE (HD); affected	46,XX
RG-194	HUNTINGTON DISEASE (HD), affected	46,XY
RG-233	HEMOGLOBIN-BETA LOCUS (HBB), affected (HbS/HbS - sickle cell anemia)	46,XX
RG-245	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), carrier (XXY)	47,XXY
RG-246	EMERY-DREIFUSS MUSCULAR DYSTROPHY; X-LINKED(EDMD), affected (XY)	46,XY
RG-271	TORSION DYSTONIA 1, AUTOSOMAL DOMINANT(DYT1), affected (N/GAGdel)	46,XY
RG-283	MUSCULAR DYSTROPHY, DUCHENNE TYPE (DMD), affected male	46,XY
RG-288	CYSTIC FIBROSIS(CF),affected (AF508/AF508)	46,XY
RG-289	CYSTIC FIBROSIS(CF), affected (AF5O8/AF508)	46,XX
RG-301	MUSCULAR DYSTROPHY, DUCHENNE TYPE(DMD) affected male	46,XY
RG-302	MUSCULAR DYSTROPHY, DUCHENNE TYPE(DMD), carrier	46,XX
RG-315	NEUROFIBROMATOSIS, TYPE 1 (NFl),affected (R19 47X / N)	46,XY
RG-316	TUBEROUS SCLEROSIS TYPE 1(TSC1), affected (N / IVS7+1 G-»A)	
RG-320	TUBEROUS SCLEROSIS TYPE l(TSCl),affected (N / IVS7+1 G->A)	
RG-326	POPLITEAL PTERYGIUM SYNDROME (PPS),affected (R84H / N)	46,XY
RG-328	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46/XY
RG-330	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHMD1A), affected	46,XY
RG-333	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHMD1A), affected	46,XX
RG-356	HEMOGLOBIN ALPHA LOCUS(HBA), affected (- - / - -)	46,XX
RG-357	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected (XY)	46,XY
RG-358	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected (XY)	46,XY
RG-399	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY lA(FSHMDlA), affected	46,XX
RG-401	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46,XX
RG-402	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46,XX
RG-403	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY lA(FSHMDlA), affected	
RG-404	SPINAL MUSCULAR ATROPHY, TYPE 1 (SMA1), affected	46,XY
RG-406	TORSION DYSTONIA 1, AUTOSOMAL DOMINANT (DYT1), affected (N/GAG del)	
RG-413	BREAST CANCER, FAMILIAL (BRCA2),affected (N/IVS7 GT del) & MULTIPLE ENDOCRINE NEOPLASIA, TYPE l(MENl),affected(N/3036 4bp del)	
RG-414	MULTIPLE ENDOCRINE NEOPLASIA, TYPE l(MENl),affected (N/3036 4bp del)	
RG-415	HUNTINGTON DISEASE(HD), affected	
RG-416	CYSTIC FIBROSIS(CF), affected (AF508 / 1717-1 G>A)	
RG-417	CYSTIC FIBROSIS(CF), affected (AF508 / 1717-1 G>A)	
RG-418	HEMOGLOBIN-BETA LOCUS (HBB), affected (cd8+G /619del)	
RG-420	HEMOGLOBIN-BETA LOCUS(HBB) affected (cd8+G/619del)	
RG-422	CYSTIC FIBROSIS(CF),affected (N1303K/ AF508)	
RG-423	CYSTIC FIBROSIS(CF), carrier (AF508)	
RG-424	MULTIPLE ENDOCRINE NEOPLASIA TYPE 2(MEN2B),affected(M918T/N)	

RG-426	PELIZAEUS-MERZBACHER DISEASE (PMLD),affected	
RG-428	TUBEROUS SCLEROSIS TYPE 1(TSC1), affected (N/IVS7+1G->A)	
RG-222	Normal embryo (wish not to freeze, donated to hESC-research)	
RG-230	Normal embryo, family with Anemia Fanconi (non-match) (donated to hESC-research)	46,XX
RG-249	Normal embryo, family with NEMO GENE and flanking STRs (non-match) (donated to hESC-research)	
RG-308	Normal female (wish not to freeze, donated to hESC-research)	46,XX
RG-313	Normal female (wish not to freeze, donated to hESC-research)	46,XX

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 content, for the human embryos to be used for research purposes.

Date

Svetlana Rechitsky, PhD (RGI) RG1 IVF Laboratory
Director at the time of donation

As the Principle Investigator of Reproductive Genetics Institute, I, Nikolai S Strelchenko, PhD, hereby certify that the derivation of the cell lines listed in table below:

ID	Name of Disorder	Karyotype
RG-148	DYSTROPHIA MYOTONICA1 (DM1), affected	46,XY
RG-153	DYSTROPHIA MYOTONICA1 (DM1), affected	46,XX
RG-170	MUSCULAR DYSTROPHY, BECKER TYPE (BMD), affected male	46,XY
RG-186	HUNTINGTON DISEASE (HD), affected	46,XX
RG-194	HUNTINGTON DISEASE (HD), affected	46,XY
RG-233	HEMOGLOBIN-BETA LOCUS (HBB), affected (HbS/HbS - sickle cell anemia)	46,.XX
RG-245	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), carrier (XXY)	47,XXY
RG-246	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected (XY)	46,XY
RG-271	TORSION DYSTONIA 1, AUTOSOMAL DOMINANT(DYTl), affected (N/GAGdel)	46,XY
RG-283	MUSCULAR DYSTROPHY, DUCHENNE TYPE (DMD), affected male	46,XY
RG-288	CYSTIC FIBROSIS(CF) affected (AF508/AF508)	46,XY
RG-289	CYSTIC FIBROSIS(CF), affected (AF508/AF508)	46/XX
RG-301	MUSCULAR DYSTROPHY, DUCHENNE TYPE(DMD) affected male	46,XY
RG-302	MUSCULAR DYSTROPHY, DUCHENNE TYPE(DMD), carrier	46,XX
RG-315	NEUROFIBROMATOSIS, TYPE 1 (NFl),affected (R19 47X/ N)	46,XY
RG-316	TUBEROUS SCLEROSIS TYPE i(TSCl), affected (N / IVS7+1 G->A)	
RG-320	TUBEROUS SCLEROSIS TYPE l(TSCl),affected (N / IVS7+1 G->A)	
RG-326	POPLITEAL PTERYGIUM SYNDROME (PPS),affected (R84H / N)	46,XY
RG-328	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46,XY
RG-330	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHMD1A), affected	46,XY
RG-333	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A (FSHMD1A), affected	46,XX
RG-356	HEMOGLOBIN ALPHA LOCUS(HBA), affected (- - / - -)	46,XX
RG-357	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected (XY)	46,XY
RG-358	EMERY-DREIFUSS MUSCULAR DYSTROPHY, X-LINKED (EDMD), affected (XY)	46,XY
RG-399	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY lA(FSHMDlA), affected	46,XX
RG-401	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46,XX
RG-402	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A(FSHMD1A), affected	46,XX
RG-403	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY lA(FSHMDlA), affected	
RG-404	SPINAL MUSCULAR ATROPHY, TYPE 1 (SMA1), affected	46,XY
RG-406	TORSION DYSTONIA 1, AUTOSOMAL DOMINANT (DYTl), affected (N/ GAG del)	
RG-413	BREAST CANCER, FAMILIAL (BRCA2),affected (N/IVS7 GT del) & MULTIPLE ENDOCRINE NEOPLASIA, TYPE l(MENl),affected(N/3036 4bp del)	
RG-414	MULTIPLE ENDOCRINE NEOPLASIA, TYPE l(MENl),affected (N/3036 4bp del)	
RG-415	HUNTINGTON DISEASE(HD), affected	
RG-416	CYSTIC FIBROSIS(CF), affected (AF508 / 1717-1 G>A)	
RG-417	CYSTIC FIBROSIS(CF), affected (AF508 / 1717-1 G>A)	
RG-418	HEMOGLOBIN-BETA LOCUS (HBB), affected (cd8+G /619del)	
RG-420	HEMOGLOBIN-BETA LOCUS(HBB) affected (cd8+G/619del)	
RG-422	CYSTIC FIBROSIS(CF),affected (N1303K/AF508)	
RG-423	CYSTIC FIBROSIS(CF), carrier (AF508)	
RG-424	MULTIPLE ENDOCRINE NEOPLASIA TYPE 2(MEN2B),affected(M918T/N)	
RG-426	PELIZAEUS-MERZBACHER DISEASE (PMLD),affected	
RG-428	TUBEROUS SCLEROSIS TYPE 1{TSC1), affected (N / IVS7+1 G->A)	

RG-222	Normal embryo (wish not to freeze, donated to hESC-research)	
RG-230	Normal embryo, family with Anemia Fanconi (non-match) (donated to hESC-research)	46,XX
RG-249	Normal embryo, family with NEMO GENE and flanking STRs (non-match) (donated to hESC-research)	
RG-308	Normal female (wish not to freeze, donated to hESC-research)	46,XX
RG-313	Normal female (wish not to freeze, donated to hESC-research)	46,XX

were conducted under IRB review and therefore meets the requirements detailed in 45 C.F. R. 46, Subpart A.

10/9/2009

Date /

Nikolai S Strelchenko, PhD Principle Investigator Blank
of Board Approved Consent Form
Letter of RGI Investigational Review Board Minutes May 21, 2003 (copy enclosed)
Letter of RGI Investigational Review Board Minutes March 24, 2004 (copy enclosed)
IRB Letter Approval (copy enclosed)

line - *Vol 10. No 1. 2005 105-110 Reproductive BioMedicine Online; www.rbmonline.com/Article/1590 on web 24 November 2004*

Article

Human embryonic stem cell lines with genetic disorders

Dr Yury Verlinsky is a graduate, post-graduate and PhD of Kharkov University of the former USSR. His research interests include cytogenetics, embryology and prenatal and preimplantation genetics. He introduced polar body testing for preimplantation genetic diagnosis and developed the methods for karyotyping second polar body and individual blastomeres. He has published over 100 papers, as well as three books on preimplantation genetics.

Dr Yury Verlinsky

Y Verlinsky, N Strelchenko, V Kukharepko, S Rechitsky, O Verlinsky, V Galat, A Kuiiev[1] Reproductive Genetics Institute, 2825 North Halsted Street, Chicago, IL 60657, USA
'Correspondence: Tel: +1 773 4724900; Fax: +1 773 8715221; e-mail: anverkuliev@hotmail.com

Abstract

A previous study described the establishment of human embryonic stem cell (ESC) lines from different sources of embryonic material, including morula, whole blastocyst and isolated inner cell mass. Using these methods, a repository of ESC lines has been established with different genetic abnormalities, which provides an unlimited source of disease cells in culture for undertaking research on the primary disturbances of the cellular processes in the genetically abnormal cells. ESC lines with genetic disorders were derived from the mutant embryos detected and avoided from transfer in the ongoing practice of preimplantation genetic diagnosis (PGD). The current repository contains 18 ESC lines with genetic disorders, including adrenoleukodystrophy, Duchenne and Becker muscular dystrophy, Fanconi anaemia, complementation group A, fragile-X syndrome, Huntington disease (three lines), Marfan syndrome, myotonic dystrophy (two lines), neurofibromatosis type I (five lines) and thalassaemia (two lines). These ESC lines are presently used for research purposes and may be available on request.

Keywords: embryonic stern cells (ESC), ESC lines with genetic disorders, preimplantation genetic diagnosis, repository of human ESC lines, single gene disorders

Introduction

A new method has been described for the derivation of human embryonic stem cell (ESC) lines from morula, and a repository of normal ESC lines from different sources of embryonic material has been established, including morula, whole blastocyst and isolated inner cell mass (Strelchenko *et al.*, 2004). These lines were characterized by a set of ESC criteria, including the presence of Oct-4, TRA-2-39, high molecular weight glycoproteins (antibodies TRA-l-60,TRA-l-80), stage-specific embryonic antigens (SSEA-3, SSEA-4), and euploid karyotype.

These developments made it possible to initiate the establishment of a repository of ESC lines from embryos with different genetic abnormalities, which will have an important role in the study of the primary disturbances of the cellular processes in the mutant cells to identify the molecular mechanisms that might be blocked to prevent the disease progression. Therefore, there is an obvious need for establishment of ESC lines derived from embryos with genetic and chromosomal abnormalities, to provide the basis for understanding of the mechanisms of the phenotypic realization of genetic defects and for the development of new approaches for their possible treatment.

The establishment of ES cell lines with genetic disorders has become possible with the introduction of preimplantation genetic diagnosis (PGD), enabling physicians to avoid transfer of the affected embryos, which then provide a valuable source for ESC lines with genetic abnormalities. This also provides a unique opportunity for investigating the potential of establishing ESC lines depending on the genotype. This paper presents the first description of the repository of human ESC lines with genetic disorders.

Materials and methods

The preimplantation embryos for the establishment of ESC lines with genetic disorders were obtained from PGD cycles, which

were performed either by the first and second polar body (PBl and PB2) removal or embryo biopsy, as described elsewhere (Verlinsky and Kuliev, 2000). Following multiplex PCR analysis of either PBl and PB2, or single blastomeres (Rechitsky *et al.,* 2001, 2002), the unaffected embryos were transferred back to patients, while the mutant ones were used for confirmation of PGD or donated for research, according to informed consent approved by the IRB of Reproductive Genetics Institute. In some cases, the embryos were obtained after PGD combined with HLA typing, as described elsewhere (Rechitsky *et al*., 2004).

Depending on the developmental stage of these donated mutant embryos, different techniques for the establishment of ESC lines were used, as described previously (Strelchenko *et al.,* 2004). The initial disaggregation of the cells (passage 0) was performed approximately 8-14 days after growth in feeder layer, by treating the cells with EDTA and cutting and transferring the soft cell clumps into a new dish with feeder layer. Fast proliferating colonies with ES-like morphology were isolated and propagated further. Within the next two to five passages, the uniform proliferating cells were selected, and colonies of established ESC lines were passaged using EDTA, followed by the harvesting procedure with a cell lifter, as described previously (Strelchenko et al.,2004).

The cell lines were tested for the following ES cell markers: alkaline phosphatase, stage-specific antigens SSEA-3 and SSEA-4, high molecular weight glycoproteins TRA-1-60 and TRA-1-80, and Oct-4, detected with polyclonal antibodies, as well as by Gene Choice One Tube RT-PCR kit (Vector Laboratories Inc., Burlingame, CA, USA), as described previously (Strelchenko *et al.,* 2004).

The established human ES-cell lines were maintained *in vitro* from 10 to 15 passages before freezing in sufficient amounts.

Results

The list of 18 ESC lines obtained from the embryos with genetic disorders is presented in **Table** 1. This repository contains three ESC lines with autosomal recessive disorders, including thalassaemia and Fanconi anaemia, complementation group A (FANCA), four ESC lines with X-linked disorders, including adrenoreukodystrophy (ABCD1), fragile site mental retardation (FMR1), Duchenne and Becker type muscular dystrophy (DMD; BMD), and 11 ESC lines with autosomal dominant conditions, including five ESC lines with dynamic mutations.

Only three ESC lines of the repository represent unaffected carriers, including one ESC line with IVSI-110 beta-thalassaemia mutation, one with/a 14 bp deletion in FANCA, and one with DMD. The remaining 15 ESC lines were affected. One of the ESC lines with beta-thalassaemia-has a double heterozygous genotype (Cd 39/IVSI-llO), with anTVSL-110 mutation inherited from the mother, and Cd 39 inherited from the father. As mentioned above, the largest group of ESC lines was obtained from PGD for dominant mutations, which, in addition to five ESC lines with dynamic mutations, contains five ESC lines with neurofibromatosis type 1 (NF1), obtained from NF1 embryos detected in PGD for NF1, described earlier (Rechitsky *et al.,* 2002; Verlinsky *et al.* 2002), While 17 ESC lines were derived from PGD practice, one ESC line (DMD) was established by request of a couple, and originated from the cohort of spare donated embryos, the transfer of two of which resulted in the birth of DMD twins.

Table 1 presents the results of PGD for only those conditions with which the above ESC lines were obtained. As seen from the data, the unaffected embryos were selected for transfer in all but two PGD cycles, with the affected embryos available as a source for the establishment of ESC lines with genetic disorders in each of the cycles shown in **Table 1.**

Figure 1 presents the results of PGD cycles resulting in the establishment of ESC line with Huntington disease (HD), a severe late onset autosomal dominant neurodegenerative disorder, which is one of the common indications for PGD. As can be seen from the presented pedigree, because of the female partner being a carrier of exon 1 expansion of the HD gene located on chromosome 4pl3.3, PGD was performed using PB1 and PB2 analysis, which predicted eight oocytes with the expanded allele and nine mutation-free oocytes. Two embryos resulting from these mutation-free oocytes (oocytes 5 and 10) were transferred, with the remaining mutation-free Ones that reached blastocyst stage being frozen for future use by' the couple. Three of eight mutant embryos were donated for research and used for establishment of ESC lines, resulting in two ESC lines, which apparently originated from the oocytes 12 and 17, as demonstrated by linked marker analysis, also confirming the presence of the expanded allele in exon 1 (hESC-186 and hESC-194). In addition, another ESC line with HD was obtained from PGD for the paternally derived mutation in the other PGD cycle and was also confirmed to contain the paternal expanded allele **(Table 1).**

Figure 2 presents the results of PGD for adrenoleukodystrophy (ALD) performed in combination with HLA typing, resulting in the establishment of ESC with this condition. The couple had two previous children affected with ALD (one of whom died), caused by the 1801 deletion of AG of *ABCD1* gene located in the X-chromosome (Xq28), inherited from the mother. Because the mutation leads to a progressive multifocal demyelination of central nervous system with adrenocortical insufficiency in boys, effectively treated only by HLA-compatible stem: cell transplantation, the couple requested PGD combined with preimplantation HLA typing. PGD was performed by blastomere biopsy, which predicted one unaffected male, one unaffected female and one affected male embryo, which was the only one that was HLA-matched to the affected sibling. This embryo was donated for research and used for the establishment of ESC line, confirmed to contain an 1801 deletion of AG in the *ABCD1* gene.

The other ESC line Obtained from PGD combined with HLA typing was the ESC line with double heterozygous thalassaemia, which was performed by blastomere biopsy and multiplex: PCR analysis, involving simultaneous testing of both mutation and HLA markers (Rechitsky *et al;*, 2004). Of .10 embryos tested, three were affected, one contained only one chromosome 11 with codon 39 mutation and the remaining six were carriers of one of the mutations: tested. Only one of these embryos was a full HLA match and was transferred. Of the six affected embryos, one embryo that was also an HLA non-match was donated for research and resulted in the establishment of an ESC line. The follow-up testing showed that the cells were double heterozygous affected for Cd39 and r/SI-110 mutations. As mentioned,

Table 1. List of embryonic stem cell lines derived from affected embryos or carriers of the mutations following PGD.

Disease	*Family genotype*	*Inheritance*	*Oocyte/embrvo*				*Stem cell line genotype*	*No. embryos received/lines obtained*
			Total	*Norma*		*Affected*		
				Total	*Transferred*			
Thalassaemia (beta locus)	Cd39/N (paternal) IVSI-110/N (maternal)	Autosomal recessive	10	6	l[b]	4	IVSI-110/cd39 (Affected)	1
Thalassaemia (beta locus)	619 bp DEL/N (paternal) recessive IVSI-1 N (maternal)	Autosomal	9	7	l[b]	2 (carrier)	IVSI-l/N	1
Fanconi anaemia, complementation group A	14 bp DEL/N (paternal); Exon 1, T-»A/N (maternal)	Autosomal recessive	**7**	5	l[b]	2	14 bp DEL/N (carrier)	1
Dystrophia myotonica 1 (expansion)	Expansion (maternal)	Autosomal dominant	[4]d	0	0	4	EXP/N (Affected)	2
Huntington disease (expansion)	Expansion (maternal)	Autosomal dominant	15	9	2	6	EXP/ N (affected)	2
Huntington disease (expansion)	Expansion (paternal)	Autosomal dominant	7	4	2	3	EXP/N (affected)	
Marfan syndrome	Maternal G7712A/N	Autosomal dominant	18	9	2	9	G7712A/N (affected)	
Neurofibromatosis, type I	Exon 29(A)/ N (maternal)	Autosomal dominant	16	9	3[a]	7	Exon 29(A)/N (affected)	
Adrenoleuko-dystrophy	1801 DELAG/ N (maternal)	X-linked	3	2[b]	0	1	1801 DELAG/N (affected)	
Fragile site mental retardation 1 (expansion)	Expansion (maternal)	X-linked	22	13	6[a]	9	EXP/N (affected)	
Muscular dystrophy, Becker type (deletion)	Deletion (maternal)	X-linked	8	7	[4]a	1	DEL; male (affected)	
Muscular dystrophy, Duchenne type (deletion)	Deletion (maternal)	X-linked	3[C]	1	NA	NA	DEL/N; female, (carrier)	
Total	**NA**	**NA**	122	72	22	48	NA	18

including frozen transfer.
"Mutation analysis combined with HLA genotyping.
[c]Spare embryos with unknown genotype obtained from donor programme, resulting in the birth of twins with DMD.
"Two embryos were chromosomally abnormal (monosomy 19).

another ESC line with thalassaemia was obtained from a heterozygous embryo donated at PGD by a couple with two different thalassaemia mutations (619 bp del/IVSI-110), and was confirmed to carry one copy of the IVSI-110 mutation.

