PGD: FROM DIAGNOSIS TO THERAPY

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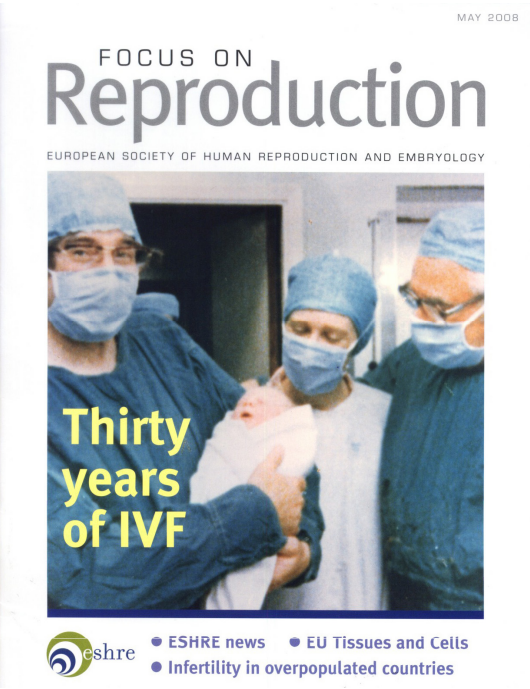
www.sismer.it



Since the birth of the first baby conceived using IVF techniques in 1978 over three million babies have been born worldwide as the result of ART.

The initial goal was treatment of infertility. However, assisted reproduction techniques are no longer used only to help infertile couples, but they have a great significance in the field of therapeutic medicine.

PGD
WHY TO GO FOR IT?

"IVF aims at having a child, PGD aims at having a healthy child and PGD/HLA testing aims at having a healthy and helpful child".

UNESCO's report on preimplantation genetic diagnosis (PGD) and Germ-Line Intervention, 2003.

PGD FOR HLA - MATCHING

- **Healthy embryos are selected for transfer**

avoids the need for termination of an ongoing pregnancy in cases of an affected fetus

- **HLA – matching with an affected child**

Vol. 285 No. 24, June 27, 2001
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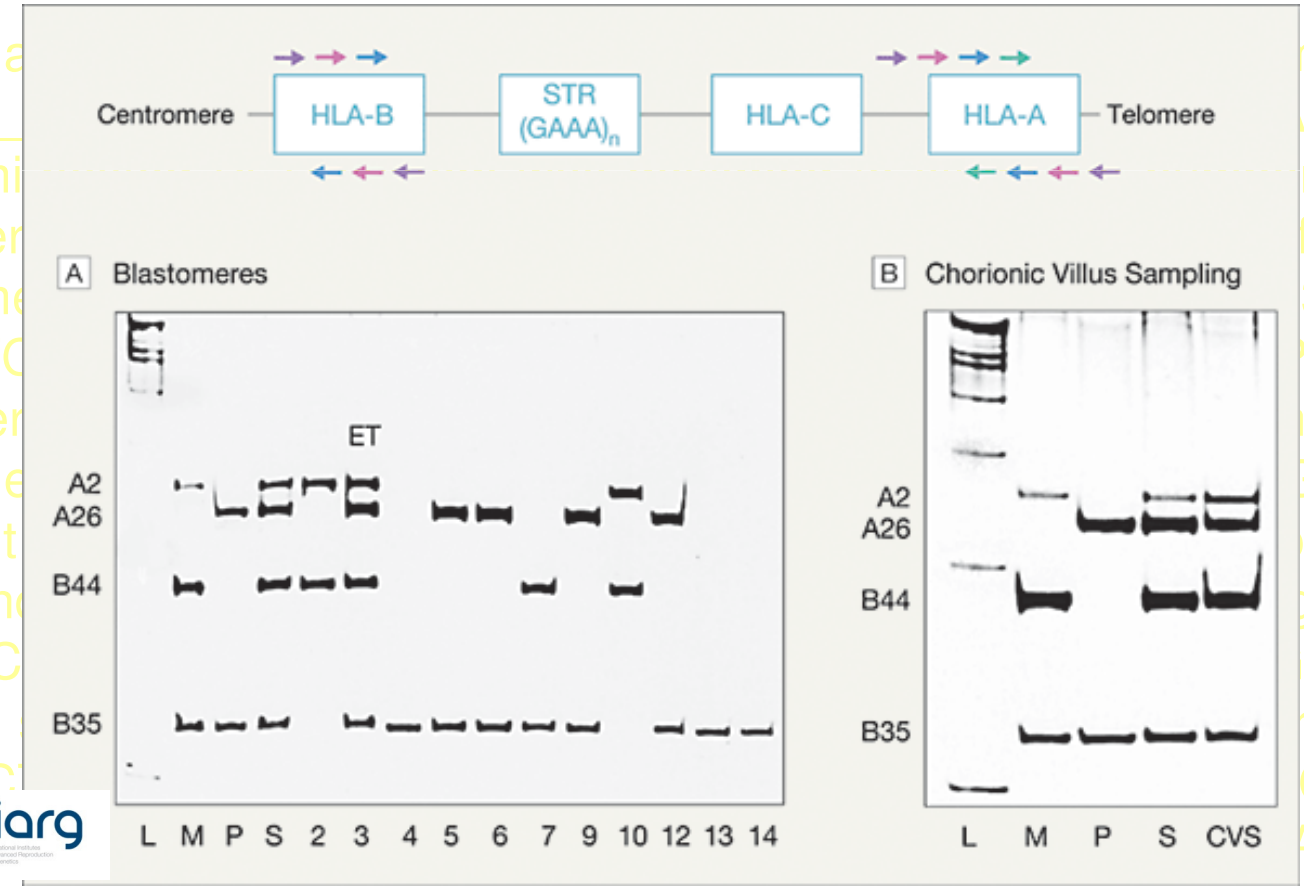
Brief Report

Preimplantation Diagnosis for Fanconi Anemia Combined With HLA Matching

Yury Verlinsky, PhD; Svetlana Rechitsky, PhD; William Schoolcraft, MD; Charles Strom, MD,PhD; Anver Kuliev, MD,PhD

JAMA. 2001;285:3130-3133.



