X CONGRESSO PAULISTA DE MEDICINA REPRODUTIVA

SPMR

I JORNADA INTERNACIONAL DE ESPECIALIDADES DA SOGESP

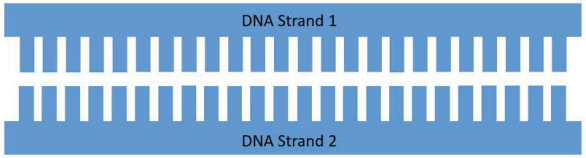
SOGESP Nova SOGESP cada vez mais FORTE

O Diagnóstico Genético Pré-Implantacional em Doenças Monogênicas

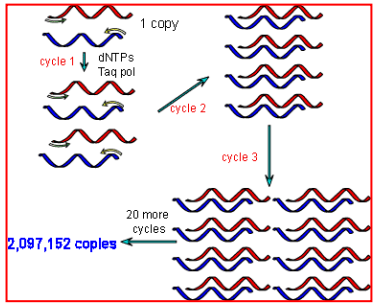
FERTILITY

Edson Borges Jr.

Step 1 of PCR is Denaturation
Takes place at 95*C to break
Hydrogen bonds between DNA
strands



Blastômero com núcleo visível



PGD-PCR

Alterações gênicas

- **Doenças Autossômicas Dominantes**
  - Distrofia Miotônica
  - Doença de Huntington
- **Doenças Autossômicas Recessivas**
  - Fibrose Cística
  - b-talassemia
  - Atrofia Muscular Espinhal
- **Doenças Ligadas ao Cromossomo X**
  - Síndrome do X-frágil
  - Distrofia Muscular de Duchenne
  - Hemofilia

FERTILITY

Human Reproduction, Vol.30, No.8 pp. 1763–1789, 2015
Advanced Access publication on June 12, 2015 doi:10.1093/humrep/dev122

human reproduction ESHRE PAGES

ESHRE PGD Consortium data collection XIII: cycles from January to December 2010 with pregnancy follow-up to October 2011†

M. De Rycke[1,*], F. Belva[1], V. Goossens[2], C. Moutou[3], S.B. SenGupta[4], J. Traeger-Synodinos[5], and E. Coonen[6]

- Thirteen sets of data on 45,073cycles / 7,751 babies born
- 62 registered centers
- Inherited chromosomal abnormalities: 6,968 cycles
- **Monogenic disorders: 9,267 cycles**
- Sexing for X-linked diseases: 1,484 cycles / social: 753 cycles
- Aneuploidy screening: 26,737 cycles
- FISH: 78% (I-XII) – 68% (XIII) – array: 355 cycles (6%)

Table IVb Cycles performed for single gene disorders using PCR, data collection XIII.

Indication	X-linked		Autosomal recessive		Autosomal dominant		HLA				Other		Total	
		%		%		%	Only		+ monogenic disease			%		%
Cycles to OR	283		437		740		36		72		6		1574	
Number infertile	51		131		133		0		3		2		320	20
Female age (years)	30		34		34		33		35		33		33	
Biopsy method														
PB	10		9		13								32	
Cleavage aspiration	260	94	392	92	699	96	17	47	64	89	6	100.0	1438	93
Cleavage extrusion	3		16		4		9						32	
Blastocyst	1	0.4	4	1	7	1	10	28	8	11			30	2
PB + embryo	3		5		3								11	
Biopsy policy														
1 cell biopsy	96	35	266	62	229	32	24	67	44	61			659	43
2 cell biopsy	124	45	94	22	334	46	2	6	18	25	6	100.0	578	37
1 or 2 cell biopsy	38	14	47	11	133	18			2	3			220	14
>2 cells (including TE)	6	2	5	1	14	2	10	28	8	11			43	3
1 and 2 PB	5	2	7	1	4	0.6							16	1
1 and 2 PB and cell	3	1	5	1	3	0.4							11	0.7
1 PB	5	2	2	0.5	9	1							16	1

Doenças Monogênicas PGD Consortium

FERTILITY

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human reproduction ESHRE PAGES