Five ESC lines were established from PGD for NFl, resulting in the birth of healthy unaffected twins, described previously (Verlinsky *et al.*, 2002). Of 16 embryos available for analysis, seven embryos with mutation Trp-»Ter (TGG-»TGA) in exon 29 of NFl gene were detected, which were donated for research and used for the establishment of five ESC lines with NFl.

The details of the remaining seven ESC lines are presented in **Table 1,** showing that only one ESC line with DMD was obtained from a source other than PGD. This ESC line was derived from one of three spare embryos left over from a cohort of donor embryos, the transfer of which resulted in the birth of DMD twins. The testing of the established ESC showed a female carrier genotype.

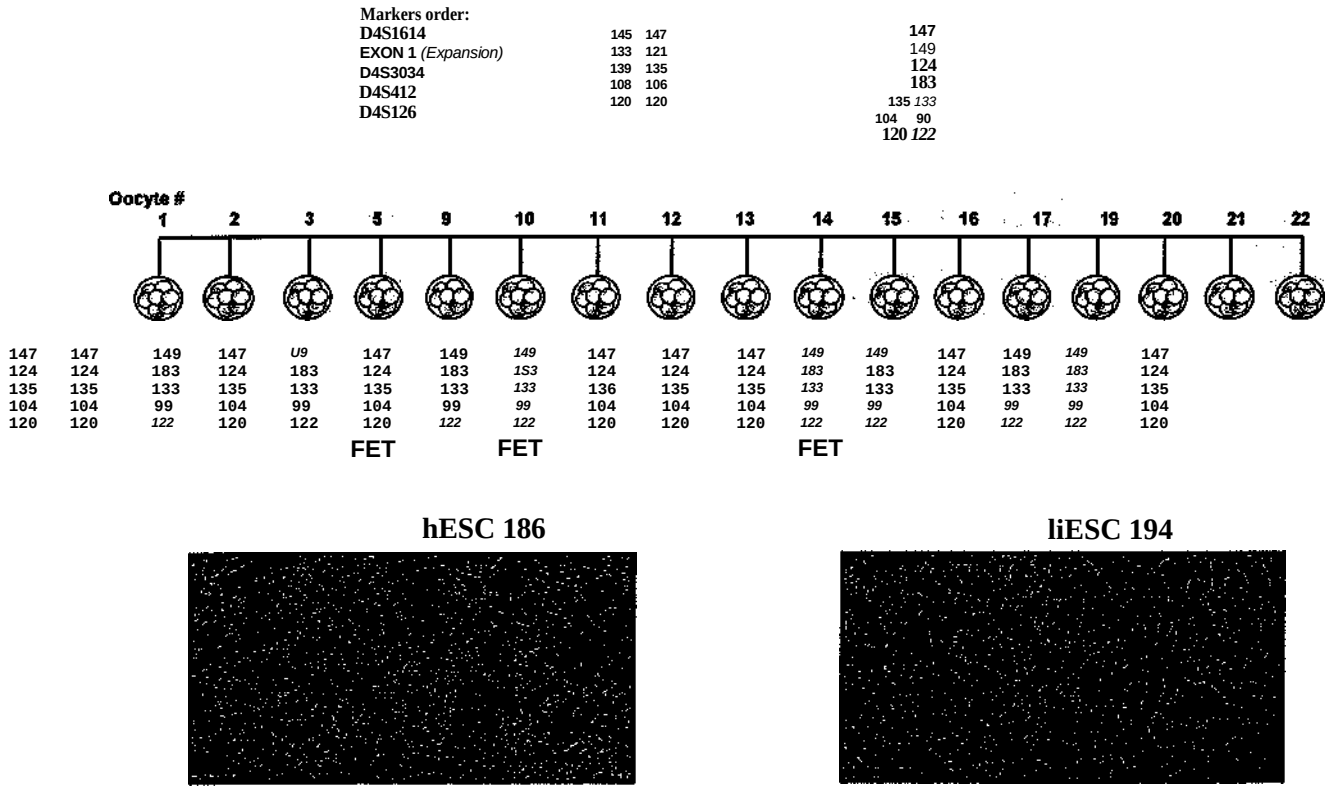


Figure 1. Embryonic stem cell line with Huntington disease obtained from PGD source. Upper panel: family pedigree With paternal and maternal haplotypes (marker order is given on left), with mother carrying an exon 1 expansion of HD gene. Middle panel: results of sequential PBl and PB2 analysis of 17 oocytes, nine of which were predicted to be mutation-free, and the other eight affected. Two embryos resulting from the mutation free oocytes were transferred back to the patient in the subsequent cycle following freezing (FET), while three affected embryos resulting from mutant oocytes were donated for research and used for establishment of ES cell lines. Lower panel: established ESC lines (hESC 186 and hESG 194), which according to marker analysis were derived from embryos resulting from oocytes 12 and 17.

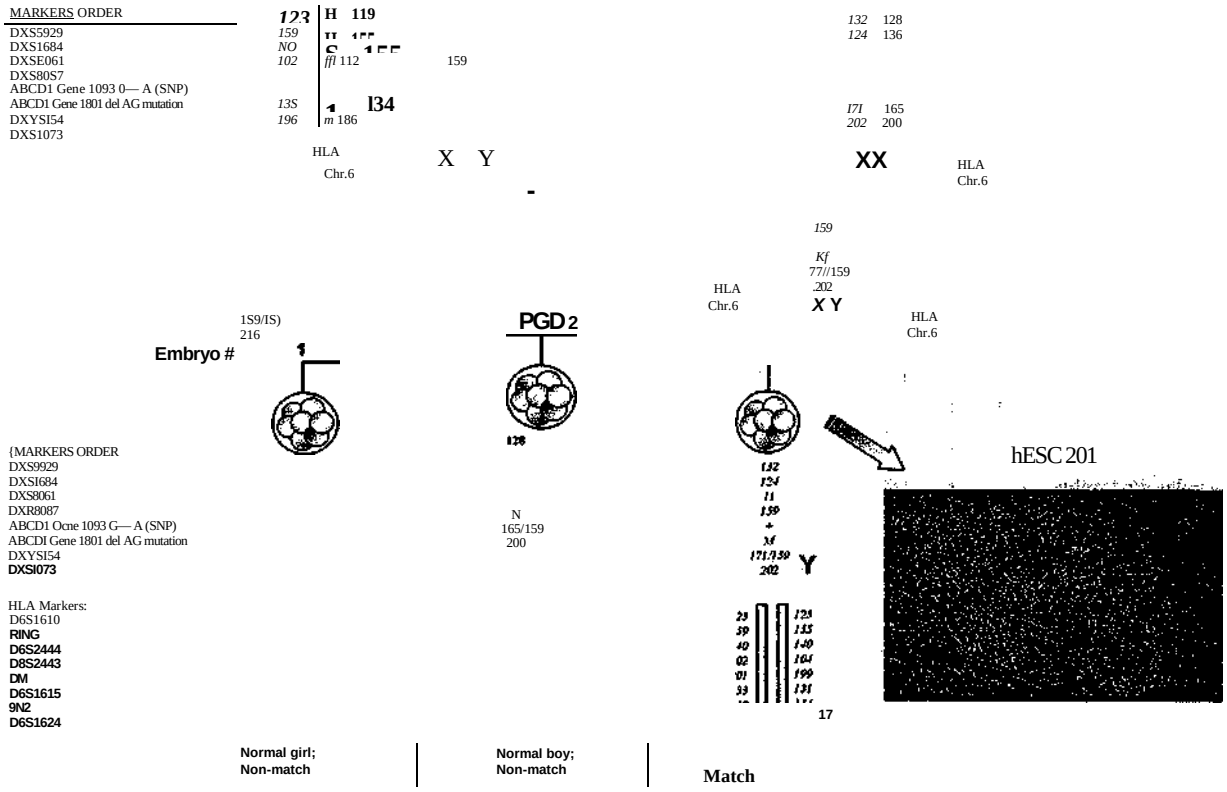


Figure 2. Embryonic stem cell line with X-linked adrenoleukodystrophy following PGD combined with HLA typing. Upper panel: family pedigree with paternal and maternal haplotypes (marker order is given on left); the other is carrier of *ABCD1* gene 1801 del AG mutation; HLA haplotypes are shown as darker and lighter bars, darker corresponding to haplotypes of sibling with ALD, shown on the right. One of the affected boys died due to absence of HLA compatible donor available (on the left). Lower panel: results of blastomere biopsy analysis of three embryos, two of which were predicted to be unaffected, but were not HLA matched to the affected sibling, and one was HLA matched but affected (embryo 3). This embryo was donated for research and was used for the establishment of ESC line (hESC 201) (lower right), which was confirmed to contain the deletion inherited from the mother.

Table 2. Characterization of ESC line with genetic disorders. NT = not tested.

Genetic disease	*hESC line ID*	*Passage no.*	*AP*	*SS3*	*SS4*	*T60*	*T80*	*T39*	*Oct4*
Thalassaemia (beta locus)	164	10	+	+	+	+	+	+	+
Thalassaemia (beta locus) carrier	158	7	+	+	+	+	+	+	+
Fanconi anaemia, complementation group A carrier	128	4	+	+	+	+	+	+	+
Dystrophia myotonica 1 (expansion)	148	8	+	+	+	+	+	+	+
Dystrophia myotonica 1 (expansion)	153	8,11	+	+	+	+	+	+	+
Huntington disease	186	6	+	+	+	+	+	+	+
(expansion)	194	4	+	+	+	+	+	+	+
Huntington disease (expansion)	187	5	+	+	+	+	+	+	+
Marfan syndrome	154	6	+		+	+	+	+	+
Neurofibromatosis, type I	137	5	+	+	+	+	+	+	+
	138	9	+	+	+	+	+	+	+
	139	3	+	+	+	+	+	+	+
	140	8	+	+	+	+	+	+	+
	141	7	+	+	+	+	+	+	+
Adrenoleukodystrophy	201	6	+	+	+	+	+	+	+
Fragile site mental retardation 1 (expansion)	125	5	+	+	+	+	+	+	+
Muscular dystrophy, Becker type (deletion)	170	7	+	NT	NT	NT	NT	NT	NT
Muscular dystrophy, Duchenne type (deletion) carrier	180	4	+	+	+	+	+	+	+

Discussion

The presented 18 ESC lines represent currently the world's first repository of ESC lines with genetic disorders, which are being used for research purposes and are also available on request (RGI Inc., Chicago, IL, USA). These ESC lines were characterized by ESC criteria, including the presence of Oct-4, TRA-2-39, high molecular weight glycoproteins (antibodies TRA 1-60, TRA-1-80), and the stage-specific embryonic antigens (SSEA-3, SSEA-4) as shown in Table 2. As euploid karyotype is-one of the important criteria of ESC, karyotyping of the ESC lines is currently underway at different passages, taking into consideration the previously demonstrated possibility of * chromosomal abnormalities occurring during the process of extended culture of ESC lines (Draper *et al.*, 2004) and consequently not retaining a diploid karyotype (Thomson *etal.*, 1998).

As previously suggested (Pickering *et al.*, 2003), the data show that PGD may provide a valuable source for obtaining ESC lines, including those with genetic disorders. Preliminary results suggest that genetic defects may not affect the efficiency of the establishment of ESC lines, their ESC marker expression or morphology. Therefore, ESC lines with genetic disorders will serve as an unlimited cell source for the study of mechanisms of cellular pathology and the development of possible treatment regimens.

References

Draper JS, Smith K, Gokhale P *et al.* 2004 Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nature Biotechnology* 22,53-54. Pickering S, Braude P, Patel M *et al.* 2003 Preimplantation genetic diagnosis as a novel source of embryos for stem cell research. *Reproductive BioMedicine Online* 7,353-364. Rechitsky S, Verlinsky O, Amet T *et al.* 2001 Reliability of preimplantation diagnosis for single gene disorders. *Molecular and Cellular Endocrinology* **183,** S65-S68. Rechitsky S, Verlinsky O, Chistokina A *etal.* 2002 Preimplantation genetic diagnosis for cancer predisposition. *Reproductive BioMedicine Online* 5,148-155. Rechitsky S, Kuliev A, Tur-Kaspa I *et al.* 2004 Preimplantation genetic diagnosis with HLA matching. *Reproductive BioMedicine Online* 9, 210-221. Strelchenko N, Verlinsky O, Kukharenko V, Verlinsky Y 2004 Morula-derived human embryonic stem cells. *Reproductive BioMedicine Online* 9,623-629. Thompson JA, Itskovitz-Eldor J, Shapiro SS *et al.* 1998 Embryonic stem cell lines derived from human blastocysts. *Science* 282,1145-1147. Verlinsky Y, Kuliev A 2000 *Atlas of Preimplantation Genetic Diagnosis.* Parthenon, New York, London. Verlinsky Y, Rechitsky S, Verlinsky O *et al.* 2002 Preimplantation genetic diagnosis for neurofibromatosis. *Reproductive BioMedicine Online* 4,218-222.

Received 15 October 2004; refereed 5 November 2004; accepted 12 November 2004.

nline - *Vol 13 No 4. 2006 547-550 Reproductive BioMedicine Online; www.rbmonline.com/Article/2437 on web 1 August 2006*

Article

Repository of human embryonic stem cell lines and development of individual specific lines using stembrid technology

Dr Yury Veriinsky is a graduate, post-graduate and PhD of Kharkov University of the former USSR. His research interests include cytogenetics, embryology and prenatal and preimplantation genetics. He introduced polar body testing for preimplantation genetic diagnosis and developed the methods for karyotyping second polar body and individual blastomeres. He has published over 100 papers, as well as three books on preimplantation genetics.

Dr Yury Veriinsky

Y VerlinskyN Strelchenko, V Kukharenko, A Shkumatov, S Rechitsky, O Veriinsky, A Kuliev[1]
Reproductive Genetics Institute, Chicago, IL, USA
Correspondence: Tel: +1 773 4724900; Fax: +1 773 8715221; e-mail: anverkuliev@hotmail.com

Abstract

A human embryonic stem cell (HESC) line repository has been established, containing HESC lines with normal and abnormal genotypes, providing the source for studying the primary mechanisms of genetic disorders at the cellular level. Because the outcome of HESC transplantation treatment depends on access to human leukocyte antigen identical stem cells, the development of individual specific HESC was initiated, using the original stembrid technology, which is based on the hybridization of adult somatic cells with cytoplast of HESC lines. The data presented here demonstrate feasibility of this approach in the future development of HESC transplantation treatment of genetic and acquired disorders. The established HESC repository presently contains 166 HESC lines, including 127 with normal genotype and 39 with genetic and chromosomal disorders.

Keywords: genetic disorders, human embryonic stem cells, reprogramming, stembrid

Introduction

Preliminary data have been presented on the establishment of a repository of human embryonic stem cell (HESC) lines from embryos with normal and abnormal genotypes, obtained from the ongoing practice of preimplantation genetic diagnosis (PGD) (Strelchenko *et al*, 2004; Verlinsky *et al.*, 2005; Verlinsky and Kuliev, 2006). These HESC lines were characterized by a set of ESC criteria, including the presence of octamer-binding transcription factor-4 (Oct-4), tumour rejection antigen-2-39 (TRA-2-39), high molecular weight glycoproteins (antibodies TRA-1-60, TRA-1-81), stage-specific embryonic antigens (SSEA-3, SSEA-4) and euploid karyotype.

In addition to HESC lines with normal genotypes, a number of HESC lines with different genetic disorders were reported, which have an important role in understanding the mechanisms of the phenotypic realization of genetic defects and for the development of new approaches for their possible treatment. Initially, no HESC lines with chromosomal disorders were isolated, with all the HESC lines showing a normal chromosomal set (Verlinsky *et al.*, 2005), but, as will be presented below, such lines have now been established. A few HESC lines with genetic disorders were reported previously, including those with cystic fibrosis, trisomy 13 and triploidy (Heins *et al.*, 2004; Pickering *et al.*, 2005), while an increasing number of HESC lines with normal genotype is being established, recently reviewed in detail (Guhr *et al.*, 2006).

The feasibility of the reprogramming of adult somatic cells into HESC with the use of HESC cytoplasm was also tested as a possible approach for obtaining individual specific HESC lines (Strelchenko *et al.*, 2006). This was previously attempted by Cowan and colleagues (Cowan *et al.*, 2005), who, however, were unable to avoid the presence of the HESC DNA in the resulting hybrid tetraploid cells.,

The present paper updates the repository of HESC lines with normal and abnormal genotypes, and presents developments in the establishment of individual specific HESC using HESC cytoplasm, representing the new stembrid technology.

Repository of HESC lines with normal and abnormal genotypes

The first HESC line was established in 1998 (Thomson *ei al.*,-1998). The present and other techniques were used for the establishment of HESC lines, described previously (Strelchenko *et al.,* 2004; Verlinsky *et al.,* 2005,) with a success rate of 20-25%. The established HESC lines were tested for alkaline phosphatase, stage-specific antigens SSEA-3 and SSEA-4, high molecular weight glycoproteins or tumour rejection antigens, TRA-1-60 and TRA-1-80, and Oct-4. The HESC lines were maintained *in vitro* for up to and over a dozen passages before freezing in sufficient amounts. The list of HESC lines presently contains 166 HESC lines, including 127 with normal genotype, 32 with different genetic disorders and eight with chromosomal abnormalities, which are frozen and available at different passages **(Table 1).** A repository of HESC lines with genetic disorders includes eight HESC lines derived from the embryos with autosomal recessive disorders, including five with beta-globin mutations (two thalassaemia mutation carriers and two affected with thalassaemia and one sickle cell disease), one with Fanconi anaemia, complementation group A, and one each with cystic fibrosis and spinal muscular atrophy. Ten HESC lines were derived from the embryos with X-linked disorders, including adrenoleukodystrophy, two with fragile site mental retardation (one affected male and one carrier female), and seven with Duchenne (one affected and two carriers), Becker and Emery-Dreifuss (one affected and one carrier) type muscular dystrophy. Fourteen HESC lines were derived from the embryos with autosomal dominant conditions, including six with neurofibromatosis type 1, one with Marfan syndrome, one with torsion dystonia, two with myotonic muscular dystrophy, and four with Huntington's disease, the latter six representing dynamic mutations, partially described previously (Verlinsky *et al.,2005).*

The collection of HESC lines with chromosomal disorders is presented for the first time, and contains two lines with translocations, one with trisomy 14, one with triploidy and four with aneuploidy of sex chromosomes, including 45, X, 47, XXX, and two with 47, XXY, one of which was derived from the same embryo that was the source of the HESC line with Emery-Dreifuss (carrier) type muscular dystrophy **(Table** 1).

As can be seen from a recent review of the present status in the establishment of HESC (Guhr et al., 2006), the presented repository of 166 HESC lines is the world's largest collection of HESC lines available for stern cell research. As mentioned, there were previous reports of the establishment of three HESC lines with cystic fibrosis, trisomy 13, and triploidy (Heins *et al,* 2004; Pickering *et al,* 2005), so the presented collection also represents the only available repository of HESC lines with genetic and chromosomal disorders as the basis for research on the primary mechanisms of the realization of genetic abnormalities.

Development of individual specific HESC

The extensive experience of stem cell transplantation treatment shows that the success rate has depended heavily on the finding of a human leukocyte antigen (HLA) matched donor (Gaziev and Lucarelli, 2005; La Nasa *et al.,* 2005). Therefore, the potential of the future cellular therapeutic interventions for many congenital and acquired disorders will depend on the production of the HLA identical patient-specific HESC lines. Although such a possibility was reported to be feasible (Cowan *et al.,* 2005; Stojkovic *et* ***oil.,*** 2005; Strelchenko *et al,* 2006), many problems still remain unresolved, the key issue being the ability to reprogramme embryonic stem cells into different

Table 1. List of human embryonic stem cell (hESC) lines with genetic and chromosomal disorders.