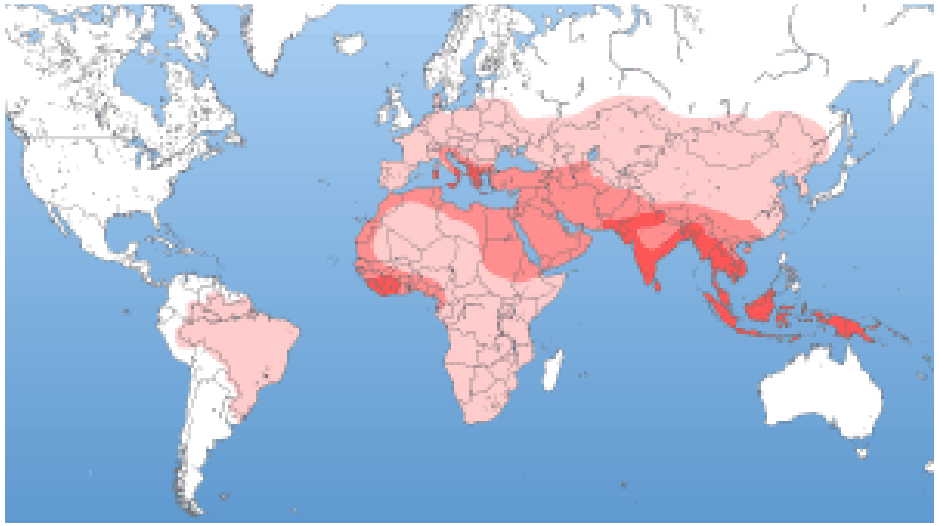
Of 30 embryos tested in 4 IVF attempts, 6 were homozygous affected and 24 were unaffected. Five of these embryos were also found to be HLA-compatible, of which 2 were transferred in the first and 1 in each of the other 3 cycles, resulting in a pregnancy and birth of an unaffected child in the last cycle

INDICATIONS FOR PREIMPLANTATION HLA MATCHING

- **Haematopoietic disorders requiring HLA compatible HSC donor**

  - Thalassemia
  - Fanconi anaemia
  - Wiskott-Aldrich syndrome
  - Diamond-Blackfand Anemia
  - X-linked Hyper IgM Syndrome
  - X-linked adrenoleukodystrophy
  - X-linked Hypohidrotic Ectodermal Dysplasia with immune deficiency
  - Aplastic anemia

- **Diseases like Acute Lymphoid Leukemia, in which HLA matching becomes the primary indication**