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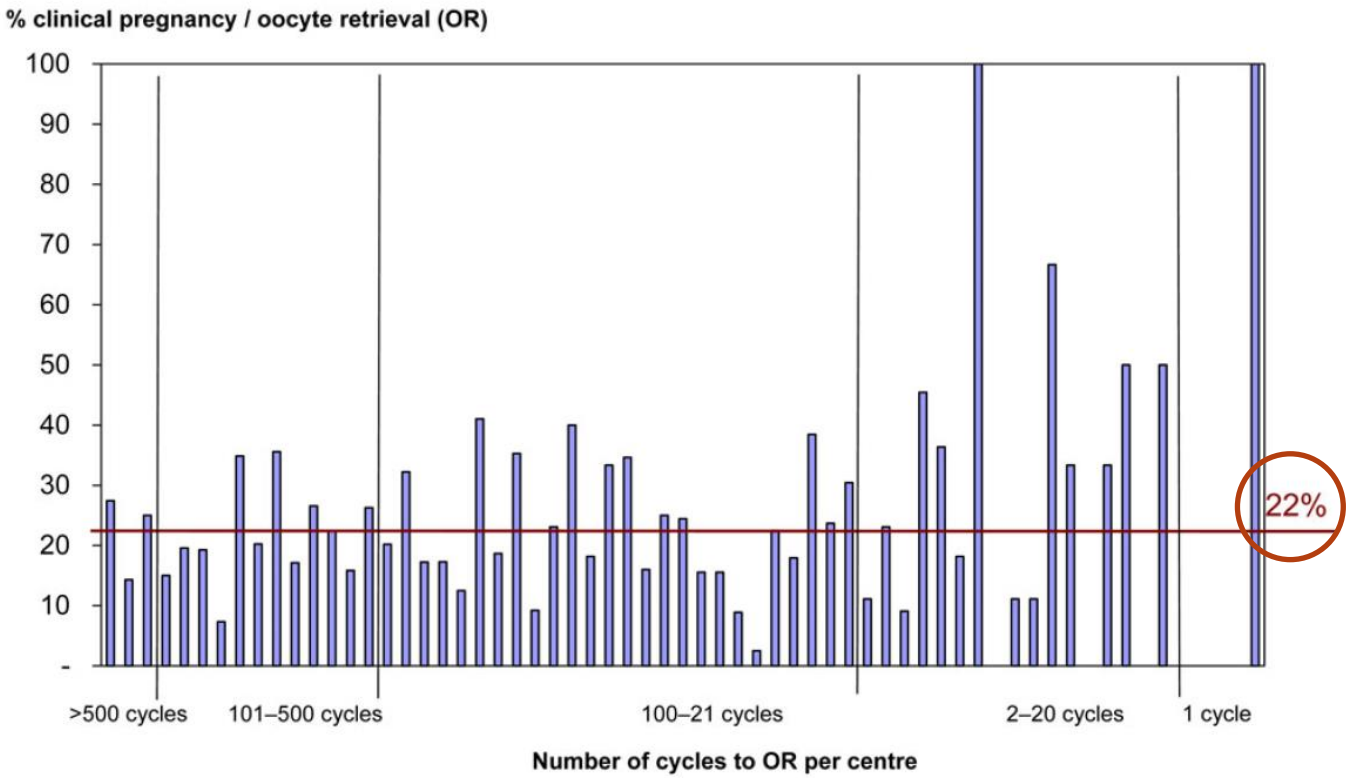

Figure 1 Clinical pregnancy rates per centre. OR: oocyte retrieval.

Dados FERTILITY OUT/2016: Doenças avaliadas	Casos	%
Acidúria metilmalônica	2	3,2%
Amiotrofia espinhal	3	4,8%
Anemia falciforme	11	17,7%
Ataxia espinocerebelar	7	11,3%
Doença de Huntington	1	1,6%
Doença de Von Recklinghausen (Neurofibromatose)	1	1,6%
Fibrose cística	2	3,2%
Gangliosidose	2	3,2%
Hemofilia	2	3,2%
HLA	3	4,8%
HLA associado à outras doenças	(20)	-
Leucoencefalopatia com substância branca evanescente	1	1,6%
Neutropenia	2	3,2%
Imunodeficiência combinada grave (SCID)	5	8,1%
Rins policísticos	1	1,6%
Síndrome de Marfan	1	1,6%
Síndrome de Meckel-Grubel	1	1,6%
Síndrome de Wolfran	1	1,6%
Talassemia	11	17,7%
X-frágil	5	8,1%
TOTAL	***62***	