Genetic disorder
Autosomal dominant
Huntington's disease, affected, expansion *(n* = 4)
Marfan syndrome, affected, G7712A/N
Myotonic muscular dystrophy, affected, expansion (h = 2)
Neurofibromatosis, type I affected *in* = 6)
Torsion dystonia, DYTl, affected, exon 7 GAG deletion
Subtotal: *n* =14
Autosomal recessive
Beta-thalassaemia, affected, IVSI-110/CD39
Beta-thalassaemia, affected, IVSII-1/Unknown
Beta-thalassaemia, carrier, IVSII-1/N
Beta-thalassaemia, carrier, IVSI-110/N
Cystic fibrosis, CFTR, affected, Rl 17C/W128X
Fanconi anaemia-A, 14-bp deletion, carrier
Sickle cell disease
Spinal muscular atrophy; exon 7 deletion
Subtotal: *n* = 8
X-linked
Adrenoleukodystrophy, X-linked, affected
Becker muscular dystrophy, affected, del
Duchenne muscular dystrophy, affected, del
Duchenne muscular dystrophy, carrier, del/N n=2
Emery-Dreifuss muscular dystrophy, X-linked, affected male
Emery-Dreifuss muscular dystrophy, X-linked, carrier"
Fragile-X syndrome, affected male, expansion
Fragile-X syndrome, female carrier
Ocular albinism, X-linked, affected
Subtotal: *n* = 10
Chromosomal disorders
69,XXX
47.XXY
46,XX,der(4)t(4:13)
46,XX,t(10:22)(q25;ql3)
47,XX,+14
45,X
47,XXY[a]
47.XXX
Subtotal: n = 8

Total: 39 hESC lines

The same hESC line (with XXY and Emery-Dreifuss muscular dystrophy).

specialized cells, and turning differentiated specialized somatic cells into ES cells.

Nuclear reprogramming of human fibroblasts has recently been performed through hybridization between fibroblasts and HESC (Cowan *et al.,* 2005), and fusion of HESC cytoplasts with adult lymphocytes and fibroblasts (Strelchenko *et al.,* 2006). Although HESC were demonstrated to reprogramme adult somatic cells, the resulting hybrid cells contained tetraploid DNA, including the contribution from both HESC and donor somatic cells (Cowan *et al.,* 2005), while the isolated colonies of the resulting HESC cybrids appeared to contain the cells with recipient nuclei, in addition to the cells with somatic donor cell nuclei (Strelchenko *et al.,* 2006).

The design of stembrid technology, in which female HESC are used for reprogramming of male adult lymphocytes or fibroblasts, is presented in Figure 1, with its outcome evaluated by the appearance of cells with male karyotype and Oct-4 and TRA-2—39 markers in the resulting proliferating colonies. The details Of the suppression of HESC nuclei, enucleation of HESC, and fusion of HESC cytoplasts with somatic cells were[1]: described earlier (Strelchenko *et al,* 2006). Isolated colonies of typical HESC morphology obtained after 7-10 days of culture were transferred into separate wells of a 48-well dish and cultured for 2 weeks before being passaged and tested for stemmness by fluorescein isothiocyanate for TRA-2—39 and tetramethylrhodamine B isothiocyanate for Oct-4, followed by analysis by fluorescence in-situ hybridization (FISH) (Strelchenko *et al,* 2006).

The somatic cell nuclei fused with HESC cytoplasts were shown to proceed with cell division, resulting in the establishment of cybrid cells with male karyotype. It was demonstrated that the mitotic donor (lymphocytes) nuclei in cytoplasts start synthesizing Oct-4, not typical for the donor nuclei, with its gradual increase despite originating from differentiated cells. Similarly, the colonies derived from the differentiated cell nuclei fusion with cytoplasts of HESC also expressed Oct-4/TRA-2-39, while FISH analysis showed that the resulting colonies contained cells with XY karyotype, mixed with recipient XX nuclei. All isolated colonies were positively tested also for SSEA3, SSEA4, TRA-1-60, TRA-1-80 as well as Oct-4/TRA-l-39. The cybrid cells demonstrated the typical HESC morphology and stemmness, as shown by positive Oct-4 and alkaline phosphatase. This provides the evidence of the replacement of HESC nuclei by the nuclei of somatic cells.

The results suggest feasibility of a complete replacement of HESC nuclei by the nuclei of donor somatic cells. However, only a small proportion of cells were derived from donor lymphocytes or fibroblasts, with no pure population of cybrid ESC yet isolated. In addition to these cybrid cells, the hybrids between the donor and non-enucleated HESC were obtained with XY/XX, XXYY/XX and XXY/XX karyotypes.

The obtained cybrid cells, with complete replacement of HESC nuclei by the nuclei of somatic cells, also demonstrated the typical SC morphology and the presence of Oct-4 and L-alkaline phosphatase, confirming the 'stemmness' of the resulting cybrids. However, the efficacy of the method is still

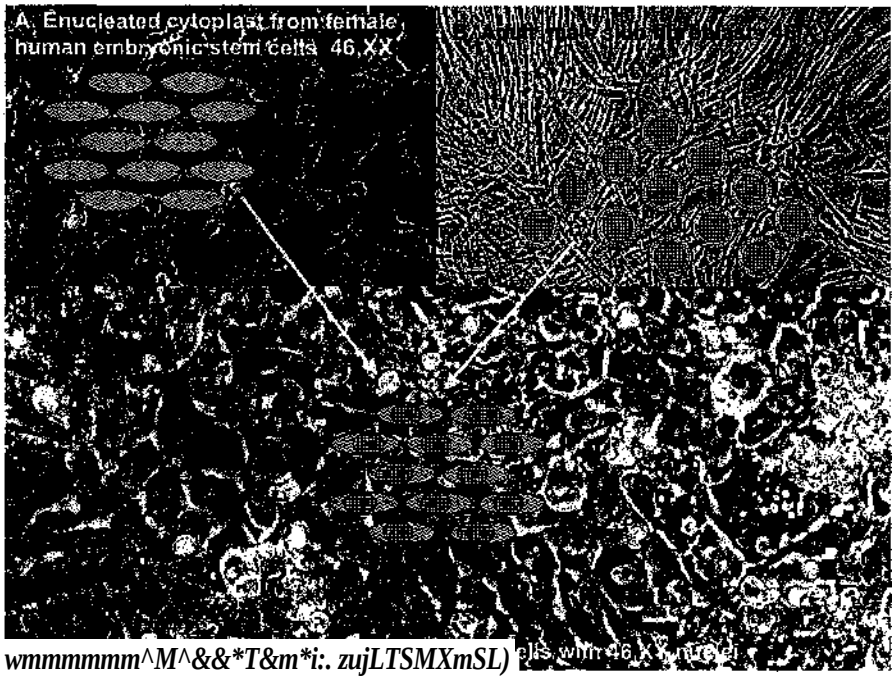


Figure 1. Stembrid technology schematic representation shown on the image of the corresponding growing cells. (A) Enucleated cytoplast obtained from female (46,XX) human embryonic stem cells (hESC). (B) Growing adult male (46.XY) skin fibroblasts. (C) The resulting colony of HESC, containing cybrid cells with male (46.XY) nuclei reprogrammed by cytoplasm of hESC. Some of these nuclei contain two nucleoli characteristic for ESC.

not sufficiently high, requiring considerable improvement before it could be considered for obtaining individual specific HESG, with the required somatic cell nuclei, as no methods are yet available for isolation and purification of HESC cybrid colonies. The further improvement of this stembrid technology may be useful for the construction of individual specific HESC lines, which provides the prospect of future stem cell therapy, also avoiding the controversy of the use of human oocytes for production of HESC.

References

Cowan CA, Atienza J, Melton DA, Eggan K 2005 Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science* **309,**1369-1373; Gaziev J, Lucarelli G 2005 Stem cell transplantation for thalassaemia. *Reproductive BioMedicine Online* **10,** 111-115. Guhr A, Kurtz A, Freidgen K, Loser P 2006 Current state of human embryonic stem cell research: an overview of cell lines and their usage in experimental work. *Stem Cells*, Published Online June 15, 2006. Heins N, Englund MCO, Sjoblorn C *et at.* 2004 Derivation, characterization, and differentiation of human embryonic stem cells. *Stem Cells* 22,367-376. La Nasa G, Argiolu F, Giardini C *etal.* 2005 Unrelated bone marrow transplantation for beta-thalassaemia patients. The experience of the Italian bone marrow transplantation group. *Annals of the New York Academy of Sciences* **10S4,** 186-195.

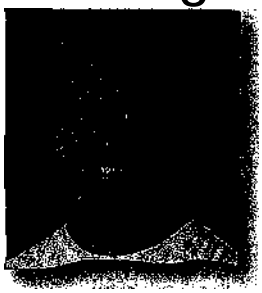
Pickering S, Minger SL, Patel KJ *et al;* 2005 Generation of a human embryonic stem cell line encoding the cystic fibrosis mutation Delta F-508 using preimplantation genetic diagnosis. *Reproductive BioMedicineOnline*lfl,390r-397. Stojkovic D, Stojkovic P, Leavy C *et al.* 2005 Derivation of a human blastocyst after heterologous nuclear transfer to donated oocytes. *Reproductive BioMedicine Online*: **11,** 226-231.
Strelchenko N, Kukharenko V, Sckumatov A *et al.* 2006 Reprogramming of human somatic cells by embryonic stem cell cytoplast *Reproductive BioMedicine Online* 12,99--103.
Strelchenko N, Verlinsky O, Kukharenko V, Verlinsky Y 2004 Morula derived human embryonic stem cells. *Reproductive BioMedicine Online* 9,. 623-629,. . Thomson JA, Itskovitz-Eldor J.Shapiro SS et al. 1998 Embryonic stem cell lines derived from human blastocysts. *Science* **282,** 1145-1147. Verlinsky Y, Kuliev A 2006 *Practical Preimplantation Genetic Diagnosis.* Springer, London Verlinsky YStrelchenko N, Kukharenko V *etal.* 2005Human embryonic stem cell lines: with genetic disorders. *Reproductive BioMedicine Online* **10,** 105-110.

Paper based on contribution; presented at the PGDIS Annual Meeting 'Nuclear transfer and reprogramming' in Belize, Central America, February 2-5, 2006.

Received 31 May 2006; refereed 21 June 2006; accepted 10 July 2006.

Article

Human embryonic stem cell models of Huntington disease



In 2006 Jonathan Niclis completed a BSc degree with Honours at Monash Immunology and Stem Cell Laboratories (MISCL), Monash University, Melbourne, Australia. He is currently undertaking a PhD at MISCL within a research group focused on the development of stem cell therapies for reproductive and genetic disorders. His current project aims to characterize human embryonic stem cells which carry the Huntington disease mutation to determine their suitability as human in-vitro models of neurodegeneration.

Mr Jonathan C Niclis

JC Niclis[1], AO Trounson[12], M Dottori[3], AM Ellisdon[4], SP Bottomley[4], Y Verlinsky[5], DS Cram[16]
[1] Monash Immunology and Stem Cell Laboratories, Monash University, Victoria, Australia; California Institute of
Regenerative Medicine, California, USA; [3]Centre for Neuroscience and Department of Pharmacology, University of Melbourne, Victoria, Australia; ""Department of Biochemistry, Monash University, Victoria, Australia; Reproductive Genetics Institute, Chicago, USA
Correspondence: e-mail: david.cram@med.monash.edu.au

Abstract

Huntington disease (HD) is an incurable late-onset neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the HD gene *(HIT).* The major hallmark of disease pathology is neurodegeneration in the brain. Currently, there are no useful in-vitro human models of HD. Recently, two human embryonic stem cell (hESC) lines carrying partial (CAG_{37}) and fully (CAG_{5l}) penetrant mutant alleles have been derived from affected **IVF** embryos identified following preimplantation genetic diagnosis (PGD). Fluorescence polymerase chain reaction (F-PCR) and Genescan analysis confirmed the original embryonic HD genotypes. Reverse transcription PCR (RT-PCR) analysis confirmed the expression of mutant transcripts and western blot analysis demonstrated expression of mutant huntingtin protein (HTT). After treatment with noggin, HD hESC formed neurospheres, which could be further differentiated into cells susceptible to neurodegeneration in HD, namely primary neurones and astrocytes. Small pool PCR analysis of neurosphere cells revealed instability of disease-length CAG repeats following differentiation. The presence of active *HTT* genes, neural differentiation capabilities and evidence of CAG repeat instability indicates these HD hESC lines may serve as valuable in-vitro human models of HD to better understand the mechanisms of neurodegeneration in patients, and for drug screening to identify new therapies for human clinical trials.

Keywords: *CAG repeats, human embryonic stem cells, Huntington disease, neural differentiation*

Introduction

Huntington disease (HD) is an autosomal dominant neurological disorder that is caused by an expanded CAG'repeat region in exon 1 of the *HTT* gene (Huntington's Disease Collaborative Research Group, 1993). HD is the most common of the nine polyglutamine (polyQ) disorders, affecting approximately 1 in 10,000 individuals worldwide. Disease onset usually occurs between 40 and 50 years of age. There are no effective treatments and death usually occurs 10-20 years after clinical onset. Studies of post-mortem brain tissue from HD patients has shown that underlying neurodegeneration is a consequence of selective neuronal loss, particularly y-aminobutyric acid (GABA)ergic medium spiny neurones (Vonsattel *et al.,* 1985; Ferrante *etal.,* 1991).

Mutant *HTT* alleles (CAG^) exhibit an age-dependent penetrance. The lowest disease range ($CAG_{3(Wg}$) is associated with partial penetrance and a later age of onset (Snell *etal.,* 1993; McNeil *etal.,* 1997) whereas CAG^ alleles are associated with full penetrance. Although wild-type repeat alleles ($CAG_{<35}$) are stable *in vivo,* mutant alleles can exhibit CAG repeat instability. Small-scale contractions of mutant alleles occur in the female germ line, whereas large expansions are commonly observed in the male germ line (Telenius *et al.,* 1993; Leeflang *et al.,* 1995). Mutant alleles also exhibit a propensity for CAG expansion within somatic cells. Expansions have been detected in the most severely affected brain regions of HD patients, with some exceeding 1000 CAG repeats (Telenius *et al.,* 1994; Kennedy

et al., 2003). Furthermore, analysis of individual laser-captured micro-dissected neurones has shown that CAG repeat gains are more prominent in susceptible neurones compared with those that are spared (Shelbourne *et al,* 2007).

The *HIT* gene encodes a 350 kDa ubiquitously expressed protein called huntingtin (HTT). Mutant HTT proteins generate intranuclear and intracytoplasmic aggregates throughout the brain, including within axons and dendrites (Davies *et al,* 1997; DiFiglia *et al.,* 1997; Scherzinger *et al.,* 1997; Becher *et al.,* 1998; Gutekunst *et al.,* 1999; Lunkes *et al.,* 2002; Shin *et al.,* 2005). Although the role of aggregates as a cell defence mechanism or as a perpetrator of pathology is debatable, it is clear that they are a major hallmark of HD. Aggregates have also been identified in a wide range of non-central nervous system tissue types that are seemingly unaffected (Sathasivam *et al.,* 1999). Another major pathological process observed in striatal neurones is the dysregulation of the transcriptome (Thomas, 2006). Mutant cytoplasmic HTT is cleaved by caspase activity to form monomeric N-terminal polyQ peptides with increased toxicity (Lunkes *et al.,* 2002; Wellington *et al,* 2002; Graham *et al.,* 2006; Pattison *et al,* 2006). Following translocation to the nucleus, the mutant polyQ peptide is believed to aberrantly modulate transcription via abnormal protein interactions with various transcription factors such as Spl (Dunah *et* al, 2002; Chen-Plotkin *et al,* 2006) and other polyglutamine-containing proteins such as wild-type huntingtin and TATA-binding protein (Huang *et al,* 1998).

Mutant HTT or N-terminal polyQ isoforms have been expressed in a range of species such as *Caenorhabditis elegans, Drosophila melanogaster* and *Mus musculus,* and most recently *Macaca mulatto,* to generate different transgenic and $knock_T$ in models of HP (Sipione and Cattaneo, 2001; Garcia-Ramos *et al.,2007;* Yang *et al.,* 2008). Animal models have added valuable knowledge to the understanding of HD pathogenesis and continue to be of great importance; however, studies to date demonstrate that these models do not precisely reflect all HD pathologies, and so far they have not led to successful human therapies. There remains a need for new in-vitro human HD models. Alternative models yet to be explored are HD human embryonic stem cells (hESC). Recently, disease-specific hESC lines have been established from affected preimplantation genetic diagnosis (PGD) embryos (Pickering *et al,* 2005; Verlinsky *et al,* 2005, 2006; Mateizel *et al,* 2006), including those carrying a mutant HTTgene (Verlinsky *etal,* 2005,2006; Mateizel *et al,* 2006). These HD hESC may be useful in-vitro disease models that replicate key HD pathologies. This paper reports on the initial characterization of two HD hESC lines (SI-186 and SI-187) carrying mutant alleles with 37 and 51 CAG repeats, respectively.

Materials and methods

hESC lines.

HD-positive stem cell lines SI-186 and SI-187 were derived from IVF embryos following PGD. Details of the IVF and PGD cycles that led to the identification of the HD embryos, as well as information on the generation and maintenance of the HD hESC lines, have been previously published (Verlinsky *et al,* 2005). Both lines were obtained as frozen stocks from Stemride International (http://www.stemride.com) under a materials transfer agreement with Monash University. Research on these lines at Monash University was approved by the Standing Committee on Ethics in Research Involving Humans (2006/063EA). Following derivation, SI-186 and SI-187 had normal female karyotypes and expressed the hESC pluripotency markers SSEA-3, SSEA-4, Oct-4, TRA-2-39, TRA-1-60 and TRA-1-80 (Verlinsky *etdl;,)2005).* Genotyping revealed that the lines were heterozygous for mutant alleles of CAG_{38} and CAG_{51}, respectively (Verlinsky *et al,* 2005). SI-186 and SI-187 were re-established at Monash University Immunology and Stem Cell Laboratories. HD-negative female control hESC lines HES2 and HES3 were derived previously from human blastocysts (Reubinoff *et al,* 2000).

Culture of hESC lines

All hESC lines were cultured as colonies in Falcon centre-well organ culture dishes (BD Biosciences, USA) in 1 ml of human embryonic stem cell (HES) medium. Base stocks of HES medium were made by combining 400 ml knockout Dulbecco's modified Eagle's medium (DMEM), 100 ml knockout serum replacement, and 5 ml nonessential amino acids (GIBCO-Invitrogen). A more complex medium for routine stem cell culture included the following supplements: Glutamax (1.9 ng/ mol), (5-mercaptoethanol (0.1 mg/mol), penicillin (24.1 units/ ml), streptomycin (24.1 ug/ml), insulin-transferrin-selenium-X (ITS-X; insulin at 9.65 ug/1, transferrin at 5.3 ug/1 and selenium at 0.00646 u,g/l) and basic fibroblast growth factor (bFGF; 4 ng/ ml). All components were purchased from Gibco-Invitrogen, Australia. The HES3 line was grown on mouse embryonic fibroblasts, which were derived from MTK/NEO CD1 mice, Monash University Physiology Department, whereas HES2, SI-186 and SI-187 lines were grown on human mammary fibroblast feeder cells (CCD919SK, ATCC Corporation). Feeder cells were metabolically inactivated by mitomycin C (10 (ug/ml) treatment for 3-4 min and plated at a density of 1.65×10^5 cells/cm^2. hESC were cultured for 7 days and colonies were mechanically passaged by cutting pieces of approximately 0.5—1.0 mm^2 and seeding 8-12 pieces onto fresh organ culture dishes, marking a single passage. The 'centre button' of colonies and other regions which displayed obvious signs of spontaneous differentiation were excluded from mechanical passaging. Colonies were grown at 37°C in 5% $C0_2$/air and HES medium was changed daily.

Neural differentiation

Neural differentiation was achieved by treatment of hESC colonies with 500 ng/ml noggin (kindly gifted from Green Chemistry, Monash University) and transfer of treated colony pieces to 96-well plates containing specific culture medium to generate neurospheres, according to protocols previously established (Pera *et al,* 2004). A proportion of neurospheres was plated and cultured for 3 days in the absence of specific growth factors on combined poly-D-lysine-coated (1:100; Sigma, MO, USA) and fibronectin-coated (1:100; Sigma) wells and immunostained for the early neuroectoderm marker PAX6 (Pera *et al.* 2004). The remaining neurospheres were differentiated in the presence of specific growth factors by plating on laminin-coated wells to produce primary neurones or plating on fibronectin-coated wells to produce astrocytes (Reubinoff *et*

al, 2001). Immunostaining for P-III-tubulin and microtubule-associated protein 2ab (MAP2ab) was used to identify primary neurones, whereas immunostaining for glial fibrillary acidic protein (GFAP) was used to identify astrocytes.