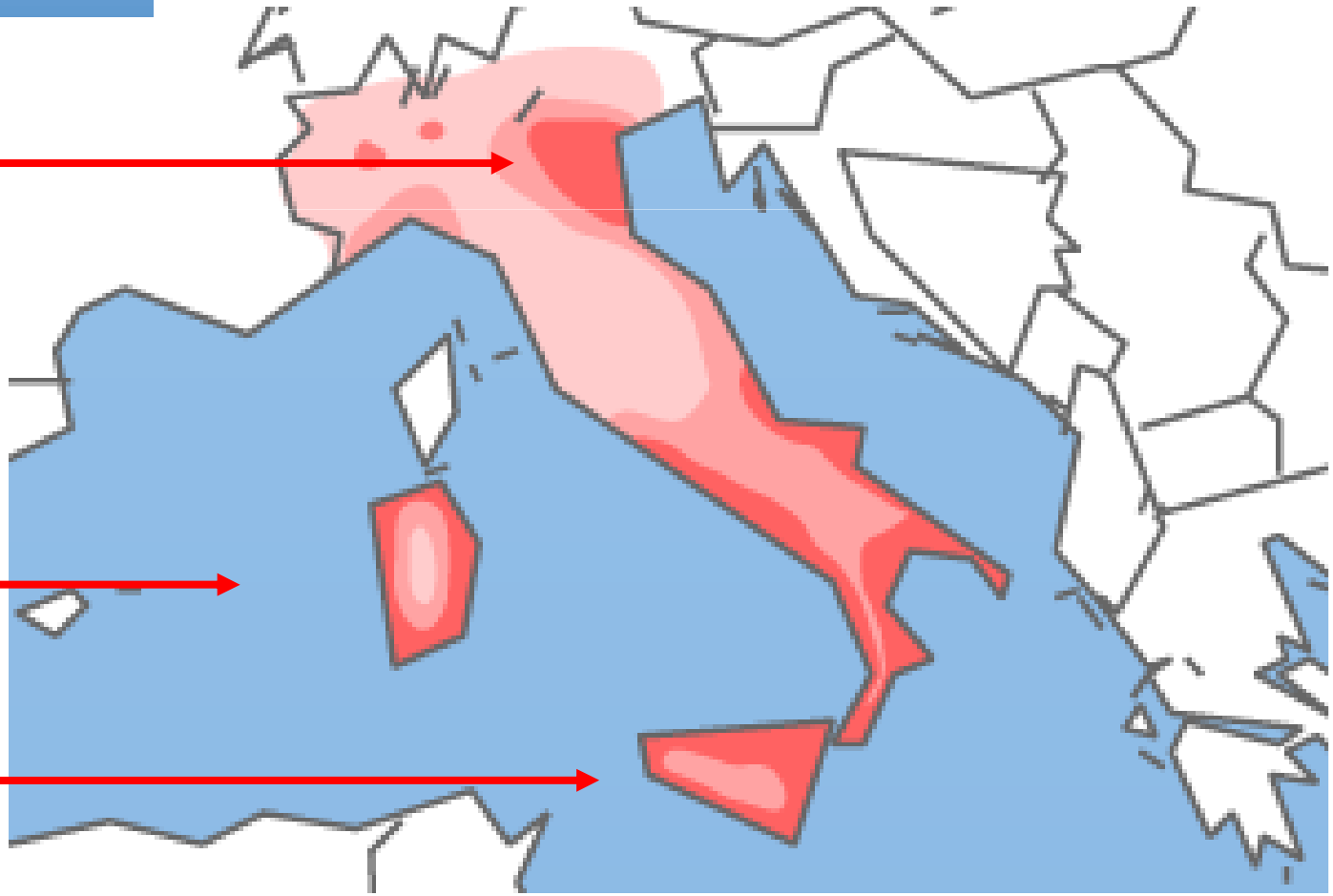
THALASSEMIA

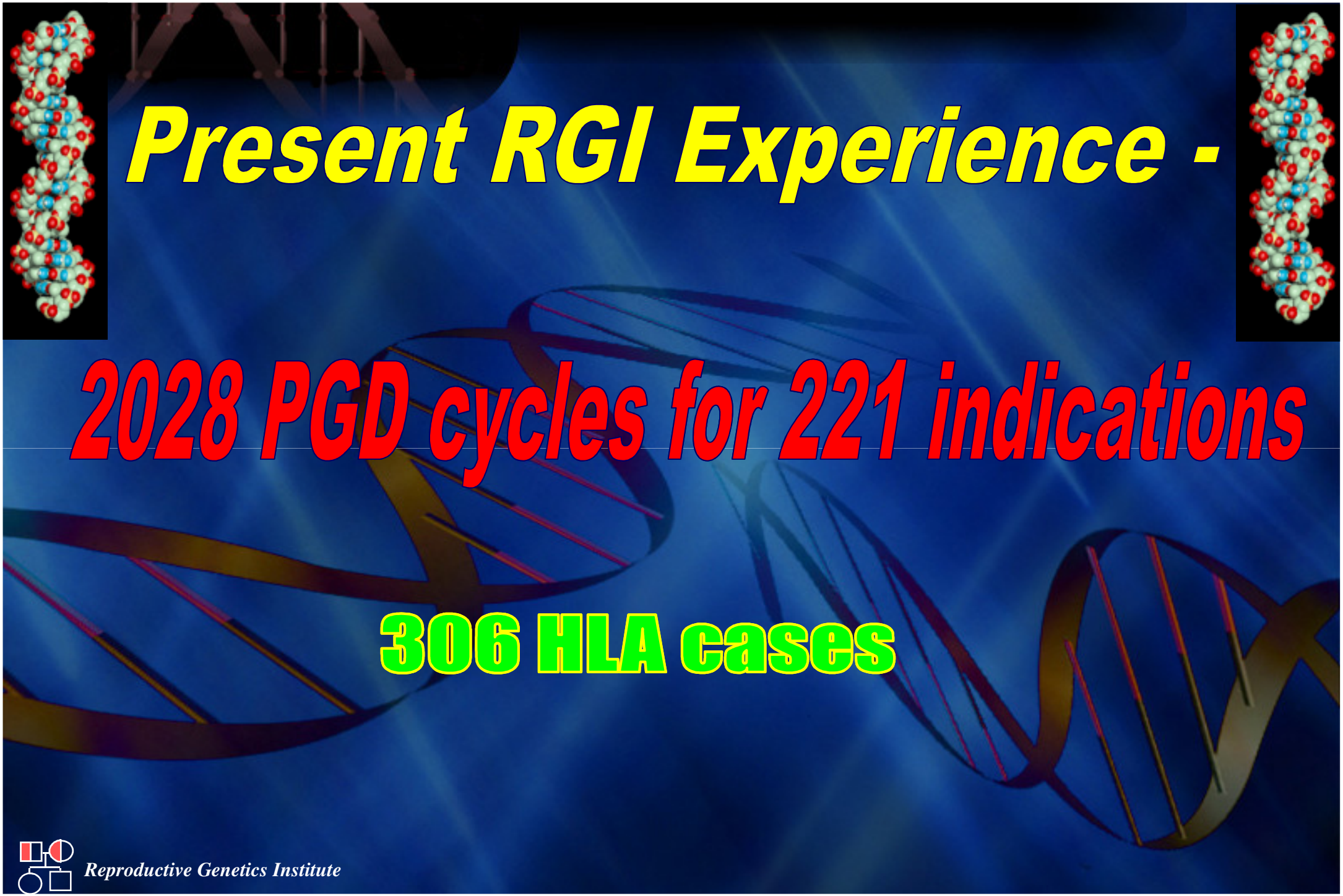
1.5% carriers in the world
→ 400.000 affected