Dados FERTILITY OUT/2016: Resultados – técnica PCR

	N	%
Nº de casos	62	-
Nº de embriões obtidos	498	-
Nº de embriões biopsiados	386	77,5%
Nº de médio de embriões biopsiados/paciente	6,2	-
Falha técnica	26	6,7%
Normais	82	21,2%
Portadores	136	35,2%

FERTILITY

Dados FERTILITY OUT/2016: PGD (Day 3) *vs* ICSI

	PGD	ICSI	ICSI_D5
Ciclos	62	3153	1339
Idade	34,9	36,1	34,8
Embriões transferidos	1,5	2,1	2,2
Taxa gestação / transferência	26,8%	38,1%	46,7%
Taxa implantação	29,6%	27,3%	33,9%
Taxa de abortamento	11,1%	13,9%	11,5%
Gestação múltipla	50,0%	29,4%	32,4%

FERTILITY

Follow-up of PGD cycles for HLA

Data analysis was performed for a total of 704 IVF-PGD cycles from 364 couples, performed between August 2001 and September 2015.
Data was "cleaned" and analysed by Athens' groups.

Data collected included all aspects of:

- Couples (fertility status, age etc etc),
- Cycles (number, embryology, genetic analysis etc)
- Outcomes (genetically suitable embryos identified, transferred, implanted, pregnancy, birth and HSCT etc).

Participating PGD centres

location	#centres
Austria	1
Belgium	3
Brazil	1
Bulgaria	1
Canada	1
CZ	1
Denmark	1
Egypt	1
Germany	3
Greece	4
Holland	1
Hungary	1
Ireland	1
Israel	2
Italy	3
Japan	4
Poland	1
Spain	3
Sweden	1
Taiwan	1
UK	4
USA	1
22	40

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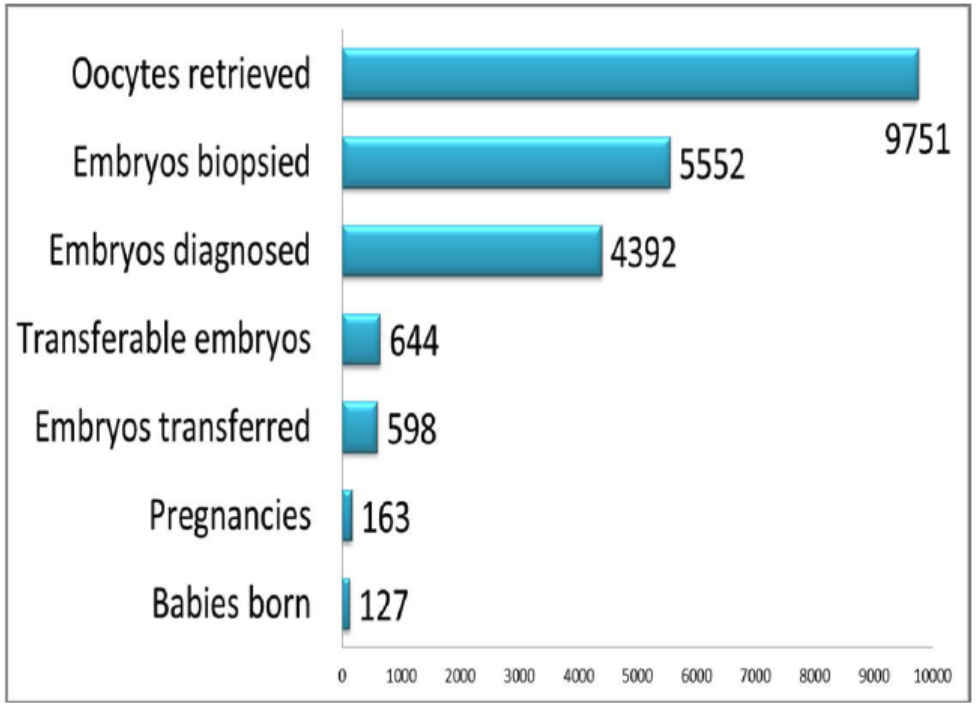
Follow-up of PGD cycles for HLA

Example of some results



Pregnancy rate/cycle initiated	23.2%
Pregnancy rate/embryo transfer	41.1%
Fetal heart beat (FHB)/cycle initiated	22.0%
FHB/embryo transfer	39.0%
FHB/embryos transferred	25.9%
Live birth rate (LBR)/cycle initiated	18.0%
Live birth rate/embryo transfer	32.0%

Main conclusions: Genetic probability and maternal age remain the major limitations to overall success.