Immunostaining for neural markers

Cells were fixed for 15 min on ice using 4% paraformaldehyde in phosphate-buffered saline containing calcium and magnesium (PBS+; Invitrogen). Cells were then permeabilized with washes of PBT (1% Triton X-100/PBS+), blocked for 1 h at room temperature in 10% fetal calf serum/PBT (Invitrogen), and then incubated with primary antibodies overnight at 4°C. Mouse monoclonal anti-PAX6 immunoglobulin (Ig)G primary antibody (1:50; Developmental Studies Hybridoma Bank, USA) was used for PAX6 neurosphere staining. Primary neurones were simultaneously stained with rabbit anti-p-III-tubulin antibodies (1:500; Chemicon, USA) and mouse anti-MAP2ab antibodies (1:200; Lab Vision Corporation, USA). Astrocytes were stained with rabbit anti-GFAP antibodies (1:500; Dako, Denmark). Isotype-matched antibodies were used as negative controls. Secondary antibodies, either Alexa Fluor goat anti-mouse IgM (568) (1:500; Invitrogen) or Alexa Fluor goat anti-rabbit IgG (488) (1:1000; Invitrogen), were applied for 1 h at room temperature. Slides were then washed and cell nuclei were stained with 4,(6-diamidino-2-phenylindole) (1:1000; Sigma). Coverslips were placed onto glass slides with Vectashield mounting medium (Australian Lab Services, Australia) and sealed with nail polish. Bound fluorescence was detected using an Olympus BX51 microscope and ULH100HG fluorescence system with appropriate filters for each fluorescence wavelength. Slides were photographed using a DP70 camera.

Flow-activated cell sorting

Colonies of hESC were harvested from organ culture dishes and placed in TrypLE Express (Invitrogen) at 37°C for 10 min to generate a disassociated cell suspension. Samples were resuspended in PBS- (containing no calcium or magnesium; Invitrogen) and divided into three aliquots. Aliquot 1 was stained with a GCTM-2 IgM primary monoclonal antibody (Pera *et al.*, 1988), aliquot 2 was stained with a matched IgM negative control antibody (1:125; BD Biosciences) and aliquot 3 was left unstained. All three aliquots were left at 4°C for 30 min. Aliquots were then resuspended in 10% normal goat serum/HES medium, and Alexa Fluor goat anti-mouse IgM (647) (1:500; Invitrogen) was added to aliquots 1 and 2. Aliquots were left for 30 min at 4°C, protected from light. Aliquot 3 was resuspended in PBS+, while aliquots 1 and 2 were resuspended in propidium iodide (PI; 1:2000; Sigma). Cells were sorted using a FG500 flow cytometer, (Cytomics FC500; Beckman Coulter, USA).; Sorting was performed for a Pi-negative population and GCTM-2-positive population, with the threshold set to sort five cells directly into 200 ul polymerase chain reaction (PCR) tubes containing 2.5 ul lysis buffer (400 mmol/1 KOH, 100 mmol/1 dithiothreitol). Sorting was also conducted on neurosphere cells, stained with PI (1:2000). The Pi-negative population was directly sorted into 200 ul PCR tubes containing 2.5 (ul lysis buffer, with the threshold set for one cell per tube. Samples were frozen at -80°C prior to PCR analysis.

Purification of genomic DNA

Genomic DNA (gDNA) from each hESC line was extracted and purified from 15-25 colonies using a DNeasy Tissue Kit (Qiagen, Germany). The yield of gDNA was determined by UV spectrometry using a NanoDrop spectrometer (NanoDrop Spectrophotometer, ND-1000; BioLab, Australia).

Fluorescent PCR

The CAG repeat region in exon 1 of the *HTT* gene was amplified by fluorescence-PCR (F-PCR) using HU3 and HU4 primers (Sermon *et al.*, 1995). The HU4 sense primer was labelled with 6-carboxyfluorescein (6-FAM; Applied Biosystems, USA). Each F-PCR reaction master mix (total volume 45 ul) comprised 27.6 ul H_20, 10 ul GC Rich Solution, 5 ul l0x $MgCl_2$ buffer, 0.4 ul Fast Start *Taq* Polymerase, 1 ul dNTP (Fast Start *Taq* Polymerase Kit; Roche Diagnostics, Australia) and 1 ul of a 20 pmol/1 HU3 and HU4 primer solution. The DNA template in the F-PCR reaction was either 1 ul gDNA (=200 ng) or fluorescence-activated cell sorted (FACS) samples. FACS cells collected in 2.5 ul lysis buffer were prepared by heating at 65°C for 10 min and then neutralized with 2.5 ul neutralization buffer (0.27 mol/ml Tris-HCl, 0.11 mol/ml KC1, 1 mol/ml HC1). F-PCR thermocycling conditions were as follows: reactions were initiated with one cycle at 95°C for 3 min, followed by 50 cycles at 95°C for 45 s, 61 °C for 60 s, 72°C for 90 s, and they were terminated with one cycle at 72°C for 5 min. Aliquots (10 ul) of the F-PCR reaction products were analysed on 2% ethidium bromide agarose gels to visualize amplified DNA products.

Calculation of CAG repeat numbers

F-PCR reactions were also analysed on an ABI Prism 3100 DNA Sequencer (Applied Biosystems), Following Genescan software analysis (Applied .Biosystems), labelled F-PCR product fragment sizes were automatically calculated using the internal 6-carboxy-X-rhodamine (ROX) size standards. The number of the CAG repeats (A) was then calculated using the formula A = (o-flanking conserved regions)/3, where 5 = F-PCR product length.

Reverse transcription PCR

Total RNA was extracted from 15-20 hESC colonies using the RNeasy Mini Kit (Qiagen) following the protocols recommended by the manufacturer. RNA samples (80 ng) were converted to cDNA in two separate reactions, one containing reverse transcriptase (RT+) the other without reverse transcriptase (RT-), as per manufacturer guidelines (Superscript III First Strand Synthesis System for RT-PCR; Invitrogen). A2 ul aliquot (10%) of the cDNA product from RT+ and RT- reactions was subjected to PCR as described above, and reaction products were separated on 296 ethidium bromide agarose gels.

Western blotting

Approximately 60 hESC colonies from each line were centrifuged at 15,500 *g* for 10 min at 4°C and resuspended in 30 ul radio-immunoprecipitation assay (RIPA) buffer (98.4%

PBS- 1% Nonidet P-40/Igepal CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS)), and 3 ul protease inhibitors (Roche EDTA-free Complete Inhibitor; Roche Diagnostics) was added immediately. Samples were sonicated and incubated for 45 min on ice. Following centrifugation at 15,500 *g* for 10 rnin at 4°C, the supernatants were collected. A BCA Protein Assay (Thermo Scientific, Australia) estimated sample protein concentrations and 100 ug total protein was mixed with an equal volume of 2x sample buffer (0.125 mol/l Tris-HCl, 4% SDS, 20% v/v glycerol, 0.2 mol/l dithiothreitol, 0.02% bromophenol blue, pH 6.8) and then denatured at 98°C for 5 min. Samples were loaded onto a 3-8% gradient polyacrylamide Tris-acetate gel (Invitrogen) and electrophoresed for 2 h at 150 V at 4°C.

The polyacrylamide Tris-acetate gel was then placed in contact with a nitrocellulose membrane (Protran 0.45 um pore size; Whatman, Schleicher and Schuell BioScience, Germany) and blotting was performed for 2 h at 250 V at 4°C. The nitrocellulose membrane was blocked in blocking buffer'(Tris-buffered saline + 5% low fat milk powder) for 1 h. and incubated overnight at 4°C with a 1:20,000 dilution of anti-mouse 1C2 antibody (Chemicon) that only binds to disease-length polyQ tracts (Trottier *et al.*, 1995). A 1:5000 dilution of horseradish-peroxidase-conjugated secondary antibody (Chemicon) was applied for 1 h at room temperature to detect bound 1C2 antibody and conjugates were revealed by enzymatic chemiluminescence (ECL+; GE Healthcare, Australia).

Results

Molecular characterization of HD hESC lines

HD hESC lines SI-186 and SI-187 were re-established at Monash University Immunology and Stem Cell Laboratories on CCD feeder cells at passages 24 and 4, respectively. All studies were performed on hESC colonies from relatively early passages (SI-187, passages 6-28; SI-186, passages 24-44; hES2/3, passages 28-64). Karyotypes were firstly assessed in lines SI-186 and SI-187; to determine whether chromosome abnormalities had occurred in early undifferentiated cultures. At passage 6 all 20 SI-187 cells analysed had a normal female 46,XX karyotype. For SI-186 however, at passage 29, 6/16 cells, tested had a normal female 46,XX karyotype whereas the remaining 10 cells were trisomic for chromosome 12 (data not shown). Despite differences in karyotype, SI-186 and SI-187 colonies displayed normal stem cell morphology and exhibited similar growth rates following continuous passage. Control HES2 and HES3 lines exhibited normal 46,XX female karyotypes.

Using F-PCR and Genescan analysis, all hESC lines and the CCD feeder cells were genotyped at the *HTT* locus to confirm their allelic CAG repeat sizes (Figure 1), As expected, SI-186 ($CAG_{37/15}$) and SI-187 ($CAG_{51/19}$) were heterozygous carrying both a wild-type and a mutant allele. The SI-187 genotype at

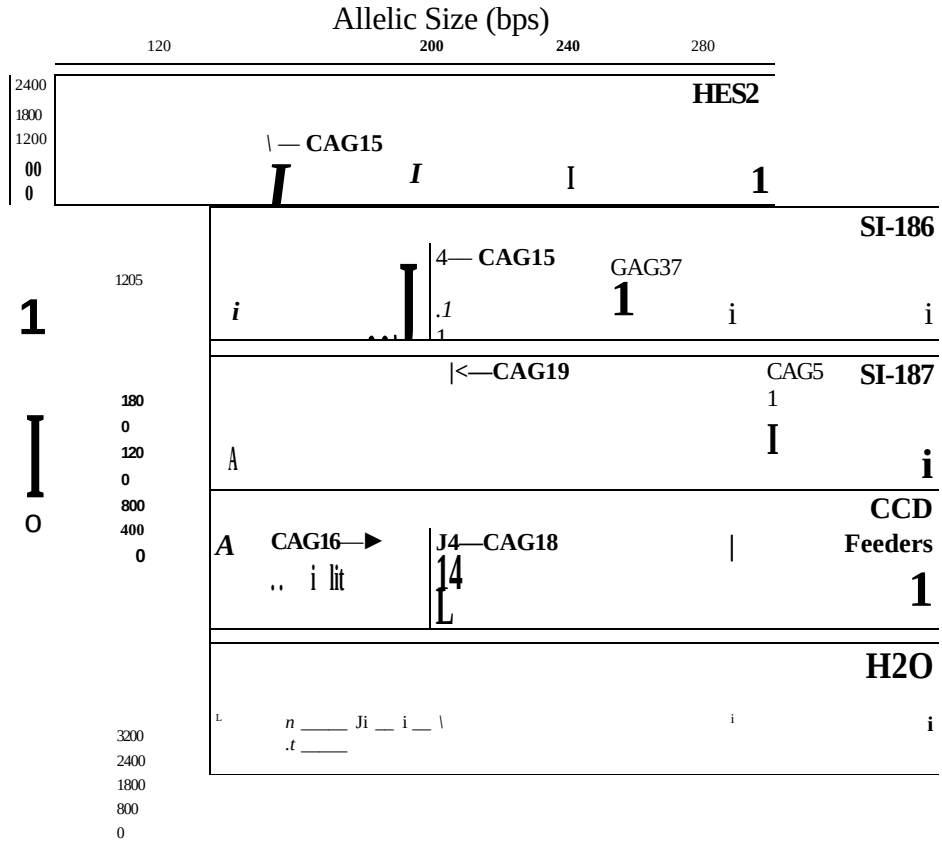


Figure 1. Genescan fluorescence polymerase chain reaction (F-PCR) product readouts. Alleles are represented by a series of open peaks of decreasing intensity to the left, with the true allele represented by the rightmost, peak (arrow); remainder peaks represent artificial stutter alleles. CAG repeat numbers labelled on arrows were calculated from F-PCR product lengths. Closed peaks common to all profiles indicate Genescan internal standards; bps = base pairs.

the *HTT* locus was identical to that of the original IVF embryo. The genotype of SI-186 at the *HTT* locus was almost identical to the original IVF embryo; varying by one CAG repeat: The minor discrepancy between the CAG repeat lengths between the original embryo and resulting stem cell line was probably due to different Genescan sizing methods used by the two laboratories. The wild-type status of CCD feeder cells, ($CAG_{I6/18}$) and HES2 ($CAG_{15/15}$) and HES3 ($CAG_{15/15}$) control lines was also confirmed. Apart from the normal allelic stutter patterns there was no evidence in the SI-186 or S1-187 Genescan profiles of variability in the fluorescence peaks representing the mutant alleles, indicating CAG repeat stability.

Neural differentiation of HD hESC lines

Differentiation of the HD lines SI-186 and SI-187 was assessed by culturing cells in standard neural differentiation conditions. Neural differentiation via neurosphere intermediates was specifically directed towards the formation of mature neurones and astrocytes that are known to be associated with HD pathology. Both SI-186 and SI-87 lines; were capable of producing spherical neurospheres of similar morphological quality to those formed by the wild-type lines HES2/HES3 **(Figure 2A-C);** Under the light microscope, neurospheres derived from SI-186; SI-187and wild-type HES2/HES3 lines displayed characteristic 'rosette' structures **(Figure 2A-C).** In addition SI-187 and HES2 neurospheres stained positive for the expression of PAX6, an early neuroectodermal marker **(Figure** 2D, G). SI-186 and wild-type-derived neurospheres plated on a laminin substrate with specific growth factors formed primary neurones that stained positive for p-III-tubulin and MAP2ab **(Figure 3).** Levels and spatial distribution of P-III-tubulin and MAP2ab staining were similar between the mutant and wild-type lines **(Figure** 3C, G). SI-186 and HE52 neurospheres plated on a fibronectin substrate with specific growth factors generated GFAP-positive astrocytes of similar morphology and number **(Figure 4).**

Expression of the *HTT* gene

The transcriptional status of the *HTT* gene was assessed by qualitative RT-PCR and gel electrophoresis **(Figure** 5A). Both SI-186 and SI-187 RT+ reactions produced two distinct bands of the expected sizes: one representing the mutant allele and the other representing the wild-type allele, indicating transcription of the *HTT* gene in undifferentiated cultures. Control hESC lines HES2 and HES3 and the CCD feeders produced a single band, representing similar-sized wild-type alleles and demonstrating expression of only wild-type transcripts. Western blot assays were used to further evaluate expression of the mutant HTT protein. Total soluble protein fractions separated using gel electrophoresis were probed with the 1C2 antibody which only reacts with proteins containing expanded polyQ tracts. Consequently, only the mutant length HTT was detectable. SI-187 undifferentiated cells produced a strong band at the expected size of 360 kDa, indicating expression of mutant HTT protein **(Figure 5B).** In preliminary studies, slot blot filter retardation assays (Scherzinger *et ah,* 1997) using the 1C2 antibody were unable to detect the presence of insoluble HTT aggregates in undifferentiated SI-186 or SI-187 cells (data not shown).

Stability of the mutant CAG repeat allele

The 200 ng of starting DNA template used for genotyping **(Figure 1)** may mask detection of any low-level CAG repeat instability. Consequently, small pool F-PCR (SP F-PCR) was conducted on undifferentiated SI-186 cells (31 pools of five cells) and SI-187 cells (27 pools of five cells). No changes at the single cell level were observed in the wild-type or mutant allele CAG repeat lengths in either undifferentiated cell line **(Figure 6A, B).** CAG repeat stability was assessed further in SI-186 neurosphere cells using SP F-PCR. Of six pooled samples analysed, one pool displayed a six CAG repeat expansion (data not shown) and a second pool displayed a five CAG repeat expansion in the mutant allele **(Figure 6C).**

Discussion

This report describes what is thought to be the first characterization studies of two HD hESC- lines derived from affected PGD embryos. Both lines were shown to express the *HTT* gene at the mRNA and protein levels, and thus have the genetic potential to develop HD pathology *in vitro.* In addition, the HD hESC lines were capable of neuronal differentiation into two neural lineages that are known to be affected by the disease process. Chromosomal analysis demonstrated a normal female karyotype (46,XX) for the fully penetrant SI-187 line and a mosaic female karyotype (46,XX, 47,XX +12) for the partially penetrant SI-186 line. Trisomy 12 is a recurrent abnormality in hESCs which is believed to provide a selective growth advantage *in vitro* (Draper *et al.,* 2004). Nevertheless, both SI-186 and SI-187 undifferentiated colonies displayed stem cell morphologies similar to those of the well-characterized control lines HES2 and HES3 (Reubinoff *et al.,* 2000). Further, the karyotypic abnormality in the SI-186 line did not hinder neural differentiation capabilities; however, investigations involving SI-186 beyond this initial characterization will require the derivation of a euploid clonal line.

The capacity of different hESC lines to undergo neural differentiation as a default pathway is well recognized (Reubinoff *et al.,* 2001). In a recent study of neural differentiation (Wu *et al.,* 2007), variation in the efficiency of neurosphere formation, quality and rosette formation was observed across a number of independently isolated hESC lines. These observations suggest inherent pluripotency differences exist between individual hESC lines; and may originate from different genetic and epigenetic backgrounds, the quality of the original blastocyst from which a line was derived, the stem cell protocol used, variation in fibroblast feeder layer cells or even extended passage. Importantly in this study, both HD hESC lines produced neurospheres of similar morphology (spherical and containing rosettes) to that of the wild-type HES2 and HES3 lines, and positive staining was observed for the neuroectodermal maker PAX6. Further, the SI-186 line produced primary neurones and astrocytes at a similar frequency to the control HES3 line. These findings suggest that the HD mutation does not prevent HD hESC lines from undergoing induced neural differentiation *in vitro.* On the basis of these observations, it is likely that these HD hESC lines will be useful models to generate specific neuronal subtypes; such as GABAergic medium spiny neurones that are susceptible to neurodegeneration or dopaminergic neurones that are more resistant to neurodegeneration. The

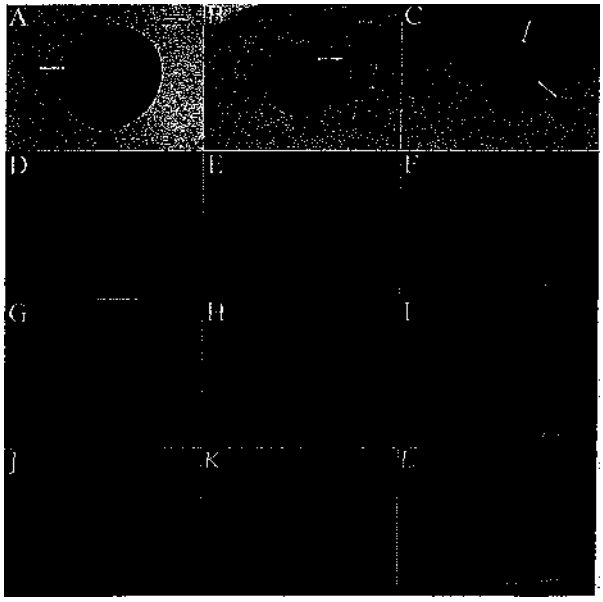


Figure 2. Neurosphere formation. Neurospheres generated in suspension from the wild-type HES2 (A), Huntington disease (HD) SI-186 (B), and HD SI-187 lines (C), containing rosettes (arrows). Pax-6 immunostaining (red) detected neuroectodermal cells in HD SI-187 (D) and wild-type (G) plated neurospheres, with corresponding 4,(6-diamidino-2-phenylindole) (blue) staining (E, H) and overlays (F, I). (J-L) Isotype negative controls. For images A-C, bar = 200 urn, for D-L, bar = 2 mm.