12% carriers
→ 700 affected

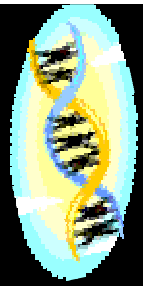
12.5% carriers
→ 1.600 affected

7% carriers
→ 1.300 affected




Present RGI Experience -
2028 PGD cycles for 221 indications
306 HLA cases
Reproductive Genetics Institute

Overall Results and Outcome of Preimplantation Diagnosis for Single Gene Disorders & Preimplantation HLA testing
RGI Experience



Testing	Patient/ Cycle	# of Transfers	# Embryos Transferred	Pregnancy / Birth
HLA	127 / 297	194	301	58 / 50 30%
		39.4 delivery rate / patients		
Single Gene Disorders	1012 / 1731	1490	2958	619 / 592 (51)* 41.5%
TOTAL	1139 / 2028	1684	3259	677 / 639 (51)* 40.2%

Reproductive Genetics Institute *rgi@flash.net*

*ongoing pregnancies

Rechitsky and Kuliev, RBM Online, 20:S1, 2010

Human Reproduction, Vol.24, No.3 pp. 732–740, 2009
Advanced Access publication on December 5, 2008 doi:10.1093/humrep/den423

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

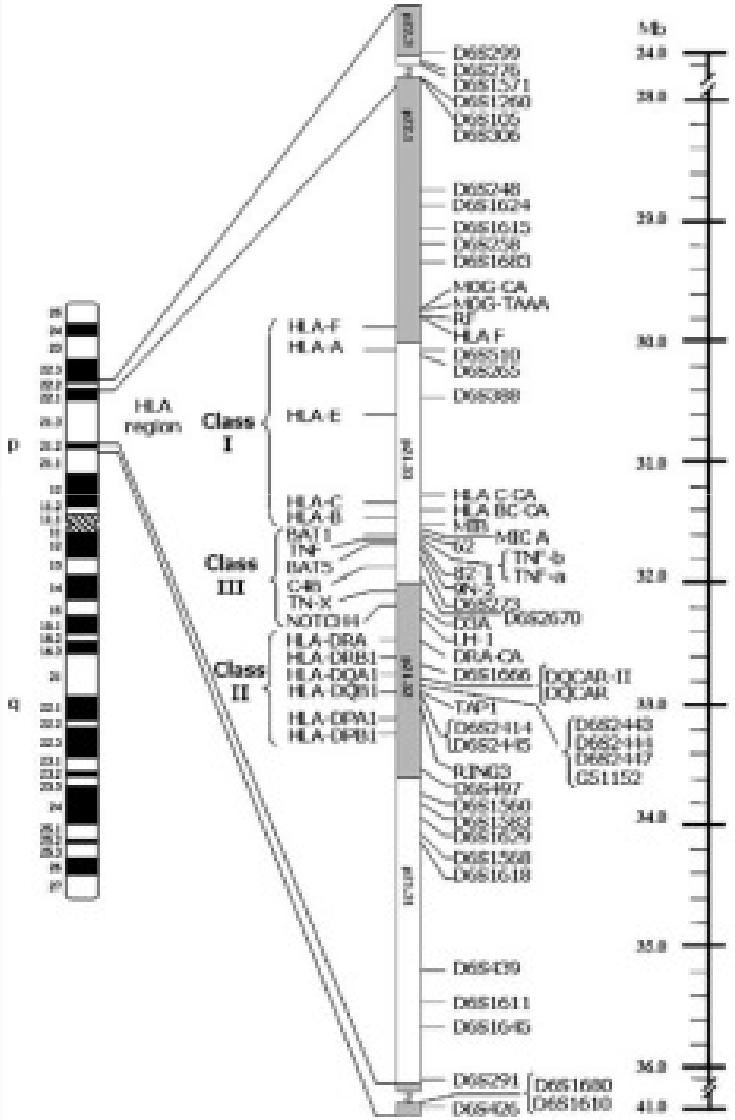


Figure 1 STR markers in the HLA locus

The experience of two European preimplantation genetic diagnosis centres on human leukocyte antigen typing

Hilde Van de Velde[1,5], Martine De Rycke[2], Caroline De Man[2], Kim De Hauwere[2], Francesco Fiorentino[3], Semra Kahraman[3], Guido Pennings[4], Willem Verpoest[1], Paul Devroey[1], and Inge Liebaers[2]

[1]Centre for Reproductive Medicine, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium [2]Centre for Medical Genetics, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium [3]Centre for Preimplantation Genetic Diagnosis, "GENOMA"—Molecular Genetics Laboratories, Rome, Italy [4]Department of Philosophy and Moral Science, Bioethics Institute Ghent, Ghent University, Blandijnberg, 2, 9000 Ghent, Belgium

139 couples → 284 cycles

No. analyzed embryos	**2205**
No. diagnosed embryos (%)	**2093 (94.9)**
No. embryos with conclusive HLA diagnosis (%)	**1898 (90.7)**