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Dados FERTILITY OUT/2016: Resultados – técnica HLA

Doenças associadas	N
Apenas HLA	3
Anemia falciforme	10
Talassemia	7
Neutropenia	2
Imunodeficiência combinada grave (SCID)	1
Total	**23**

FERTILITY

Dados FERTILITY OUT/2016: Resultados – técnica HLA

	N	%
Nº de casos	23	-
Nº de embriões biopsiados	163	-
Falha técnica	9	5,5%
Compatíveis	27	16,6%

FERTILITY

Dados FERTILITY OUT/2016: HLA (Day 3) *vs* ICSI

	HLA	ICSI	ICSI_D5
Ciclos	23	3153	1339
Idade	33,7	36,1	34,8
Embriões transferidos	1,1	2,1	2,2
Taxa gestação / transferência	28,6%	38,1%	46,7%
Taxa implantação	33,3%	27,3%	33,9%
Taxa de abortamento	25,0%	13,9%	11,5%
Gestação múltipla	33,3%	29,4%	32,4%

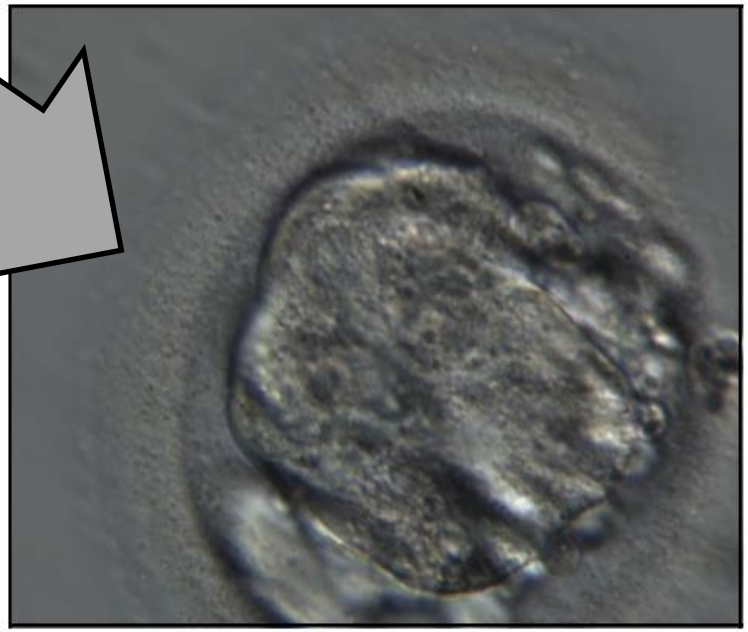
FERTILITY

J Assist Reprod Genet (2012) 29:1305–1309
DOI 10.1007/s10815-012-9862-3

GENETICS

Preimplantation diagnosis for β-thalassemia combined with HLA matching: first "savior sibling" is born after embryo selection in Brazil

This article presents the first Brazilian clinical experience demonstrating feasibility of combined PGD and HLA matching for β-thalassemia major, designed to preselect for transfer only those unaffected embryos that are HLA antigen compatible with a sibling needing cord blood transplantation.