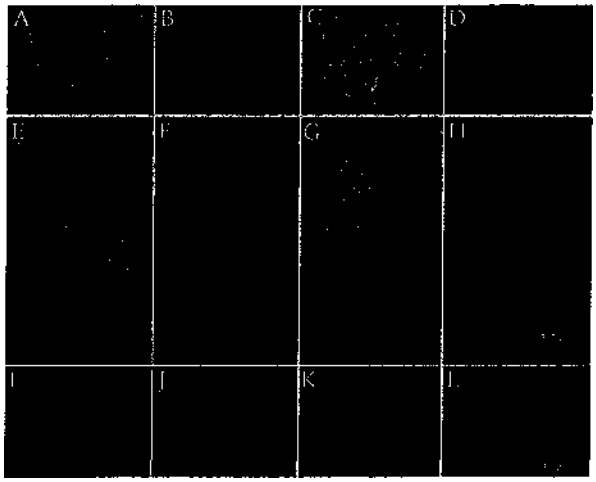


Figure 3. Neuronal differentiation. Neural-directed wild-type HES3 (A-D) and Huntington disease (HD) SI-186 (E-H) plated neurospheres immunostained to detect primary neurones using p-III-tubulin (green) and microtubule-associated protein 2ab (MAP2ab) (red), and nuclei stained using 4,(6-diamidino-2-phenylindole) (blue). (C, G) Overlays illustrate the co-localization of MAP2ab and (3-III-tubulin, within primary neurones. (I-L) Negative controls. For images A-H, K, L, bar = 200 um, for I, J, bar = 500 um,

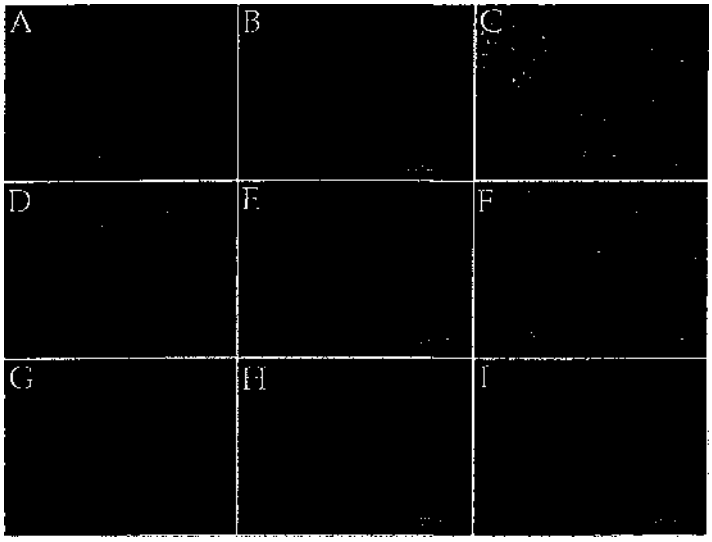


Figure 4. Glial differentiation. Glial-directed wild-type HES3 (A-C) and Huntington disease (HD) SI-186 (D-F) plated neurospheres immunostained to detect astrocytes using glial fibrillary acidic protein (green) and nuclei stained using 4,(6-diamidino-2-phenylindole) (blue). (C, F) Overlays illustrate the cytoplasmic location of GFAP. (G—I) Negative controls. For all images, bar = 200 urn.

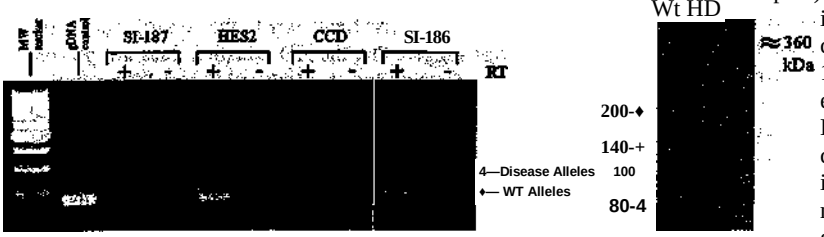


Figure 5. Reverse transcriptase-polymerase chain reaction and western blot. (A) Reverse transcriptase RT+ and RT- *HIT* mRNA samples visualized on a 2% agarose gel. Positions of wild-type (WT; =170 base pairs) and disease (>210 base pairs) alleles are indicated. (B) Western blot chemiluminescence staining using the 1C2 antibody demonstrates exclusive expression of the mutant protein in the Huntington disease (HD) positive SI-187 cells. Molecular mass markers (kDa) are indicated on the left. CCD = human mammary fibroblast feeder cells; gDNA=genomic DNA.

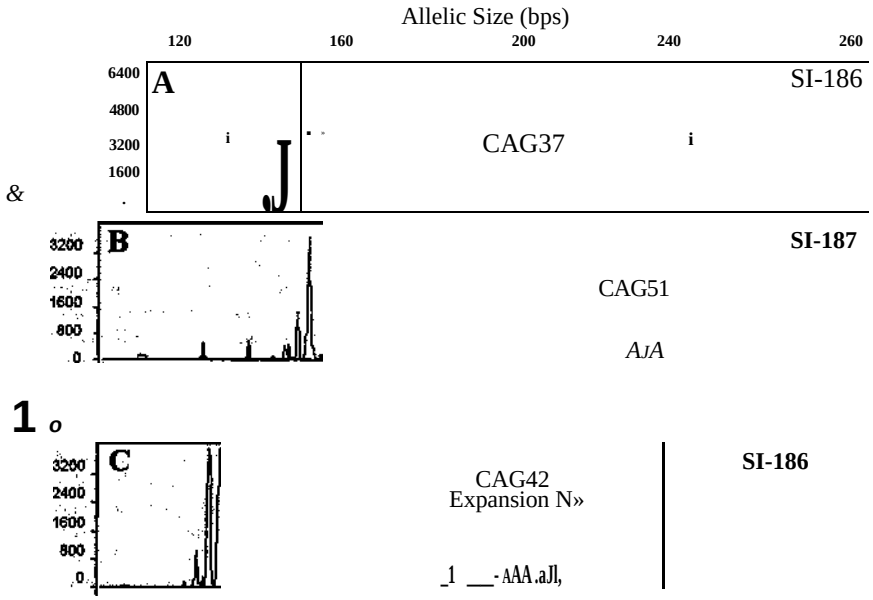


Figure 6. CAG repeat instability assessment. (A) Small pool fluorescent polymerase chain reaction (SP F-PGR) of Stl86 and (B) SI-187 undifferentiated cells shows no change in mutant allele length from progenitor length. (C) SP F-PCR of neurosphere differentiated SI-186 cells detects an expansion of five CAG repeats in the mutant allele. Closed peaks common to all profiles indicate Genescan internal standards. True product sizes are indicated by arrows; bps = base pairs.

ability to generate sufficient numbers of these neuronal cell types will provide the basis for detailed functional studies to investigate the mechanism(s) of HD pathogenesis *in vitro.*

Given the progressive nature and late age of onset of clinical HD, it could be argued that the relatively early passages of the undifferentiated HD hESC makes them unlikely to display the major hallmarks of disease pathology such as CAG expansion, transcriptome dysregulation or HTT aggregate formation. In this regard, analysis of SI-186 and SI-187 single cells by SP F-PCR did not detect any variation in their mutant allele CAG repeat tracts. Interestingly, however, when HD hESC were differentiated to neural progenitors, there was a high incidence of minor CAG expansions of the mutant allele at the single-cell level. Although the CAG repeat expansions were short compared with the very large CAG expansions seen in neurones of patients with HD (Kennedy *et al,* 2003; Shelboume *et al,* 2007); this finding indicates that the neural progenitors derived from these HD HESC may be more prone to CAG repeat instability within the *HTT* gene. So far, even though the HT*T* gene was shown to be transcribed and translated in undifferentiated HD hESC, no HTT aggregates have been detected by slot blot analysis. Further studies of neuronal cultures using more sensitive techniques such as electron and confocal microscopy are warranted to establish the conditions that favour the formation of HTT aggregates.

This study provides the first example of two novel in-vitro human stem cell models of HD that will complement existing animal HD models to further the understanding of the pathways that lead to neurodegeneration in human patients. Further investigation of CAG repeat expansion and the identification transcriptional dysregulation and/or aggregate formation will also enable detailed molecular and cellular manipulation of the model to identify the underlying mechanism(s) that initiate and perpetuate these disease pathologies. Due to the innate ability of hESC lines to differentiate into any cell of the body, it will be possible to use these HD stem cell models to address the conundrum as to why some neural cell types are particularly susceptible to apoptosis while other neural and non-central nervous system cells which express mutant *HIT* and form aggregates are resilient to disease. The availability of well-characterized HD hESC in-vitro models, which closely reflect clinical HD pathology, will also allow high-throughput drug screening which is currently precluded in animal models due to high costs. Many novel libraries containing small molecules, peptides or small inhibitory RNAs will in the future be screened to identify those that either reduce or prevent the initiation of HD pathology. By this approach, it will be possible to identify more potent drugs or new therapeutic strategies for human clinical trials.

Acknowledgements

The authors thank Karen Koh, Linh Nguyen and Paolo Carai for the re-establishment of hESC lines and assistance with their growth and maintenance. Jonathan Niclis was supported by an Australian Postgraduate Award PhD scholarship from the Federal Government of Australia.

References

Becher MW, Kotzuk JA, Sharp AH *et al.* 1998 Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and IT 15 CAG triplet repeat length. *Neurobiology of Disease* 4,387-397.

Chen-Plotkin AS, Sadri-Vakili G, Yohrling GJ *et al.* 2006 Decreased association of the transcription factor Spl with genes downregulated in Huntington's disease. *Neurobiology of Disease* 22,233-241.

Davies SW, Turmaine M, Cozens BA *et al.* 1997 Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* 90,537-548.

DiFiglia M, Sapp E, Chase KO *et al.* 1997 Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* **277,**1990-1993. Draper JS, Smith K, GokhaleP *et al.* 2004 Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nature Biotechnology* 22, 53-54. Dunah AW, Jeong H, Griffin A *et al.* 2002 Spl and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science* **296,**2238-2243, Ferrante RJ, Kowall NW, Richardson EP Jr. 1991 Proliferative and degenerative changes in striatal spiny neurons in Huntington's disease: a combined study using the section-Golgi method and calbindin D28k immunocytochemistry. *Journal of Neuroscience* **11,**3877-3887. Garcia-Ramos R, del Val-Fernandez J, Catalan-AIonso MJ *et al,* 2007 Experimental models of Huntington's disease. *Revista de Neurologia* **45,**437-441. Graham RK, Deng Y, Slow EJ *et al.* 2006 Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. *Cell* **125,** 1179-1191. Gutekunst CA, Li SH, Yi H *et al.* 1999 Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. *Journal of Neuroscience* 19,2522-2534. HuangCC, FaberPW; PersichettiF *et al.* 1998 Amyloid formation by: mutant huntingtin: threshold, progressivity and recruitment of normal polyglutamine proteins. *Somatic Cell Molecular Genetics* 24,217-233. Kennedy L, Evans E, Chen CM *et al.* 2003 Dramatic tissue-specific mutation length increases are an early molecular event in Huntington disease pathogenesis. *Human Molecular Genetics* **12,** 3359-3367; Leeflang EP, Zhang L, Tavare S *etal.* 1995 Single sperm analysis of the trinucleotide repeats in the Huntington's disease gene: quantification of the mutation frequency spectrum. *Human Molecular Genetics* 4,1519-1526, Lunkes A, Lindenberg.KS, Ben-HaiemL *et al.* 2002 Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. *Molecular Cell* **10,** 259-269. Mateizel-I; De Temmerman N, Ullmann U *et al.* 2006 Derivation of human embryonic stem cell lines from embryos obtained after IVF and after PGD for monogenic disorders. *Human Reproduction* ***21,*** 503-511., McNeil SM, Novelletto A, Srinidhi J *et al.* 1997 Reduced penetrance of the Huntington's disease mutation. *Human Molecular Genetics* 6,775-779. Pattison LR, Kotter MR, Fraga D, Bonelli RM 2006 Apoptotic cascades as possible targets for inhibiting cell death in Huntington's disease. *Journal of Neurology* **253,**1137-1342. Pera MF, Andrade J, Houssami S *et al.* 2004 Regulation of human embryonic stem cell differentiation by BMP2 and its antagonist noggin. Journal *of Cell Science* **117,**1-12. Pera MF, Blasco-Lafita MJ,Cooper S *et al.* 1988 Analysis of cell-differentiation lineage in human teratomas using new monoclonal antibodies to cytostructural antigens of embryonal carcinoma cells. *Differentiation* 39,139-149. Pickering SJ, Minger SL, Patel M *et al.* 2005 Generation of a human embryonic stem cell line encoding the cystic fibrosis mutation deltaF508, using preimplantation genetic diagnosis. *Reproductive BioMedicine Online* 10,390-397. Reubinoff BE, Itsykon P,.Turetsky T *et al.* 2001 Neural progenitors from human embryonic stem cells. *Nature* **19,**1134-1140. Reubinoff BE, Pera MF, Fong CY *et al.* 2000 Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro. Nature Biotechnology* 18,399-404. Sathasivam K, Hobbs C, Turmaine M *et al.* 1999 Formation of polyglutamine inclusions in non-CNS tissue. *Human Molecular Genetics* 8, 813-822. Scherzinger E, Lurz R, Turmaine M *et al.* 1997 Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates *in vitro* and *in vivo. Cell* 90,549-558.

Sermon K, Goossens V, Seneca S *et al.* 1995 Preimplantation diagnosis for HD: clinical application and analysis of the HD expansion in affected embryos. *Prenatal Diagnosis* **18,**1427-1436.

Shelbourne PF, Keller-McGandy C, Bi WL *et al.* 2007 Triplet repeat mutation length gains correlate with cell-type specific vulnerability in Huntington disease brain. *Human Molecular Genetics* **16,** 1133-1142. Shin JY, Fang ZH, Yu ZX et *al.* 2005 Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. *Journal of Cell Biology* **171,**1001-1012. Sipione S, Cattaneo E 2001 Modeling Huntington's disease in cells, flies and mice. *Molecular Neurobiology* **23,** 21—51. Snell RG, MacMillan JC, Cheadle JP *et al.* 1993;Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease.;*Nature Genetics* 4,393-397. Telenius H, Kremer B, Goldberg YP *et al.* 1994 Somatic and gonadal mosaicism of the Huntington disease gene CAG repeat in brain and sperm. *Nature Genetics* 6,409-414. Telenius H, Kremer HP, Theilmann J *et al.* 1993. Molecular analysis of juvenile Huntington disease: the major influence on (CAG)n repeat length is the sex of the affected parent. *Human Molecular Genetics* 2,1535-1540. The Huntington's Disease Collaborative Research Group; 1993 A hovel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes, Ce// **72,**971-983. Thomas EA 2006 Striatal specificity of gene expression dysregulation in Huntington's Disease. *Journal of Neuroscience Research* 84, 1151-1164. Trottier Y, Lutz Y, Stevanin G *et al.* 1995 Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias.Nature **378,**403-406, VerlinskyY; Strelchenko N, Kukharenko V *et al.* 2006 Repository of human embryonic stem cell lines and development of individual specific lines using stembrid technology. *Reproductive BioMedicine Online* 13, 547-550. Verlinsky Y, Strelchenko N, Rechitsky S *et al.* 2005 Human embryonic stem cell lines with genetic disorders. *Reproductive BioMedicine Online* 10,105-110. Vonsattel JP, Myers RH, Steven TJ *et al.* 1985 Neuropathological classification of Huntington's disease. *Journal of Neuropathology and Experimental Neurology* 44,559-577. Wellington CL, Ellerby LM, Gutekunst CA *et al.* 2002 Caspase cleavage of mutant huntingtin precedes neurodegeneration in Huntington's disease. *Journal of Neuroscience* **22,**7862-7872. Yang, SH, Cheng PH, Banta H *et al.* 2008 Towards a transgenic model of Huntington's disease in a non-human primate. *Nature* **453,** 921-924. Wu H, Xu J, Pang ZP *et al.* 2007 Integrative genomic and functional analyses reveal neuronal subtype differentiation bias in human embryonic stem cell lines. *Proceedings of the National Academy of Sciences of the USA* **104,**13821-13826,

Declaration: The authors report no financial or commercial conflicts of interest.

Received 18 July 2008; refereed 18 August 2008; accepted 16 February 2009.

Reproductive Genetics Institute

56

www.reproductivegenetics.com
E-mail: rgiworld@gmail.com
FAX: 773-871-5221

Director: ***Yury Verlinsky, Ph.D.***

2825 North Halsted St., Chicago, IL 60657

Tel.: 773-472-4900

MEDICAL DIRECTOR
Joe Leigh Simpson. M.D.

PRENATAL GENETICS & FETAL MEDICINE
Norman Ginsberg. M.D.

CYTOGENETICS
Garry KalmanovichB.S.
Zev Zlatopolsky, U.S.
YuryVerlinsky. Ph. D.

CLINICAL AND MOLECULAR GENETICS
Christina M. Lavin, M.S.. C.GC.
Lama Eldahdah, M.S.. C.G.C.
Dana Pauling. M.S.. C.G.C.
Gloria Enriquez, B.S. Lisa Kinsley. M.S.

PREIMPLANTATION MOLECULAR GENETICS
Svetlana Rechinsky. Ph.D Oleg Verlinsky, B.A. Tatiana Sharapova, M.S Irina Barsky, M.S. Julija Sivakava. B.S. Tatsiana Pakhalchuk. M.S. Ekaterina Pomerantsera. Ph. D.

PREIMPLANTATION CYTOGENETICS (F.I.S.H.)
Yuri llkevitch. Ph.D.
Zev Zlatopolsky, B.S.
Irina Kirillova, Ph. D.
Svetlana Lerner M.S.

EXPERIMENTAL EMBRYOLOGY
Nikolai Strelchenko. Ph. D.

ASSISTED REPRODUCTION TECHNOLOGY AND MICROMANIPULATION
Ilan Tur-Kaspa, M.D. Nathan Katz, Ph. D. Nataliya Tkachenko. PH. D. Svetlana Lerner, M.S. Natalie llkevitch, M.S. Georg Wolf, B.S.

TISSUE & STEM CELL BANK
Anver Kuliev, Ph.D. Valeri Koukharenko, Ph. D.

Letter for the Signature of the Signing Official for Certification and Assurance Required for hESC Registry Request -NIH Form 2890.

Sandra Concialdi. R.DMS. *Date*: September 23,2009

NIH Stem Cell Registry:

I hereby certify that the statements in the Request for Human Embryonic Stem Cell Lines to be Approved for Use in NIH Funded Research (NIH Form *2890)*, submitted by Strelchenko Nick, 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, Section 1001).

1 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:

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 1 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.

Director of Reproductive Genetics Institute
Oleg Verlinsky

	Gadbois, Ellen (NIH/OD) (E)
To:	Dean, Betsy (NTH/OD) (E): Marino, Susan (NIH/NINDS) (E)
Subject:	Fw: New hESC Registry Application Request #2009-ACD-006
Date:	Friday, November 20, 2009 12:29:58 PM
Attachments:	RG228 Consent for embryo donation.pdf RG228 Consent for clinical care.pdf RG308 Consent for embryo donation.pdf RG308 Consent for clinical care.pdf

Fyi

From: Oleg Verlinsky <verlin@rcn.com>
To: Gadbois, Ellen (NIH/OD) [E]
Sent: Fri Nov 20 11:45:29 2009
Subject: FW: New hESC Registry Application Request #2009-ACD-006

Dear Mr. Gadons,

Thanks you for your message.

Please find attached the requested documentation, including the following:

(1) Signed, dated consent forms for lines both for clinical care and for donation of the embryos for research.

(2) As for clarification regarding the date(s) of IRB approval of the research protocol and consent form, it was was originally approved on June 28, 2002, reviewed the proposed changes to the Consent form, in IRB Meeting on May 21, 2003, and then finally approved by IRB on March 24, 2004. These are still valid in our Institution, until next modified by IRB.

(3) As can be seen from the attached consent forms above, the donation of the remaining embryos to another couple was the option at the time of consent and was, of course, explained to the embryo donors.

(4) Please note that there is no restriction on transfer or sale of RGI hESC lines outside of RGI, and they have already been supplied both in USA and European countries.

(5) The statement "We further agree that we, our heirs, successors, relatives, representatives and/or agents will not bring any action in law or in equity, or in any administrative setting, related to our participation in this study." in consent form under the Discoveries and Patents section was introduced by RGI Lawyers, in order to avoid possible lawsuits.

We hope the above information is useful for further review by the Working Group.

---- Original Message ------
From: Gadbois. Ellen (NIH/OD) [E]
To: verlin@rcn.com ; nstrelch@wisc.edu ; mail@reproductivegenetics.com
Cc: HESCREGISTRY (NIH/NIDCD); Gadbois, Ellen (NIH/OD) [E]
Sent: Thursday, November 12, 2009 10:10 AM
Subject: RE: New hESC Registry Application Request #2009-ACD-006

Dear Mr. Verlinsky,

Thank you for your two submissions of information about human embryonic stem cell lines on October 14, 2009 (2009-ACD-006 and 2009-ACD-007). The ACD Working Group for Human Embryonic Stem Cell Eligibility Review has conducted an initial review of your submissions.