2093

No. embryos with conclusive HLA diagnosis (%)	1898 (90.7)
HLA identical (%)	401 (21.1)
HLA identical healthy (%)	238/1559 (15.3)
HLA non identical embryos (%)	1497 (78.9)
Embryos with recombination (%)	41 (2.2)
Abnormal embryos (%)	195 (9.3)

139 couples → 284 cycles

→ 165 transfers (58%)
→ 45 term pregnancies (27%)
→ 51 babies born

10 successful HSC transplantations

Van de Velde et al., Hum Reprod 2009

iiarg

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ISO 9001:2008

Clinical results: transfers

Reported in Van de Velde et al, 2009

	HLA+PGD	HLA-only	Total	
No. of couples treated	124	33	157	107
Maternal age	31.6 ± 4.8	37.3 ± 3.6	32.6 ± 5.1	
No. of cycles performed	241	60	301	199
Per couple	1.8 ± 1.2	2.1 ± 1.7	1.9 ± 1.3	
No. of transfers (%)	165 (68.4)	45 (75.0)	210 (69.8)	
No. of embryos transferred	253	75	328	
Mean no. of embryos transferred	1.0 ± 1.7	1.2 ± 0.9	1.1 ± 0.7	

Genoma, Rome (updated 03.2009)

Data modified from: Fiorentino *et al.* (2004) Mol Hum Reprod 10: 445-460; Fiorentino *et al.* (2005) Eur.J.Hum Genet. 13: 953-958

Clinical results: pregnancies and babies

	HLA+PGD	HLA-only	Total	
Maternal age	31.6 ± 4.8	37.3 ± 3.6	32.6 ± 5.1	
No. of clinical pregnancies	62	17	79	
Clinical per cycle	25.7%	28.3%	26.2%	
Clinical per transfer	37.6%	37.7%	37.6%	
Miscarriages	11	4	15	
No. of embryos implanted	78	18	96	
Implantation rate	30.8%	24.0%	29.3%	
No. of pregnancies went to term	51	13	64	45
No. of babies born	55	13	68	42
Live birth rate per cycle	21.2%	21.7%	21.3%	

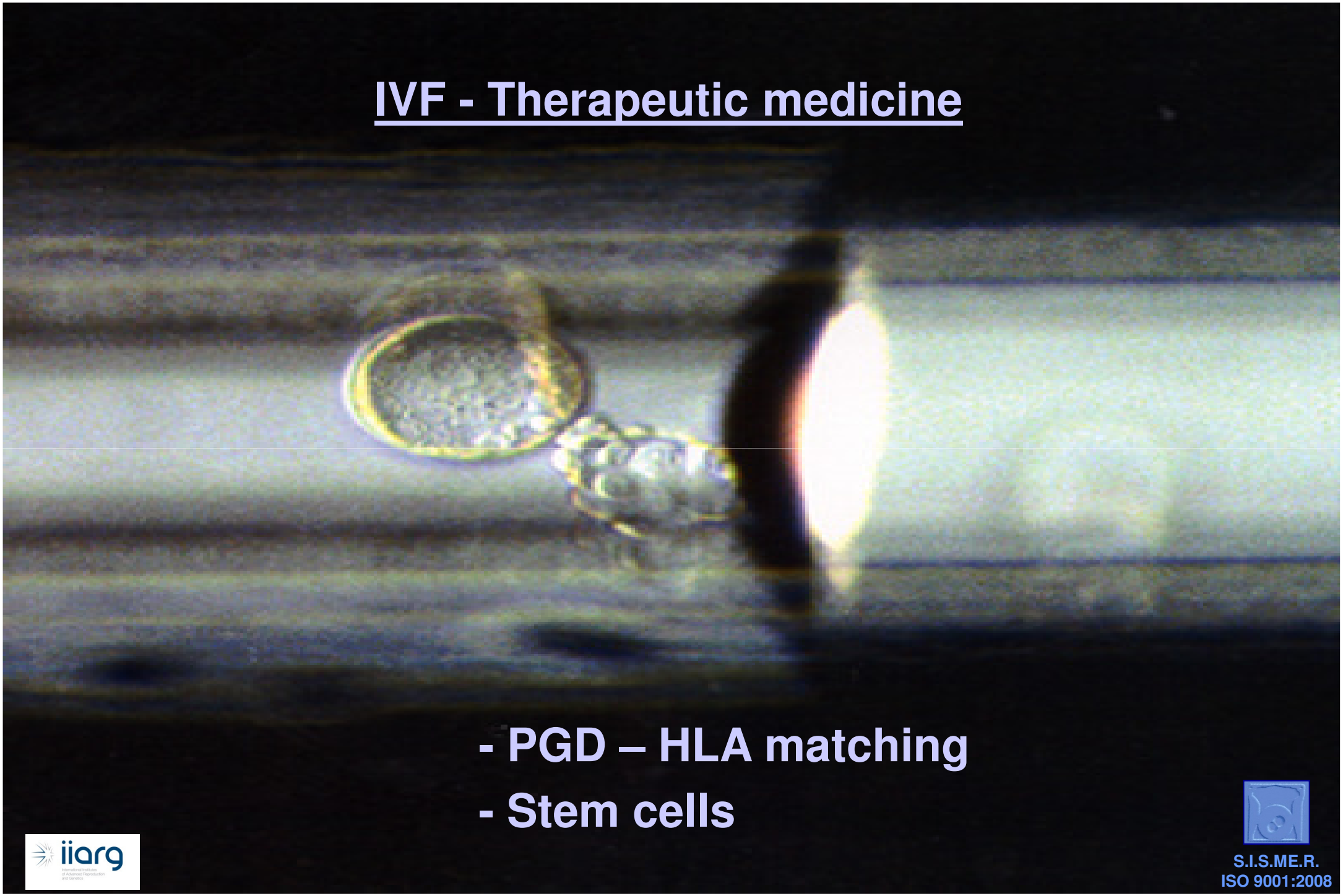
Genoma, Rome (updated 03.2009)