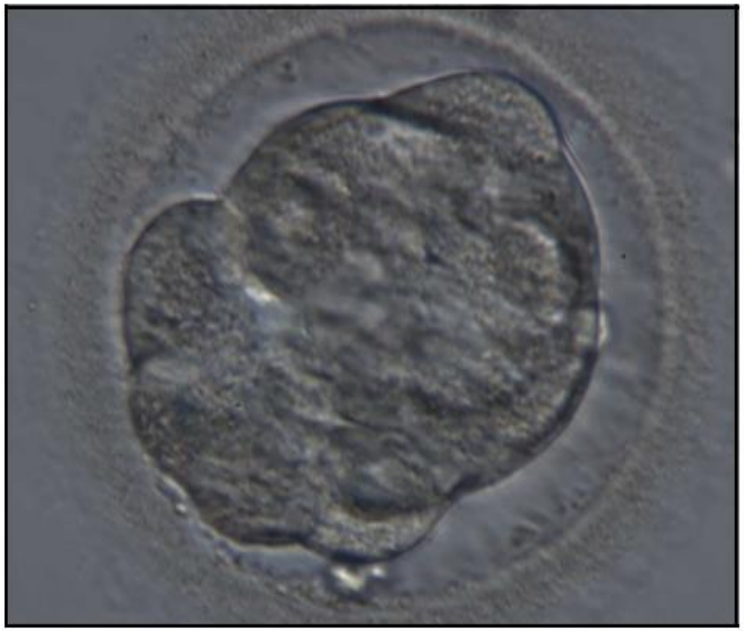


Timestamp

13/06/2011 07:53:52

Comment

Embrião 13 - Transferido
Dia +5 - Compactando (Compacto na transferência)
Resultado de PGD: Normal, HLA compatível

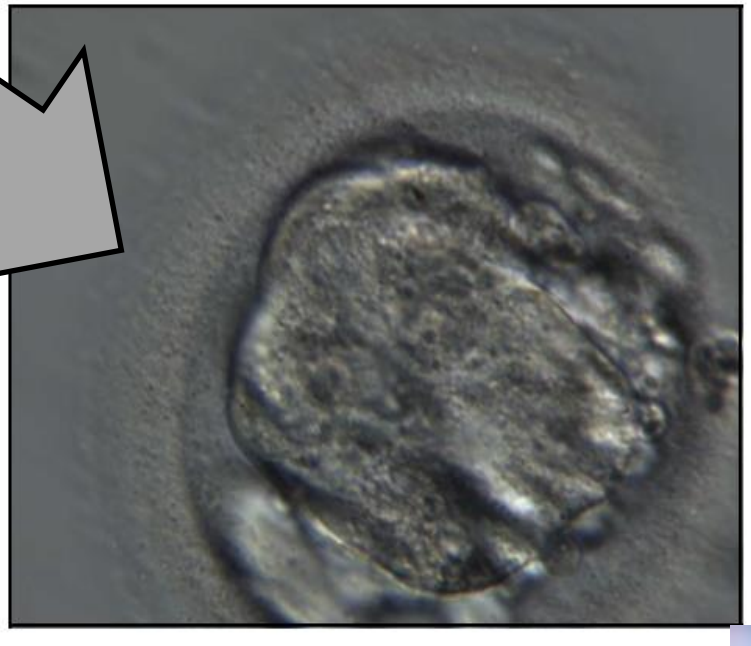


Timestamp

13/06/2011 07:55:41

Comment

Embrião 15 - Transferido
Dia +5 - Compacto
Resultado de PGD: Traço materno, HLA compatível



Timestamp

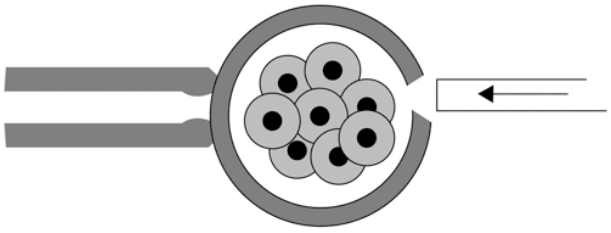
13/06/2011 07:53:52

Comment

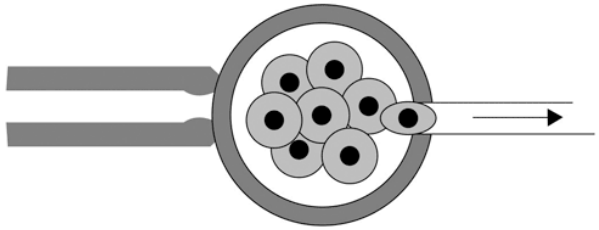
Embrião 13 - Transferido
Dia +5 - Compactando
(Compacto na
transferência)
Resultado de PGD:
Normal, HLA compatível