The Working Group requests that you provide NIH with additional documents that will assist in further review:

(as examples):

- For the donations of the embryos that were used to derive the lines
  - o The signed, dated consent form for clinical care
  - o The signed, dated consent form for donation of the embryos for research Please redact any signatures of patients or other patient identifiers from these documents.

- Clarification regarding the date(s) of IRB approval of the research protocol and consent form. The Brief Description of Supporting Information document states, "This research protocol was originally approved on June 28, 2002 and reapproved on March 24, 2004." Please provide information addressing the continuity in IRB approval of the protocol and consent form. Please provide all dates of approval, the duration for which approval is (or was, from 2002 until the present) valid in your institution, and dates of revision of protocol and/or consent form.

- Any information about whether donation of remaining embryos to another couple was an option at the times of consent for donation of embryos for research, and if so, whether that was explained to the embryo donors.

- Clarification regarding whether there are any restrictions on transfer or sale of these hESC lines to researchers outside of RGI.

- The consent form under the Discoveries and Patents section states, "We further agree that we, our heirs, successors, relatives, representatives and/or agents will not bring any action in law or in equity, or in any administrative setting, related to our participation in this study." Please address the rationale by RGI for this language and any documented evaluation by the RGI IRB regarding this language.

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 in planning further review by the Working Group.

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

Sincerely, Ellen L.
Gadbois

From: HESCREGISTRY (NIH/NIDCD)
Sent: Wednesday, October 14, 2009 7:21 PM
To: verlin@rcn.com; nstrelch@wisc.edu; mail@reproductivegenetics.com
Subject: New hESC Registry Application Request #2009-ACD-006

To: Verlinsky Oleg (Signing Official)
Strelchenko Nick (Submitter)

This is to confirm that the hESC Registry Application request, as detailed below, has just been submitted and is pending review by the Working Group of the Advisory Committee to the Director (ACD). You can expect to hear back from us about the status of your application soon.

While pending review, the name of the stem cell line (Question 6) will appear on the public NIH Human Embryonic Stem Cell Registry for Submitted hESC Lines Pending Review.

After review, your organization name, name of the stem cell line(s) and the method of NIH review (Administrative Review or review by the Working Group of the Advisory Committee to the Director) will be posted on the Web page of hESC Lines Reviewed for NIH Funding Eligibility.

If the cell line is approved for inclusion on the NIH Registry, information entered into Questions 6-9 will be posted on the NIH Human Embryonic Stem Cell Registry for hESC Lines Eligible for NIH Funding.

Note that NIH will not be able to change your selection (Administrative or ACD review) or any of the application data or attachments, in the submitted application. If changes are necessary, you may send an email to the hESC Registry Help Desk requesting that the application be deleted, then you may resubmit, as appropriate.

If you have any questions about the hESC Registry Application process, please contact us.

Thank you,

hESC Registry Help Desk

Female Patient Name Social Security #

Partner's Name -. . . - _Social Security #

Institute for Human Reproduction (IHR)
Han Tur-Kaspa, MD, Director
Norber Gleicher, **MD**

ASSISTED REPRODUCTIVE TECHNOLOGIES PROGRAM Description, Explanation and Informed Consent

We_____ , _____ have been informed that our infertility may be treatable by Assisted Reproductive Technologies (A.R.T.). The members of The Institute for Human Reproduction (MR) staff, including physicians, nurses, procedure staff, clerical and laboratory personnel are known as the ART team. We understand that by signing this form, we evidence our consent to the use, by the ART team, of assisted reproductive technology procedures in connection with our participation in the Assisted Reproductive Technologies Program.

DESCRIPTION AND EXPLANATION OF PROGRAM

We understand that the ART program involves the use of the following procedures:

> Determination by standard infertility tests that we are suitable candidates for ART. These tests may include, but are not limited to blood tests, ultrasound tests, specialized x-rays to view the uterine cavity and sperm function tests.

> Use of "fertility drugs" to cause the development of more than one oocyte at a predictable time. Fertility drugs include clomiphene citrate, human menopausal gonadotropin (Repronex , Pergonal, Humegon), highly purified human follicle stimulating hormone (Metrodin, Fertinex), recombinant human follicle stimulating hormone (Gonal; F, Follistim), leuprolide acetate (Lupron) so called antagonists (Antagon) and human chorionic gonadotropins (Profasi, Novarel).

> Treatment with antibiotics and/or glucocorticoids (steroids), to reduce inflammation and infection.

> Ultrasound examinations, combined with blood tests, to determine the expected time of oocyte maturation (progress of development) and ovulation. The ultrasound exams are performed transvaginally, with exceptions at times for abdominal scanning.

> Collection of oocytes from the Woman's ovary(ies) by placing a needle into the ovary and aspiration of follicular contents using ultrasound guidance. This is done while the woman is sedated (asleep) by an anesthesiologist.

> If applicable, collection and preparation of sperm obtained from the male partner by masturbation.

> Combining sperm and oocytes in an attempt to achieve fertilization in the laboratory (in vitro fertilization) or in the Woman's body (gamete intrafallopian transfer—GIFT). If reduced fertilization is expected or there is a history of fertilization failure, a micromanipulation procedure called Intracytoplasmic Sperm Injection (ICSI) may be performed in an effort to enhance fertilization.

> After fertilization occurs, embryos are allowed to develop in culture for several celt divisions until the

• embryologists and physicians determine that they are suitable for transfer to the woman's uterus or fallopian tubes. In some situations, a micromanipulation technique, called Assisted Zona Hatching (AZH), is performed prior to embryo transfer to increase the likelihood of establishing a pregnancy.

> Transfer of the embryo(s) to the woman's uterus by means of a plastic catheter (tube). In some situations, fertilized oocytes or early cleavage stage embryos are transferred to the fallopian tube(s) using either of two procedures called Zygote Intrafallopian Transfer (ZIFT) or Tubal Embryo Transfer (TET). Either of these two procedures are performed during a laparoscopy, a surgical procedure under general anesthesia.

> Obtaining blood samples, and if indicated, ultrasound examinations several times in the subsequent 8-12 weeks to determine if pregnancy has occurred and is proceeding normally.

> Treatment with progesterone to maintain early pregnancy or with human chorionic gonadotropin (HCG) to support luteal function.

RISKS/ REASONS FOR ADVERSE RESULTS

"Recent studies have suggested that IVF may be associated with an increased risk of having children of low birth weight and with twice the usual risk of birth defects. Even though we believe that these risks are due to the underlying infertility, and not the IVF procedure itself, such a direct cause arid effect relationship can at this point not be ruled out."

Each of us has been informed that neither becoming pregnant nor a successful outcome of the pregnancy can be assured as a result of assisted reproductive technology procedures. We have also been informed that the world wide pregnancy rate following ART is approximately 20%. We have been informed that the practice of medicine is, not an exact science and that no guarantees have been made to us as a result of this procedure. We have also been informed that there are many complex and sometimes unknown factors that may limit pregnancy rates in ART. Some of the known risk factors include:

1. Irritation, redness or swelling may result from the injection of fertility and other medications.
2. Hemorrhage, hematoma and/or infection may result from frequent blood drawing.
3. Medications may cause allergic reactions or anaphylactic shock.
4. Follicles containing oocytes may not develop during the treatment (monitoring) cycle or response to the drug may be inadequate and oocyte retrieval may be canceled.
5. Too many follicles may develop and a condition known as Ovarian Hyperstimulation Syndrome (OHSS) may occur.
6. Pelvic scarring or adhesions and/or technical problems may prevent retrieval of one or more oocytes.
7. Ovulation may occur prior to oocyte retrieval and oocytes may be lost, making obtaining the oocyte(s) impossible.
8. One or more oocytes may not be obtained after attempts to aspirate the follicles.
9. The oocytes may not be viable or mature.
10. Hemorrhage or infection may occur during or after the attempt to aspirate oocytes from follicles.
11. The male partner may not be able to produce a semen sample at the appropriate time or the sample may not be adequate for use.
12. Fertilization of the oocyte(s) may not occur or may occur but be abnormal.
13. Embryo development (cell division, cleavage) may not occur or occur but be abnormal.
14. Embryo transfer to the uterus or fallopian tube(s) may be technically difficult or impossible or medically contraindicated.
15. If transfer occurs, the embryo(s) may not implant and not continue to develop.
16. Administration of progesterone may cause the woman to feel pregnant. Side effects include bloating, nausea, depression, increased appetite, weight gain, fatigue, headache and sleep disorders.
17. If pregnancy occurs, it may result in a multiple pregnancy or an ectopic pregnancy or a pregnancy which ends in miscarriage.
18. If pregnancy and delivery occur, the child or children may be stillborn, have chromosomal abnormalities and/or congenital (birth) defects.
19. Psychological stress may result in anxiety and disappointment.
20. A substantial amount of time and effort is required of participants in the ART program.
21. Many of the standard products used to stimulate the development of follicles and growth of the oocytes/embryos are derived from biologic origins. While these products are manufactured under
    - the strictest guidelines of the Federal Drug Administration, the Center for Human Reproduction cannot be held responsible for unspecified product recalls.
22. The long-term effects of the administration of fertility drugs is not known. The long-term effects of fertility drugs is still being evaluated, including the potential long terms risks of ovarian and breast cancer.
23. The long term effects on children born as a result of the Assisted Reproductive Technologies is not known.
24. Equipment failure, infection and/or human error or other unforeseen circumstances may result in loss of or damage to oocyte(s), sperm and embryo(s). „
25. Unusual circumstances (e.g., lack of personnel due to severe weather conditions) may necessitate the cancellation of any or all parts of an ART cycle.

AGREEMENT AND CONSENT

We understand and agree that, if in the exercise of reasonable medical judgment, the embryologists and physicians involved in the ART Program determine that any of our sperm, oocytes or embryos are non-viable or otherwise not medically suitable for use or embryo transfer, such embryos, sperm, or oocytes will be disposed of in an ethically acceptable manner, according to the Center for Human Reproduction policies and the American Society for Reproductive Medicine ethical standards. We consent to such disposition in the circumstances described.

We have been informed that Intracytoplasmic Sperm Injection (ICSI) and Assisted Zona Hatching (AZH) are specialized procedures that may be indicated in addition to the assisted reproductive technology procedures already described.

We understand that intracytoplasmic sperm injection (ICSI) is a procedure employed when previous cycles of ART have resulted in fertilization failure or a severely reduced fertilization rate or when semen parameters suggest that this may occur during our ART cycle. The procedure involves the isolation of a single sperm cell from the man's semen specimen, followed by microsurgical injection of a single sperm into an oocyte retrieved from the woman. We have been informed that, while ICSI is no longer considered experimental by the American Society for Reproductive Medicine, the long-term effects of the procedure have not been fully evaluated. However, we acknowledge that the procedure is practiced in centers worldwide where the specialized equipment and expertise for the procedure is available and thousands of children have been born as a result of the procedure. We understand that possible adverse effects of the procedure include damage/breakage of the oocyte during the procedure or failure to isolate sperm cells. We understand that the selection of sperm for injection is arbitrary with the theoretical potential for increased risks of chromosomal abnormalities or birth defects, including eventual infertility in offspring. We understand that the use of the procedure cannot completely eliminate the risk of fertilization failure. We further understand that there may be some effects oh the offspring, which at this time cannot be determined.

We have been informed that Assisted Zona Hatching (AZH) is a micromanipulation procedure performed on embryos just prior to transfer to the uterus of the Woman. We are aware that In this procedure, small cuts are made in the outer shell of the embryo (zona pellucida) to enhance the possibility that the embryo will hatch out of its shell and implant in the uterine wall. The procedure may be indicated in situations where there has been failure (failure to conceive) in previous cycles of ART, where there is an elevated follicle stimulating hormone (FSH) level, where the woman is 39 years old or older, or her embryos are of poor quality or the zona pellucida (shell) is thickened. We acknowledge that the procedure has been demonstrated to enhance implantation in some cases and has resulted in many live births worldwide, although the long term risks are still unknown. We understand that one or more embryos may be damaged during the procedure, and that hatching may, theoretically, expose the embryo(s) to invasion by other cells or microorganisms

We understand that, in general, medical history and results of fertility evaluations, obtained prior to the ART procedure, will suggest that ICSI and/or AZH are indicated, but that in some cases, observations made by the ART Team during the cycle itself may cause reevaluation of the need to utilize these techniques. We understand and agree that, unless we have specifically indicated that we will not consent to the use of these procedures and be financially responsible for them, the utilization of these procedures is at the discretion of the ART team. We understand that the costs of these procedures are in addition to the costs of a typical ART cycle and that, if they are employed, we may be financially responsible for the procedure if insurance coverage is not available. We also understand that the procedures are utilized at the discretion of the ART team based on their assessment of medical history and/or observations made during the ART cycle.

consent to the admittance, for the purpose of observation, of other physicians and health care personnel during any medical procedures performed on us during the ART Program. All information obtained during the procedure will be handled confidentially and neither our identity nor specific medical details will be revealed without our consent. We understand that we will receive no compensation for such participation. No personal information may be provided to the media by the Center for Human Reproduction without our consent, which may compromise the confidentiality of our medical record.

Federal law requires that all ART programs report cycle specific data to the Centers for Disease Control (CDC). We understand that to collect these data, it may be necessary for the Center for Human Reproduction to contact us for follow up after completion of our ART cycle. We understand that all personal identifiers submitted with our cycle specific data will be protected under the Federal Privacy Act. However, we further understand that we can elect to not have any personal identifiers reported.

Female. _____ Partner _____ We do **NOT** authorize the use of personal identifiers in cycle specific
Initials Initials data for submission to the Centers for Disease Control.

Female __ Partner_ We authorize the use of personal identifiers in cycle specific data for
Initials Initials submission to the Centers for Disease Control.

Each of us has been informed that, if we should suffer any physical injury as a result of participation in this program, all medical facilities are available for treatment. We understand, however, that we cannot expect to receive from the ART Team, The Center for Human Reproduction, or its employees any reimbursement for hospital expenses or any financial compensation for such injury.

All of our questions regarding the Institute for Human Reproduction consent on Assisted, Reproductive Technologies Program (A.R.T.)have been answered. Each of us has read the consent and acknowledges receipt of a copy of this consent.

Date Signature Female Name - Print

Date Partner Name - Print

As one of the members of The Institute for Human Reproduction, by my signature indicate that the foregoing consent was read, discussed and signed in my presence.
egoing consent was read, discussed

Signature
Date Signature of Witness patient Witness Name -Print /

2/25/06
Date Witness Name - Print

NOTE: If you or your partner are unable to have this consent witnessed by a staff member at IHR or FULLY UNDERSTAND THE CONSENT, please notify the IHR medical staff. We will provide you with further information and a witness. If you wish to sign the consent outside of IHR, please have the consent notarized.

State I, the undersigned, a Notary Public in and for the said County in the

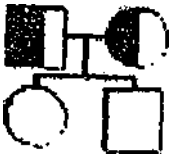
State aforesaid; DO HEREBY CERTIFY that _____
(Female Patient / Partner; personally known to me as the same persons whose names are subscribed to the foregoing document appeared before me this day in persons, and acknowledged that he and she signed, sealed and delivered the said document as his and her free and voluntary, for the use and purposes therein set forth.

ML.

Female Patient Name: . _ Social

Security

Partner's Name: __ , Social Security #:



Institute for Human Reproduction (IHR)Ilan
Tur-Kaspa, M*D,* Director
Norbert Gleicher, MD

ASSISTED REPRODUCTIVE TECHNOLOGIES PROGRAM (A.R.T.)
CRYOPRESERVATION OF HUMAN EMBRYOS

Description, Explanation and Informed Consent

We, _____ understand that cryopreservation (freezing) of human embryos is, a procedure that can be utilized to preserve embryo they may be transferred at a later date. Cryopreservation may be employed in the event that assisted reproductive technology procedures produce more embryos than may be used in a treatment cycle. It can also be employed to preserve embryos for later use if it is determined that, because of unexpected complications; it is not medically advisable to perform an embryo transfer, during the same treatment cycle as the egg retrieval that provided eggs for these procedures. We understand that executing this consent does not guarantee that embryo freezing will be performed unless the special circumstances listed above are met. It is our understanding that cryopreservation, if employed, will be performed by the embryologists at The Center for Human Reproduction.

DESCRIPTION AND EXPLANATION OF THE PROGRAM

We have been informed that to prepare embryos for cryopreservation, embryos must be treated with chemicals known as cryoprotectants. These chemicals include propylene glycol (1,2-propanediol) and glycerol. The type of cryoprotectant used depends upon the developmental stage at which the embryos are cryopreserved.

We have also been informed that embryos can be frozen at various stages of development and that embryos are routinely frozen as zygotes (a single cell with two pronuclei), early cleavage stage embryos (2 to 8 cell embryos) and blastocysts (32-64 cells).

We have been Informed that all cryopreserved embryos will be stored in liquid nitrogen (-196 degrees C) in the laboratories of the Institute for Human Reproduction (IHR) until physicians at IHR determine that conditions are appropriate for transfer of the embryos to the woman's uterus. At that time, one or more of the embryos will be thawed and examined to determine if the embryo(s) have survived (defined as at least 50% of the original embryonic cells remaining intact) the freezing and thawing process. When it is deemed medically appropriate, potentially viable embryos will be transferred to the woman's uterus by means of a plastic catheter (tube). In some situations, transfer of embryos to the uterus through the cervix may be impossible or it may be deemed by the physician that it is warranted to instead transfer the embryos after thaw into the fallopian tubes by means of laparoscopy

We understand we must agree to one of the following options:

Choice A: **We** want to cryopreserve embryos.
We understand there will be a fee (S800.00)due at the time of the in-vitro fertilization retrieval for initial freezing and 3 months of storage. Thereafter, storage fees will be billed quarterly.
**Your cryopreserved (frozen) embryos can at any time be transferred to another IVF program. Should you Instruct us to prepare them for such a transfer, a transfer fee will be assessed which will be due upon pick up of the embryos for transfer.*

Choice B: We do not want to cryopreserve embryos for our own use but would like embryos generated and frozen for anonymous donation to another couple,
There will be no storage or freezing fees due if this option is chosen. It will, however, be necessary that you and your partner have a blood test drawn in 6 months and complete a history/profile form in a timely fashion.

Choice C: We do not want to cryopreserve embryos.

RISKS/REASONS FOR ADVERSE RESULTS

We have been advised that cryopreservation of embryos has been utilized in hundreds of centers in the world where specialized equipment and expertise are available, and that thousands of pregnancies and live births of normal infants have resulted. The potential benefits from this procedure may be an increased chance of pregnancy without the necessity of multiple surgical interventions for oocyte recovery.

We understand that, if we do not consent to cryopreservation and allowing the embryology staff of the Center for Human Reproduction to attempt to fertilize all oocytes, we may be limiting our chances to achieve pregnancy. In allowing for cryopreservation of excess embryos, we may have the opportunity of additional embryo transfers at a later date, if we so choose to proceed with a frozen embryo transfer cycle sometime In the future.

We understand that freezing and long-term storage may result in damage to the embryos including damage to embryonic genetic material, loss of some embryonic cells or loss of viability of the embryo as a whole. We understand that, although thousands of children have been born worldwide as a result of this technology, there may be some effects on the offspring, which, at this time cannot be determined, including risks of chromosomal abnormalities and congenital malformations. The primary concern with the use of cryopreservation is thought to be unspecified cryo-injury; however, according to published reports, cryo-injuries are only blamed for loss of viability during the thaw procedures. The long-term risks associated with human embryo cryopreservation are, however, still unknown at present.

We understand that, as with any technique that requires mechanical support systems, equipment failure can occur. We also note that, while backup freezer systems and/or liquid nitrogen holding facilities are used in an effort to diminish the potential damage, which may be caused by any malfunction, unforeseen situations may occur.

If, after thawing, an embryo does not grow, then that embryo will not be transferred back into the woman's uterine cavity and, instead, will be disposed of in an ethically-accepted manner according to the Center for Human Reproduction Guidelines and the American Society for Reproductive Medicine Ethical Standards.

We further understand that if any of the following situations occur, the embryos will be disposed of in an ethically-accepted manner according to the Center for Human Reproduction Guidelines and the American Society for Reproductive Medicine Ethical Standards:

1. In the exercise of reasonable medical judgment, the embryologists and physicians determine that sperm, oocytes or embryos (either fresh or frozen-thawed) are non-viable or otherwise not medically suitable for use or embryo transfer.

2. If one or more of the cryopreserved embryos remain in cryopreservation after expiration of the three-year period, during which we must direct the use or disposition of cryopreserved embryos (see below for further explanation).

In addition, we agree that the staff of the Center for Human Reproduction is not obligated to transfer these embryos at any point in the future if medical evidence and/or experience indicate that the risk of transfer of frozen embryos outweighs the benefits.