Data modified from: Fiorentino *et al*. (2004) Mol Hum Reprod 10: 445-460; Fiorentino *et al*. (2005) Eur.J.Hum Genet. 13: 953-958

Indications for HLA typing – Genoma, Rome (updated 03.2009)

Indications	No. of PGD cycles	No. of couples	Clinical pregnancies	Babies born	CBT
HLA typing combined with PGD					
Sickle cell disease	8	4	3	3	2
Beta-thalassemia	215	108	53	45	7
Fanconi anemia	1	1	0	0	1
Wiskott Aldrich' syndrome	1	1	1	1	1
Chronic granulomatous disease	1	1	1	2	0
Duncan syndrome	2	1	1	1	0
Mannosidosis Alpha	2	1	0	0	0
Hurler syndrome	2	2	0	0	0
Gaucher disease	4	1	1	0	0
Bruton agammaglobulinemia	1	1	1	2	0
Glanzmann thrombasthenia	1	1	0	0	0
Adrenoleukodystrophy	3	2	1	1	0
HLA-only typing					
Acute lymphoblastic leukemia	40	29	12	10	2
Diamond Blackfan anemia	17	3	4	2	2
Histiocytosis	3	1	1	1	0
Total	**301**	**157**	**79**	**68**	**15**

IVF - Therapeutic medicine



- PGD – HLA matching
- Stem cells

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International Institutes of Advanced Reproduction and Genetics

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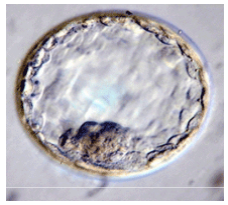
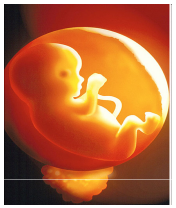
ISO 9001:2008

Different sources / Different Stem cells population

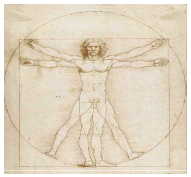
Human development



Blastocyst ICM



Foetal tissue (umbilical cord blood)



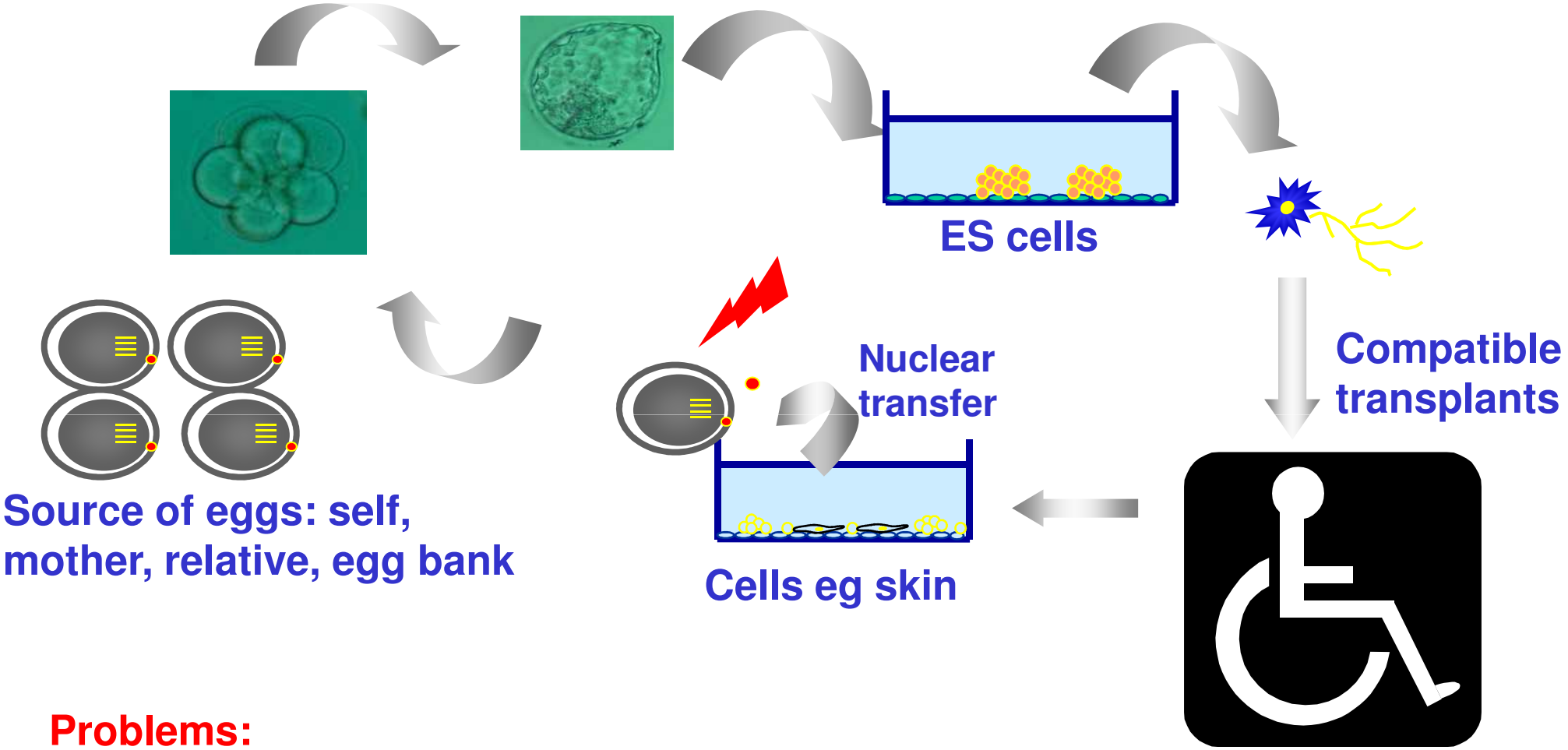
Adulthood

hESCs: Self-renewal
Plasticity
Pluripotency
Teratoma formation
High immunogenicity
Complex/aspecific differentiation

hASCs: Low proliferation
Multi/unipotent
High tolerance for transplants
Specific differentiation

Foetal Stem Cells: Reduced self-renewal
Multi/totipotency
No teratoma
No immunogenicity

Therapeutic Cloning



Problems:

Inefficient - may need large numbers (50 to several hundreds) of eggs

Technically demanding - need to be available in many or all hospitals

In vitro hES CELLS

Nuclear Reprogramming

- Somatic Cell Nuclear Transfer (SCNT)
- Induced Pluripotent Stem Cells (iPS)

In vitro hES CELLS
Differentiation

- **Cells for therapy**

  - **Parkinson's Disease and other neurodegenerative disorders**
  - **Diabetes**
  - **Cystic Fibrosis**
  - **Vascular and Heart Disease**
  - **Tissue Injuries**

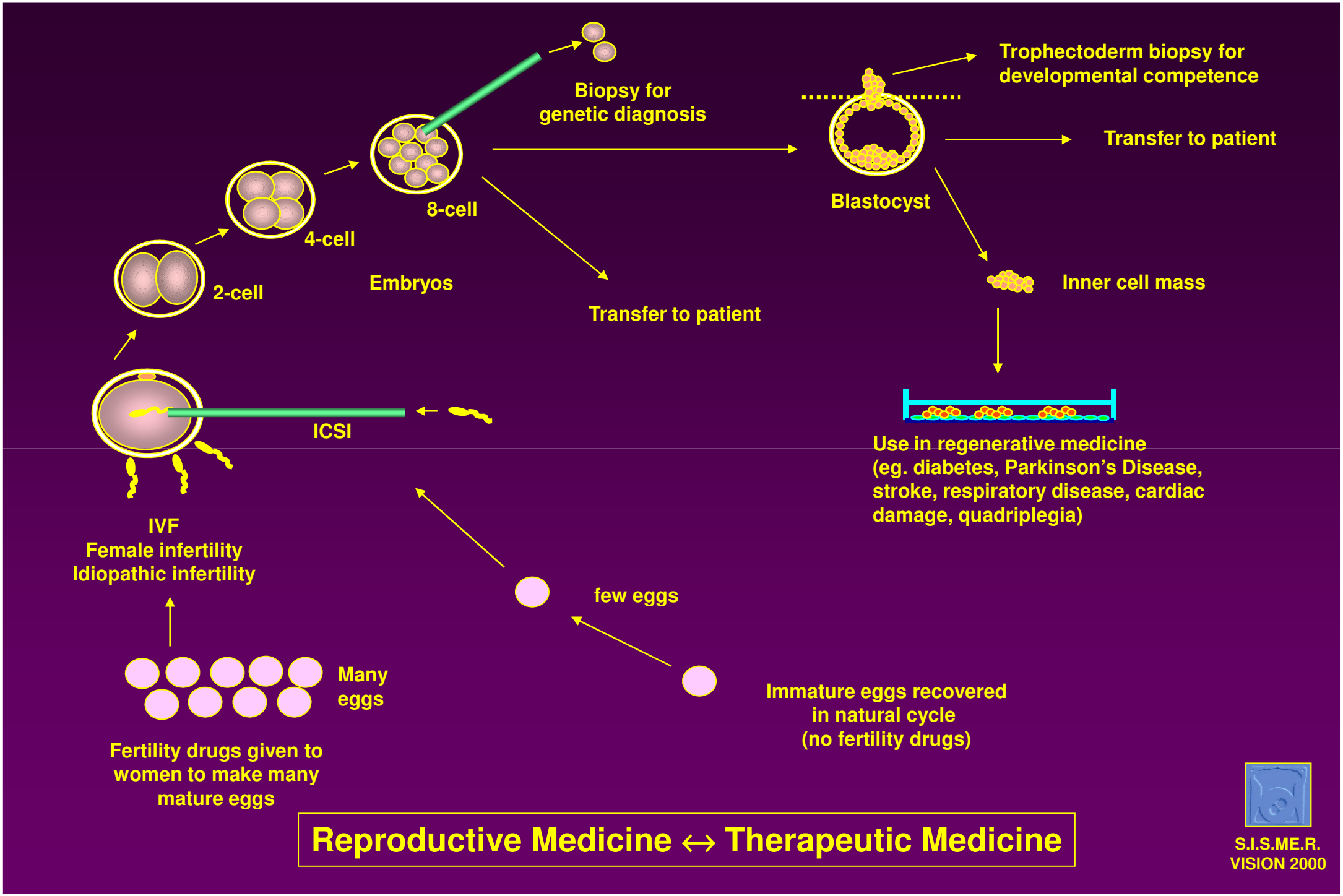
Applications for Embryonic Stem Cells and their Derivatives