USE OR DISPOSITION OF CRYOPRESERVED EMBRYOS:

We understand that the Center for Human Reproduction limits the term of storage of cryopreserved embryos to three (3) years. We understand that we must redirect the use or disposition of cryopreserved embryos no later than three (3) years after cryopreservation. **We understand that we d<u>o not</u> need to make a selection at this time regarding the use or disposition of cryopreserved embryos and that this may be decided upon at a later date.** During this 3-year period, we have the following options with respect to each cryopreserved embryo:

The thaw and, under medically appropriate circumstances, the **transfer** of these embryos to the uterus of the woman, unless we designate in writing that we wish to continue maintaining these cryopreserved embryos in storage. If we elect to maintain these embryos in storage after the initial three- (3) year period, we must state so in writing and continue to pay for storage fees.

The use of all existing embryos for Institutional Review Board-approved medical **research** according to the Center for Human Reproduction Guidelines and the American Society for Reproductive Medicine Ethical Standards.

We have been advised of the risks and benefits of donating our embryos to research. We also understand that, as of this point, the kind of research our embryos will be used for is unknown.

We acknowledge the fact that our embryos may be used for research which may result in commercial benefits. We specifically acknowledge that our donation of embryos to research may result in economic benefits to IHR, related entities or unrelated entities, which, as of this point, may not be known or are unimaginable. We expressly acknowledge that, should such economic benefit occur, we are not, nor will we under any circumstances, be entitled to any payments, royalties or ownership interests related to our initial donation. We understand that we have the option of reversing our donation at any time until the research begins. However, IHR can make no representation as to when this will be the case. We understand that, if we chose to reverse our decision to donate our embryos to research, we must so designate in writing, accompanied by a notarized witness signature to both (if applicable) of our signatures. We also understand that our decision not to donate embryos to research will in no way affect our care at IHR.

Some embryo research may lead to destruction of embryos, while other research may lead to the establishment of cell lines, which might exist for long time periods or even indefinitely. No embryo, donated to research, will, however, ever be transferred into a woman's uterus.

- The disposal of all existing embryos in an ethically accepted manner according to the Institute for Human Reproduction Guidelines and the American Society for Reproductive Medicine Ethical Standards. We understand that, should we choose this option, we must accompany this written request with a notarized witness signature affixed to both, if applicable of signatures.

- The donation of any/all existing embryos for use by an anonymous or designated recipient in accordance with the regulations and policies in force at the Institute for Human Reproduction at the time of donation. This option is called the Embryo Adoption option.

We have been advised of the risks and benefits of donating our embryos to another couple. We understand that we may rescind our decision at any time until our embryos have been given to another couple. However, IHR. cannot make a representation as to when this will occur. We understand that, should we chose to rescind our donation, notice has to be given in writing, accompanied by a notarized witness signature to both (if applicable) of our signatures. We also understand that our decision not to donate embryos to research will in no way affect our care at IHR

In the event that prior to the expiration of the three-year period, we are no longer participating in the Institute for Human Reproduction Program as a couple and the program has in its possession embryos created by reason of our participation in the program, we hereby agree that the program's disposition of such embryos shall be determined only by applicable terms of any legally binding written agreement between us, signed by each of *us* or on our behalf and delivered to the program.

In the event of divorce, and if embryos are remaining in storage, we acknowledge that by signing this consent, we cannot expect disposition of the embryo custody to be the responsibility of the Institute for Human Reproduction. We fully understand that we retain custody of any existing cryopreserved embryos, and should we pursue dissolution of our marriage, the custody of the embryos shall be decided in a court of law. Institute for Human Reproduction shall abide by the courts ruling.

In the event that one of us dies while the program has in its possession such embryos, the survivor shall have the right to determine use of disposition of such embryos, unless stated otherwise in this consent form.

In the event that both of us die while the program has in its possession such embryos, we hereby designate our wishes by initialing the one option we choose:

_____ _____ Option #1: We wish to donate the embryos to the Institute for Human Reproduction for
Female Partner disposal according to the Center guidelines.

_' _ Option #2: We wish to donate the embryos to Institute for Human Reproduction for
Female Partner research per the Center guidelines.

CHOICE A: We do agree and consent to cryopreservation and, therefore, we have to choose one of the options as stated below:

AGREEMENT AND CONSENT:

- We hereby give consent for cryopreservation of extra embryos resulting from assisted reproductive technology procedures.

- We request that these embryos be stored for subsequent transfer to the female partner's uterus or other such use as is permitted by this consent.

- We understand that embryos not claimed by us within three (3) years after the date of cryopreservation may be disposed of in an ethically acceptable manner.

. We acknowledge that we are financially responsible for the freezing and storage of these embryos and, should we fail to keep this financial account current (within 90 days), the embryos will be disposed of
written notice to us. We agree to disclose such information as is required to determine our financial status and ability to pay for cryopreservation.

- We understand that the fees associated for the cryopreservation of embryos and the storage of these embryos are usually not a covered benefit by an insurance company and will therefore be our responsibility.

We understand that delinquent accounts may be turned over to any attorney or collection agency for collection of delinquent amounts.

We acknowledge it is our responsibility to notify the Institute for Human Reproduction of any change of address.

We do agree and consent to cryopreservation and, therefore, we have to choose one of the options as stated below:

I understand that if this is my choice and I have resulting embryos in excess of the number that is designated to be returned to the female patient's uterus these embryos will be cryopreserved for our future use. I acknowledge that I have received information regarding the approximate cost of such cryopreservation and I am aware that such costs are estimates and may increase at any time. I accept and acknowledge financial responsibility for the freezing and the storage fees of any/all excess embryos.

Option # 1: Have sperm added to ALL retrieved oocytes in an attempt to create fertilization, We agree to cryopreserving any excess embryos.

Option # 2: Have sperm only added to 12 oocytes maximum in an attempt to try and avoid creating excess embryos after an embryo transfer. We agree to cryopreserving any excess embryos.

We have chosen to cryopreserve—(initial the option you both agree to):

Female ___ Partner _____ Option #1. Attempt to fertilize ALL retrieved oocytes.

Female _____ Partner _____ Option #2. Attempt to fertilize up to 12 oocytes.

CHOICE B: We have chosen to allow for cryopreservation of excess embryos NOT for our own use but for the use in IHR's Donor Embryo Program for anonymous donation to another couple. Please initial below.

AGREEMENT AND CONSENT:

- We hereby give consent for the cryopreservation of extra embryos resulting from assisted reproductive technology procedures. ■■
- We request that these embryos be donated to IHR's Embryo Adoption Program for use by an anonymous couple.

  We understand that we are consenting to relinquish all parental rights to these excess embryos.

  We understand that both partners will need to complete a donor profile that discloses family/medical history in accordance with the regulations and policies in force at the Institute for Human Reproduction at the time of donation.
- We understand that both partners will need to have blood tests 6 months after the embryos are cryopreserved in order to complete the donation of the embryos to the Embryo Adoption Program. (IHR will pay for the cost of the laboratory tests.)
- We understand there will be no storage or freezing fees due if this option is chosen and we complete our above stated obligations.

We understand that IHR has the option to dispose of all embryos according to The Institute for Human Reproduction Guidelines and The American Society of Reproductive Medicine Ethical Standards if the embryos are determined not to be acceptable for cryopreservation and/or for the Embryo Adoption Program.

I understand that, if this is my choice, and I have embryos in excess of the number that is designated to be returned to my uterus on the day of embryo transfer, these excess embryos will be cryopreserved. The cryopreserved embryos will be used in accordance to the guidelines of IHR's Embryo Adoption Program for anonymous adoption by fertility patients in the program.

We have chosen to allow cryopreservation of excess embryos for IHR's Embryo Adoption Program. Partners must initial to acknowledge you both agree.

Female Partner

CHOICE C: We do NOT agree or consent to cryopreservation and therefore we have to choose one of the options as stated below. Please initial the option you have chosen.

_____ _____
Female Partner

Option # 1: Have sperm added to only 12 oocytes in an attempt to create fertilization. If there are any excess embryos after my embryo transfer I agree to allow the Institute for Human Reproduction discard these excess embryos according to Center guidelines in an ethical manner.

_____ _____
Female Partner

Option #2: Have sperm added to _____ oocytes (MAXIMUM 6) and then transfer ALL viable embryos to the woman's uterus per the Institute for Human Reproduction guidelines.

All of our questions regarding the Institute for Human Reproduction consent on Assisted Reproductive Technologies Program (A.R.T.) Cryopreservation of Human Embryos have been answered. Each of us has read the consent and acknowledges receipt of a copy of this consent.

2/25/06
Date Signature o*f Female Patient* Female

2/25/06
Date Signature of Partner Partner
Name-Print

As one of the members of The Institute for Human Reproduction, by my signature indicate that the foregoing consent was read, discussed and signed in my presence.

Date Signature of Witness (Female Patient) Witness Name - Print

Date Signature of Witness (Partner) Witness Name - Print

NOTE: If you or your partner are unable to have this consent witnessed by a staff member at IHR or FULLY UNDERSTAND THE CONSENT, please notify the IHR medical staff. We will provide you with further information and a witness. If you wish to sign the consent outside of IHR, please have the consent notarized.

State of Ohio County of Delaware, I, the undersigned, a Notary Public in and for the said County in the

State aforesaid; DO HEREBY CERTIFY that _____ _____
(Female Patient / Partner)

personally known to me as the same persons whose names are subscribed to the foregoing document appeared before me this day in persons, and acknowledged that he and she signed, sealed and delivered the said document as his and her free and voluntary act, for the use and purposes therein set forth.

Given under my hand and official seal this 25 day of February 2006
Commission expires on: 4-25, 2010

Dailene A. Morton Notary
Public-State of Ohio (NOTARY PUBLIC)
My Commission Expires April 25,2010

FINAL-6/28/02
Revised 6/20/03

CONSENT TO DONATE HUMAN EMBRYOS FOR RESEARCH STUDY TO ESTABLISH HUMAN EMBRYONIC STEM CELL LINES

We, the undersigned husband and wife, understand that we are being invited to participate in a medical research study to establish human embryonic stem cell lines. This study is being conducted by the Reproductive Genetics Institute (RGI) and the study protocol has been approved by RGI's Institutional Review Board ORB).

PURPOSE

The purpose of this study is to create a supply of human embryonic stem cells for use in research related to human developmental biology, including research of the mechanisms of genetic expression at the cellular level. It is hoped that this research will provide information that may ultimately lead to new treatments for disease. The establishment of human embryonic stem cell lines is an experimental procedure.

BACKGROUND

Human embryonic stem cells are derived from the inner cells of preimplantation embryos. The embryo at this point in its development is microscopic in size and is made up of less than 300 cells. The scientific value of these cells is that they are .undifferentiated; i.e., they have not yet developed into a cell that has a specific biological purpose. Other stem cell lines have been created by other, researchers using animal embryos and human embryos. Many scientists are researching possible ways to direct the differentiation of stem cells to function as specific human body cells, such as heart tissue, brain tissue, and pancreatic cells etc. If the differentiation of stem cells can be directed, it may be possible to treat diseases using these new cells to replace defective cells in the human body. This treatment concept is known as cell transplantation therapy.

Human embryos are not the only potential source of stem cell lines. Other scientists, in studies unrelated to this research study, have explored, and continue to explore, establishment of stem cell lines using cells taken from umbilical cord samples and from adult marrow cells.

METHODOLOGY ..... _.....

Embryos donated by couples undergoing in vitro fertilization (IVF) will be utilized in this research study to create lines of human embryonic stem cells for further research. Any nontransferred embryos will be used for this study.

Embryos donated to this study will be allowed to develop to the blastocyst stage, which is microscopic in size and is made up of less than 300 cells. Blastocysts are incapable of further development outside of the uterus. The stem cell colonies, once created from the blastocysts will be both frozen and stored for use in research at a later time, or will be directly utilized immediately.

It is anticipated that this study will involve approximately 200 donor couples as study subjects. The duration of the study is indefinite as its goal is to establish stem cell lines for current and future use in research. This study is expected to continue for a minimum of one year and may continue well beyond the initial one-year period. Our participation as a study subject is limited to donation of embryos from this current IVF cycle for which we have no desire for embryo transfer. Our participation in this study will conclude at the time of embryo donation.

RISKS AND BENEFITS

We understand that our participation in this study is limited to donation of any untransferred embryos from this current IVF cycle. We also understand that there are no medical risks to us as participants in this study, and acknowledge that risks and benefits related to the IVF process itself have been disclosed to us. Further, we have been informed and understand that embryos donated for this research will not be available to us for IVF transfer. We have also been informed and understand that embryos donated, as such, for this research will be used exclusively by RGI and will not be transferred to or sold to anyone else.

We also understand that we will receive no direct benefit of any kind as a result of our participation in this study. Potential benefits from this study are limited to benefits which may result from advances in science, including the possibility that research on human embryonic stem cells will lead to cell transplantation therapies or other, new ways to treat disease.

ALTERNATIVES

We have been informed and understand that the following alternatives are available to us for our nontransferred embryos,

1. We can instruct RGI to release our nontransferred embryos to us,
2. We can instruct RGI to allow our nontransferred embryos to degenerate in the laboratory.
3. We can donate our nontransfered embryos to this research study.
4. We can cryopreserve our nontransferred embryos for future transfer.

CONFIDENTIALITY

We understand that the information obtained about us during this study will be treated as confidential and that our identities will not intentionally be revealed without our prior written consent, except as otherwise required by state or federal law or other governmental regulatory authority, such, as the FDA. In addition, we understand that any medical records related to this study may be inspected by RGI's Institutional Review Board.

CONTACT PERSONS

If we have any questions about this study, we may contact Dr. Yury Verlinsky at 773-472-4900. If we have any questions about our rights as participants in this research protocol, we may contact Dr. Ed Feldman, Chairperson of the Institutional Review Board at 773-472-4900.

VOLUNTARY PARTICIPATION

Our participation in this study is purely voluntary. We have received no direct or indirect payment or compensation as an incentive to participate, and we understand that we have no right to payment or compensation for our participation in this study. We also understand that there are no costs to us for our participation in this study.

We understand that we may withdraw from this study by submitting a written request to withdraw to Dr. Yury Verlinsky, Reproductive Genetics Institute, 2825 North Halsted Street, Chicago, Illinois 60657. If at the time our written request to withdraw is received, our donated embryos can be identified and have not already

been cultured for stem cell production, our donated embryos will be withdrawn from this study in accordance with our written direction. We further understand, that as of the date of this consent, our embryos that were donated to this research study from this IVF cycle will no longer be suitable for transfer.

We have been informed and understand that we may discontinue participation in this study without penalty or loss of benefits to which we are otherwise entitled. We have also been informed and understand that discontinuing our participation in this study will not affect ongoing treatment, access to services offered by RGI, or our relationship with RGI.

DISCOVERIES AND PATENTS

By choosing to enroll in this research study, we acknowledge and agree that we will receive no payment of any kind for donation of our embryos and/or for participation in this study. We further acknowledge that RGI may realize monetary or other benefits and awards from its research utilizing our donated embryos and that RGI will be the sole and exclusive owner of any such monetary or other benefits and awards. We further agree that we, bur heirs, successors, relatives, representatives and/or agents have no interest in, and will make no claim to, any monetary or other benefits and awards which RGI may derive, in whole or in part, from use of our donated embryos. We further agree that we, our heirs, successors, relatives, representatives and/or agents will not bring any action in law or in equity, or in any administrative setting, related to our participation in this study.

AGREEMENT TO PARTICIPATE

We have received a copy of this consent form and have carefully read and considered its contents. We have had an opportunity to ask questions about donation of our embryos and about the procedures, risks, potential outcomes and any additional considerations associated with this research study. We acknowledge that all of our questions have been answered to our satisfaction. We further acknowledge that we have had the opportunity to discuss any ethical concerns related to this study and that any ethical concerns have been answered to our satisfaction.

We have been informed and understand that our refusal to participate in this study will involve no penalty or loss of benefits to which we are otherwise entitled.

We have also been informed and understand that our refusal to participate in this study will not affect any ongoing treatment, access to services offered by RGI,or our relationship with RGI

By affixing our signatures, we acknowledge that we have had adequate time to reach our decision; that we voluntarily consent to donate all of our nontransferred embryos, from this current IVF cycle, Month Day Year _ , and we agree to participate in the above-described research study. We acknowledge that the procedures in this study are experimental and that there is no certainty that the results of this research will lead to medical benefits for ourselves or others.

Female Date

Male Partner Date

Printed Names

Identification Confirmed by : _____ signatures notarized)

WITNESS:

: 6/16/06

Date _____

CERTIFICATION I certify that I have consulted with the above-named male and female partner and, to the best of my knowledge, have answered their questions and explained the procedures, benefits, risks, alternatives and costs, if anv, involved in this study to their satisfaction.

Signature_

Title

Date 6/16/06

REPRODUCTIVE GENETICS INSTiTUTE-RGI

CONSENT TO IN VITRO FERTILIZATION (IVF) TREATMENT

NOTE: THIS WRITTEN CONSENT IS AN IMPORTANT DOCUMENT AND THE COPY PROVIDED TO YOU SHOULD BE RETAINED WITH OTHER VITAL RECORDS FOR FUTURE REFERENCE

(Print Female's full name)

(Print Partner's full name)

initial
Female Partner

70 I/We hereby acknowledge that l/we have received the guide for patients to Assisted Reproductive Technologies Booklet ("ASRM ART Booklet"} and have been given ample opportunity to review it. I/We have read and understand the general Information provided in such ART Booklet. I/We have conferred with my/our physician and medical team, during which time we have discussed (1) the risks and benefits of ART treatment, (2) my/our individual medical circumstances and (3) options including non-treatment and/or adoption, arid any questions l/we had were answered. We understand and agree that laboratory procedures will be conducted arid analyzed by Reproductive Genetics Institute ("RGI"). I/We hereby consent to the following procedures:

- OOCYTE (EGG) DEVELOPMENT AND MONITORING

  Stimulated Cycle oocyte (egg) development and monitoring

. TRANSVAGINAL OOCYTE (EGG)RETRIEVAL -

Transvaginal aspiration of my ovarian follicles and isolation of my oocytes (eggs) at a time to be determined by my physician

and

☐ If during the course of performing the retrieval, my/our physician determines that an ovarian cvst(s) , is present, l/we desire that cyst or cysts shall also be aspirated

- SPERM SOURCE Male Partner Fresh and/or Frozen (circle, one or both if applicable)

or

I Anonymous donor

or

Known donor _____
---------- (Name)

INSEMINATION AND CULTURE: MONITORING OF EMBRYO DEVELOPMENT

Initial
Female Partner

I/We agree to attempt to inseminate all viable oocytes

or

If all viable oocytes (eggs) are not to be inseminated, l/we agree to the insemination of _____ oocytes and discarding the remainder not inseminated
(# of oocytes)

- DISPOSITION OF OOCYTES NOT INSEMINATED

Cryopreserve viable oocytes not inseminated, if process is available. A separate consent (Form #34j will be provided

or

Donate viable oocytes not inseminated to appropriate *recipient* (may donate as fresh or frozen). THIS OPTION WILL REQUIRE YOU TO UNDERGO ADDITIONAL MEDICAL, GENETIC AND PSYCHOLOGICAL TESTING. A separate consent (Form #11) will be provided

or

Discard viable oocytes (eggs) not inseminated

or

Donate for research separate consent (Form #32) will be provided

- ADDITIONAL IN VITRO METHODS (to be determined by embryology staff and clinician post retrieval)

Assisted Hatching . _ - .

Intracytoplasmic Sperm Injection (ICSI)

. EMBRYO TRANSFER

Multiple gestations, particularly those involving three or more fetuses, pose significant potential medical risks to the patient, the pregnancy, and any resulting offspring.There is considerable evidence that the rise in multiple births over the last two decades is due in large part to assisted reproductive technologies. Accordingly, after discussion with my/our physician, at the time of transfer, I/We will decide the disposition of my/our embryos. This will be documented in the "Embryo or Gamete Transfer Sheet" signed by the physician and myself at the time of Transfer, if requested by my physician.

Initial
Female Partner

I/We agree to embryo transfer. After discussion with the physician l/we will determine the number of embryos to transfer

- DISPOSITION OF EMBRYOS NOT TRANSFERRED

Initial
Female Partner

☐ Cryopreserve viable embryos, not transferred. A separate consent (Form #2) will be provided

or

Donate embryos not transferred to appropriate recipient. (May donate as fresh or frozen). THIS, OPTION WILL REQUIRE YOU TO UNDERGO ADDITIONAL MEDICAL, GENETIC AND PSYCHOLOGICAL TESTING. A separate consent (Form #10) will be provided

or

Discard viable embryos not transferred. A separate consent (Form #8) will be provided

or

^L Donate for research. A separate consent (Form #32) will be provided

OOCYTES, ZYGOTES AND EMBRYOS DEVELOPING ABNORMALLY

it has been explained to me/us that advances in ART techniques and efforts to ensure quality control have been made by utilizing embryos dividing abnormally and/or discarded oocytes in the laboratory. I/We hereby consent to allow RGI to utilize any embryos dividing abnormally and/or discarded oocytes for these purposes, with the express understanding that none of these materials will be utilized for any other purposes, including the transfer for the sake of establishing a pregnancy. I/We also acknowledge and consent to the fact that any oocytes, zygotes and/or embryos which develop abnormally may also be discarded in accordance with laboratory procedure or may be donated to an IRB approved research project

- HIV (HUMAN IMMUNODEFICIENCY VIRUS) TEST

I/We have been counseled as to the need for, and limitations of, testing for the HIV virus (the. virus that causes AIDS), in signing this consent form, l/we consent to be tested for the HIV virus and to the release of the results to my/our physician at RGI. I also agree to inform the results of the test to my partner.

- RISKS OF PROCEDURES

I/We have been fully advised of the risks and benefits of each of the procedures initialed above, as well as ART generally, and have been informed of the available alternatives and risks and benefits of such alternatives. This Information, which is generally contained in the Assisted Reproductive Technologies Booklet, has been supplemented by my/our consultation with my/our physician and medical team. l/We understand that there are risks associated with pregnancy and especially multiple pregnancy, should it occur, and that my/our treatment during any such pregnancy will be provided by my/our obstetrician.

• FINANCIAL RESPONSIBILITY Financial responsibility for all services and medical treatments provided by RGI. and the physicians and staff, laboratory services and hospital costs associated with medical care, are the sole responsibility of the couple receiving these treatments. In the event that services of an egg (oocyte) donor, or sperm donor, or gestational carrier are necessary in the conduct of the medical therapy, the couple desirous of having the child/children bear the responsibility for the cost of all the services, medications and tests for the donor or carrier. Financial responsibility for the pregnancy and any pregnancy complications (whether of the female partner herself or of the gestational carrier) are the responsibility of the couple.

the clinical and financial staff of RGI will attempt to predict (as best they can) the cost of services before they are rendered, but the costs may vary depending on unforeseen circumstances, insurance company decisions and/or complications of the treatment. RGI reserves the right to change its charges and fees.

The financial staff of RGI works with the couple to determine possible insurance reimbursement for care rendered, but the ultimate responsibility for payment rests with the couple, not their insurance company.

Initial
Female Partner

I/We have each had an opportunity to discuss this information with the staff of RGI and all our questions to date have been answered to our satisfaction

- CLINICAL OUTCOME

l/We have also been fully advised concerning the likelihood of pregnancy and live birth outcomes in ART and understand that any statistics provided to me/us are no more than average rates of clinical outcome of other persons. I/We understand, that the chance that an ART procedure will result in a clinical pregnancy or live birth depends upon many individual factors and that there are not guarantees of success at this, or any other, ART program.

I/We understand that RGI, in keeping with federal regulations, sends data to a national registry supervised by the American Society for Reproductive Medicine, and agrees to permit this data to be verified by an audit. It is possible that at some time in the future, information regarding the treatment of a particular couple would be part of an audit No information would be released to outside agencies except the information required for the completion of the audit. In the future, federal regulations may require that patients be contacted for follow-up information.

I/We agree to the above mentioned treatment and the options l/we have indicated

Female Date 7/13/06

Partner (if applicable): Date 6-9-06

Witness: Date 6-9-06

Date

ure ID corrrirmed by:

■ Consents signed outside the practice must be notarized and dated.

The above mentioned woman/couple has been informed and counseled by me and others regarding the risks and benefits of the relevant treatment options, including non-treatment. The woman/couple appeared capable of understanding the information presented as demonstrated by our discussion and the responsive nature of the participation of the woman/couple.

Physician 6/26/06 Date

FINAL-6/28/02
Revised 6/20/03

CONSENT TO DONATE HUMAN EMBRYOS FOR RESEARCH STUDY TO ESTABLISH HUMAN EMBRYONIC STEM CELL LINES

We, the undersigned husband and wife, understand that we are being invited to participate in a medical research study to establish human embryonic stem cell lines. This study is being conducted by the Reproductive Genetics Institute (RGI) and the study protocol has been approved by RGTs Institutional Review Board (IRB).

PURPOSE

The purpose of this study is to create a supply of human embryonic stem cells for use in research related to human developmental biology, including research of the mechanisms of genetic expression at the cellular level. It is hoped that this research will provide information that may ultimately lead to new treatments for disease. The establishment of human embryonic stem cell lines is an experimental procedure.

BACKGROUND

Human embryonic stem cells are derived from the inner cells of preimplantation embryos. The embryo at this point in its development is microscopic in size and is made up of less than 300 cells. The scientific value of these cells is that they are undifferentiated; i.e., they have not yet developed into a cell that has a specific biological purpose. Other stem cell lines have been created by other researchers using animal embryos and human embryos. Many scientists are researching possible ways to direct the differentiation of stem cells to function as specific human body cells, such as heart tissue, brain tissue, and pancreatic cells etc. If the differentiation of stem cells can be directed, it may be possible to treat diseases using these new cells to replace defective cells in the human body. This treatment concept is known as cell transplantation therapy.

Human embryos are not the only potential source of stem cell lines. Other scientists, in studies unrelated to this research study, have explored, and continue to explore, establishment of stem cell lines using cells taken from umbilical cord samples and from adult marrow cells.

__ METHODOLOGY

Embryos donated by couples undergoing in vitro fertilization (IVF) will be utilized in this research study to create lines of human embryonic stem cells for further research. Any nontransferred embryos will be used for this study.

Embryos donated to this study will be allowed to develop to the blastocyst stage, which is microscopic in size and is made up of less than 300 cells. Blastocysts are incapable of further development outside of the uterus. The stem cell colonies, once created from the blastocysts will be both frozen and stored for use in research at a later time, or will be directly utilized immediately,

It is anticipated that this study will involve approximately 200 donor couples as study subjects. The duration of the study is indefinite as its goal is to establish stem cell lines for current and future use in research. This study is expected to continue for a minimum of one year and may continue well beyond the initial one-year period. Our participation as a study subject is limited to donation of embryos from this current lVF cycle for which
participation in this study will conclude at the time of embryo donation.

RISKS AND BENEFITS

We understand that our participation in this study is limited to donation of any untransferred embryos from this current IVF cycle. We also understand that there are no medical risks to us as participants in this study, and acknowledge that risks and benefits related to the IVF process itself have been disclosed to us. Further, we have been informed and understand that embryos donated for this research will not be available to us for IVF transfer. We have also been informed and understand that embryos donated, as such, for this research will be used exclusively by RGI and will not be transferred to or sold to anyone else.

We also understand that we will receive no direct benefit of any kind as a result of our participation in this study. Potential benefits from this study are limited to benefits which may result from advances in science, including the possibility that research on human embryonic stem cells will lead to cell transplantation therapies or other, new ways to treat disease.

ALTERNATIVES

We have been informed and understand that the following alternatives are available to us for our nontransferred embryos;

1. We can instruct RGI to release our nontransferred embryos to us.
2. We can instruct RGI to allow our nontransferred embryos to degenerate in the laboratory.
3. We can donate our nontransfered embryos to this research study.
4. We can cryopreserve our nontransferred embryos for future transfer.

CONFIDENTIALITY

We understand that the information obtained about us during this study will be treated as confidential and that,our identities will not intentionally be revealed without our prior written consent, except as otherwise required by state or federal law or other governmental regulatory authority, such as the FDA. In addition, we understand that any medical records related to this study may. be inspected by RGI's Institutional Review Board.

CONTACT PERSONS

If we have any questions about this study, we may contact Dr. Yury Verlinsky at 773-472-4900. If we have any questions about our rights as participants in this research protocol, we may contact Dr. Ed Feldman, Chairperson of the Institutional Review Board at 773-472-4900.

VOLUNTARY PARTICIPATION

Our participation in this study is purely voluntary. We have received no direct or indirect payment or compensation as an incentive to participate, and we understand that we nave no right to payment or compensation for our participation in this study. We also understand that there are no costs to us for our participation in this study.

We understand that we may withdraw from this study by submitting a written request to withdraw to Dr. Yury Verlinsky, Reproductive Genetics Institute, 2825 Norm Halsted Street, Chicago, Illinois 60657. If at the time our written request to withdraw is received, our donated embryos can be identified and have not already

been cultured for stem cell production, our donated embryos will be withdrawn from this study in accordance with our written direction. We further understand,.. that as of the date of this consent, our embryos that were donated to this research study from this IVF cycle will no longer be suitable for transfer.

We have been informed and understand that we may discontinue participation in this study without penalty or loss of benefits to which we are otherwise entitled. We have also been informed and understand mat discontinuing our participation in this study will not affect ongoing treatment, access to services offered by RGI, or our relationship with RGI.

DISCOVERIES AND PATENTS

By choosing to enroll in this research study, we acknowledge and agree that we will receive no payment of any kind for donation of our embryos and/or for participation in this study. We further acknowledge that RGI may realize monetary or other benefits and awards from its research utilizing our donated embryos and that RGI will be the sole and exclusive owner of any such monetary of other benefits and awards. We further agree that we, our heirs, successors, relatives; representatives and/or agents have no interest in, and will make no claim to, any monetary or other benefits and awards which RGI may derive, in whole or in part, from use of our donated embryos. We further agree that we, our heirs, successors, relatives, representatives and/or agents will not bring any action in law or hi equity, or in any administrative setting, related to our participation in this study.

AGREEMENT TO PARTICIPATE

We have received a copy of this consent form and have carefully read and considered its contents. We have had an opportunity to ask questions about donation of our embryos and about the procedures, risks, potential outcomes and any additional considerations associated with this research study. We acknowledge that all of our questions have been answered to our satisfaction. We further acknowledge that we have had the opportunity to discuss any ethical concerns related to this study and that any ethical concerns have been answered to our satisfaction.

We have been informed and understand that our refusal to participate in this study will involve no penalty or loss of benefits to which we are otherwise entitled.

We have also been informed and understand that our refusal to participate in this study will not affect any ongoing treatment, access to services offered by RGI, or. our relationship with RGI.

By affixing our signatures, we acknowledge that we have had adequate time to reach our decision; that we voluntarily consent to donate all of our nontransferred embryos, from this current IVF cycle, Month Day Year , and we agree to participate in the above-described research study. We" acknowledge that the procedures in this study are experimental and that there is no certainty that the results of this research will lead to medical benefits for ourselves or others.

7-22-06

Female Partner Date 7-22

Male Partner Date

(or signatures notarize

Printed Names

WITNESS:

Date: 7/22/06

CERTIFICATION

I certify that I have consulted with the above-named male and female partner and, to the best of mv knowledee. have answered their questions and explained the procedures, benefits, risks, alternatives and costs, if anv, involved in this study to their satisfaction.

SignatureTitle

Date 7/22/2006

From: **Oleg** Verlinsky
To: Gadbois. Ellen (NIH/OD) (E)I
Subject: RE: New hESC Registry Application Request #2009-ACD-006
Date: Monday, January 11,2010 6:36:47 PM
Attachments: Image001.png

Hello Ms Gadbois.

RGI's IRB was comprised of individuals absolutely independent of the RGI: two obstetrician (MDs), one clinical geneticist (MD), one internal medicine (MD), one anesthesiologist (MD), one master in genetic counseling, one medical laboratory technologist, one PhD in public health, one PhD in psychology, one master in public relations, one master in industrial relations, one master in nursing, and one Pastor of Lutheran Memorial Church. Affiliations included Northwestern University, University of Illinois, and Illinois Masonic Medical Center. Six of IRB members were women, and only four were from RGI, including genetic counselor, laboratory technologist, a researcher associate and secretary of IRB: Chairman of IRB was Dr. Edwin Feldman, MD (Internal Medicine) who had no any relationship to RGI. Of course, IRB was following the Code of Federal regulations, including Title 45, Public Welfare, and Part 46, Protection of Human Subjects, as well as all relevant local, state and federal laws.

We are working on the table.
Unfortunately in order to get all the clinical consents, we need to order the patient files from Iron Mountain storage. This could take up to a week or more. We will begin the order process and will enter the dates of the clinical consents as we receive the files. We will be able to able to enter some dates from the files in our facility.

To your final point, the patients coming for fertility treatments or PGD treatment make decisions at different times. Some come for IVF only and then decide to do aneuploidy PGD. Others come for Molecular PGD as in case of Cystic fibrosis, and make the decision to donate the affected embryos during the cycle. Usually the results of PGD are available on day 5 after aspiration. Some patients decide to freeze all embryos not transferred and after a successful pregnancy sign a consent to donate the embryos left in cryo storage.

Please feel free to contact me at any time if you have any questions or require clarification of any point.

Oleg Verlinsky

From: Gadbois, Ellen (NIH/OD) [E] [mailto:gadboisel@od.nih.gov]
Sent: Monday, January 11, 2010 11:44 AM
To: 'verlin@rcn.eom'
Cc: HESCREGISTRY (NIH/NIDCD); 'nstrelch@wisc.edu'
Subject: RE: New hESC Registry Application Request #2009-ACD-006

Dear Mr. Verlinsky,

Thank you again for your two submissions to NIH (2009-ACD-006 and 2009-ACD-007) and supplemental materials. The ACD Working Group for Human Embryonic Stem Cell Eligibility Review has further considered these submissions and has several additional requests.

The Working Group would like to have a better understanding of the composition and policies and procedures of the Reproductive Genetics Institute (RGI) Institutional Review Board (IRB). Could you please provide information about the members of the IRB, the relationships between individual IRB members and RGI, and the policies and procedures of the IRB (including whether, and if so, how the IRB follows 45 CFR 46)? In particular, the Working Group would appreciate any information or documentation addressing the independence of the IRB and separation of the IRB's reviews from the scientific investigators at RGI. The Working Group also requests an assurance that RGI is following all relevant local, state and federal law, as appropriate.

The Working Group also requests a table showing, for each of the hESC lines in the RGI submissions; the date of consent for clinical treatment and the date of the consent to donate remaining embryos for research.

Finally, the Working Group would appreciate a description of the, general process for obtaining consent for donations of embryos remaining after preimplantation genetic diagnosis (PGD). In particular, when are the embryos biopsied for PGD and when does the patient provide consent to donate embryos for research?

Documents should be submitted to the NIH hESC Registry mailbox (hescregistry@mailnih.gov). Please redact any signatures of patients or other patient identifiers from these documents.

Please let me know if you have any questions about this request. If you can let me know when you anticipate submitting the documents, that will help for planning further review by the Working Group.

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

From: Oleg Verlinsky [mailto:verlin@rcn.com]
Sent: Tuesday, December 15, 2009 5:25 PM
To: Gadbois, Ellen (NIH/OD) [E]
Subject: RE: New hESC Registry Application Request #2009-ACD-006

Hello Ms. Gadbois

The first protocol and consent was approved in 2002 and we began the project.

We proposed changes on May 21,2003. The final approval of these changes was passed by the IRB on March 24th, 2004. The research continued under the initial approval received in 2002 until the new changes were approved in 2004. The changes were very minor as stated in our previous E-mail, and thus did not affect our ability to do research under the 2002 protocols and consents. This is why it took almost a year to approve the changes; no one was in a hurry.
I want to assure you that at no time we conducted any research without IRB approval..

On your second point, you are correct. Our IRB does not as regular practice conduct annual re-approvals of research protocols, but does receive updates of ongoing research at its meetings.

I will call you on the 17th to help answer any questions you may have; but if you need any other information earlier, please feel free to e-mail or call me at 312-296-2006 cell or 773-472-4900 ext 2146 work.

Sincerely

Oleg Verlinsky.

From: Gadbois, Ellen (NIH/OD) [E] [mailto:gadboisel@od.nih.gov]
Sent: Tuesday, December 15, 2009 3:37 PM
To: 'Oleg Verlinsky'
Cc: HESCREGISTRY (NIH/NIDCD)
Subject: RE: New hESC Registry Application Request #2009-ACD-006

Hello Mr. Verlinsky,

Thank you for your quick reply. I'm not quite sure I understand: can you address whether between May 21,2003 and March 24,2004, the consent form was considered approved by your IRB for use? (Since there was almost a year between the changes being proposed and the approval dates, the Working Group wants to know the status of the protocol and the consent form during that period. I assume that the research was ongoing during that period.)

On the second point, it sounds like your IRB doesn't as a regular practice do annual re-approvals, but does receive updates. Can you confirm that I am understanding this correctly?

Thank you again. If you would like to talk about your submission, I am available between 9-11 a.m. EST. on Thursday December 17.

Sincerely,

Ellen Gadbois

From: Oleg Verlinsky [mailto:verlin@rcn.com]
Sent: Tuesday, December 15, 2009 4:26 PM
To: Gadbois, Ellen (NIH/OD) [E]
Subject: FW: New hESC Registry Application Request #2009-ACD-006

Dear Ms. Gadbois,

Thank you for your message, regarding the dates of approval of the: protocol and consent form. As mentioned previously, the research protocol and consent form was originally approved on June, 2002. The reasons for revision were the required changes; to be included in the process of realization of the project. For example, originally the project was planned to include 60 donor couples, and it was suggested to extend this number to 200 donor couples. In the meantime some wordings were also modified, such as the phrase" normal spare embryos and those found to have genetic abnormalities" was changed to "non-transferred embryos" etc. -overall there were five proposed changes at the IRB Meeting on May 21, 2003, which were then finally approved by IRB on March 24, 2004. As there were no changes required after that, no further revision was needed, so the consent form is still valid. The IRB provided full approval of the project; and since no changes to the protocol or consent forms were made after 2004, no special re-approval was required. IRB was updated on the status of the project at its subsequent meetings.

Should you need any further details let us know.

Oleg Verlinsky

From: Gadbois, Ellen (NIH/OD) [E] [mailto:gadboisel@od.nih.gov]
Sent: Tuesday, December 15, 2009 12:12 PM
To: 'Oleg Verlinsky'
Cc: HESCREGISTRY (NIH/NIDCD)
Subject: RE: New hESC Registry Application Request f2009-ACD-p06

Hello Mr. Verlinsky,

Thank you again for providing the additional documentation. The Working Group requests further clarification on the question regarding the IRB's approval of the protocol and consent.

Can you please verify whether the protocol and consent form were IRB-approved during the period between May 21, 2003 and March 24, 2004, or was there a lapse in IRB approval during that time? Also, has there been annual re-approval since 2004?

If you are able to provide an answer by December 18, 2009; that would be very helpful for the Working Group.

Sincerely, Ellen
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

OSPA

Gadbois, Ellen (NIH/OD) [E]

From: Oleg Verlinsky [verlin@rcn.com]
Sent: Saturday, April 03, 2010 1:28 PM
To: HESCREGISTRY (NIH/NIDCD)
Subject: RE: New hESC Registry Application Request #2009-ACD-006

Hello Miss Gadbois

T

We can try and recontact the donors of the embryos if we can get the current contact information for these patients. As a laboratory, we do not have the contact information, the physician has this information in the patient's clinical chart. We would have to ask the physician to reveal to us the information which the physician may not do due to privilege.

Sincerely

Oleg Verlinsky

From: HESCREGISTRY (NIH/NIDCD) [mailto:hescregistry@mail.nih.gov]
Sent: Thursday, April 01, 2010 12:16 PM
To: verlin@rcn.com
Cc: HESCREGISTRY (NIH/NIDCD); nstrelch@wisc.edu; mail@reproductivegenetics.com
Subject: RE: New hESC Registry Application Request #2009-ACD-006

Hello Mr. Verlinsky,

As we prepare materials for consideration by the full Advisory Committee to the Director in June, one additional question has come up. Can you clarify whether RGI would be able to recontact the donors of the embryos from which the lines listed in these two submissions (2009-ACD-006 and 2009-ACD-007) were derived?

Thank you,
Ellen 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

From: HESCREGISTRY (NIH/NIDCD)
Sent: Wednesday, October 14, 2009 7:21 PM
To: verlin@rcn.com; nstrelch@wisc.edu; mail@reproductivegenetics.com
Subject: New hESC Registry Application Request #2009-ACD-006

To: Verlinsky Oleg (Signing Official)
Strelchenko Nick (Submitter)

This is to confirm that the hESC Registry Application request, as detailed below, has just been submitted and is pending review by the Working Group of the Advisory Committee to the Director (ACD). You can expect to hear back from us about the status of your application soon.