- ES cells for research and discovery
- Progenitor cells for drug screening
- Progenitor cells for toxicology
- Gene products (proteins), growth and differentiating factors, cell surface molecules for pharmaceutical use in regenerative medicine

Embryonic Stem Cell Derivatives Applications for Cell and Tissue Therapy

- Vehicles for the delivery of gene therapies

  - correcting genetic disease
  - new immunization strategies
  - targeting cancers


Biopsy for
genetic diagnosis
Trophectoderm biopsy for
developmental competence
Transfer to patient
8-cell
4-cell
2-cell
Embryos
Blastocyst
Inner cell mass
Transfer to patient
ICSI
Use in regenerative medicine
(eg. diabetes, Parkinson's Disease,
stroke, respiratory disease, cardiac
damage, quadriplegia)
IVF
Female infertility
Idiopathic infertility
few eggs
Many
eggs
Immature eggs recovered
in natural cycle
(no fertility drugs)
Fertility drugs given to
women to make many
mature eggs
Reproductive Medicine ↔ Therapeutic Medicine
S.I.S.ME.R.
VISION 2000

RGI's Repository of Human Embryonic Stem Cells

NORMAL (258 lines)		AUTOSOMAL DOMINANT CONDITIONS (34)
CHROMOSOMAL ABNORMALITIES (14 lines)		BRCA2 (2 lines – of which 1 also has MEN1)
46X +mar	47,XX,+12	FSHD (7 lines)
46,XX,der(4)t(4;13)	47,XY,+12	Huntington Disease (7 lines)
46,XX t(14;17)	47,XX,+13	Marfan Syndrome
46,XX iso (17q)	47,XX,+14	Myotonic Dystrophy (2 lines)
47,XY+der(21)t(2,21)	47,XX,+21	Neurofibromatosis, type 1 (7 lines)
69,XXX	47,XXX	Popliteal Pterygium Syndrome
	47,XXY (2 lines)	Torsion Dystonia, DYT1 (2 lines)
X-LINKED CONDITIONS		Tuberous Sclerosis, TSC1 (2 lines)
		Treacher Collins-Franschetti syndrome (3 lines)
		AUTOSOMAL RECESSIVE CONDITIONS (24)
Adrenoleukodystrophy		Alpha Thalassemia (1 lines)
Becker Muscular Dystrophy		Beta Thalassemia (9 lines)
Duchenne Muscular Dystrophy (4 lines)		Cystic Fibrosis (8 lines)
Emery-Driefuss Muscular Dystrophy (2 lines)		Fanconi Anemia A
Fragile X syndrome (2 lines)		Sandhoff Disease (3 lines)
Ocular Albinism, X-linked (2 lines)		Spinal Muscular Atrophy (2 lines)

hESCreg

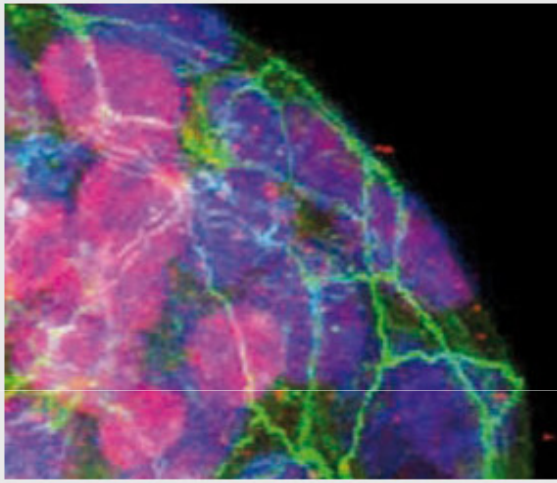
European Human Embryonic Stem Cell Registry

Welcome

Quicksearch

- Home
- Detailed Search
- Show available Cell Lines
- Show all Research Projects
- Show all Provider
- My hESCreg
  - Login
  - Register
- About hESCreg
- Project Structure
- EU hESC Research
- Legislation
- Ethics
- Newsletter
- Contact

Welcome



Register:

Cell Line
Research Project

Login to my hESCreg

Show:

Available Cell Lines
All Research Projects
All Research Provider

Welcome to the European Human Embryonic Stem Cell Registry – hESCreg

You have entered the work-in-progress site of the hESCreg database. Here you can browse the database or log in using the Username/Password combination issued to you. At the first login we advise you to change your personal Username/Password combination to one only known to yourself. If you are a provider of hES cell lines we are calling on you to complete the minimum registration information for your pre-registered cell lines. If there are any new lines please add them as well. We would greatly appreciate if you could complete this by the end of November 2007. Apart from that we are looking forward to receiving your comments or suggestions. Should you have any problems operating the database or would like to contribute to the further development of the database please direct all communication to helpdesk@hescreg.eu.

Until the official launch of hESCreg on January 18, 2008, in Berlin the interim web page at www.hescreg.eu will act as the official information resource of the initiative.

Thank you very much in advance for your contributions!