FERTILITY

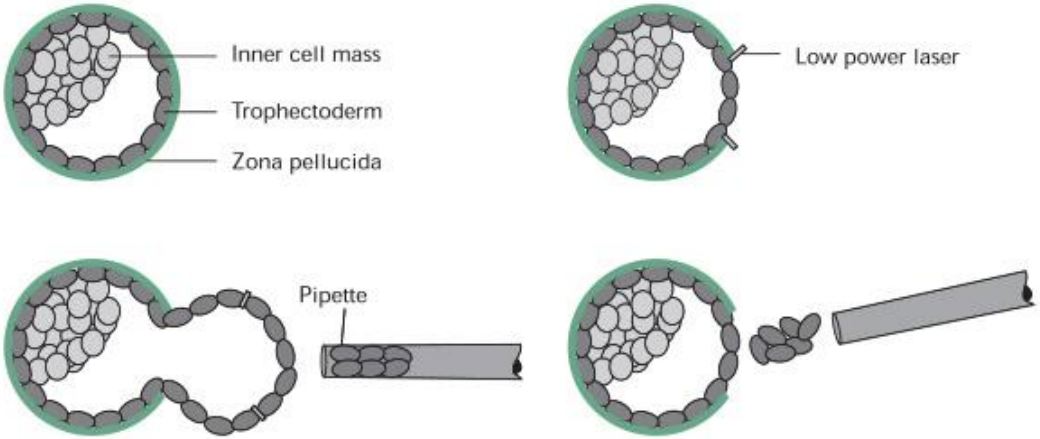
Embryo Biopsy

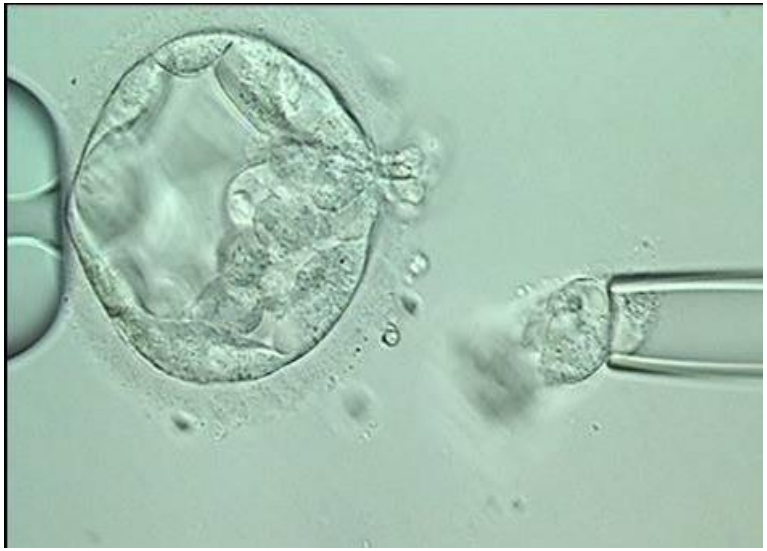


The 8-cell embryo is held by a holding pipette and a hole is made in the zona pellucida



A blastomere is aspirated into a biopsy pipette, and then can be genetically tested.





FERTILITY

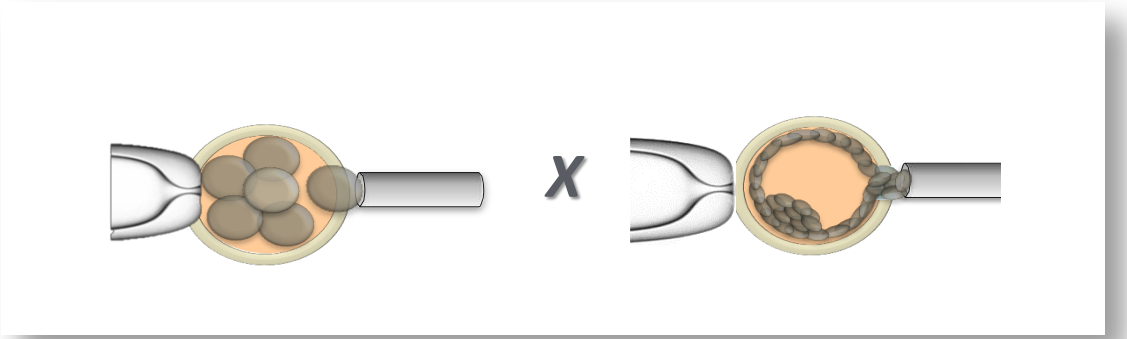
Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial

Richard T. Scott Jr., M.D.,[a,b] Kathleen M. Upham, B.S.,[a] Eric J. Forman, M.D.,[b] Tian Zhao, M.S.,[a] and Nathan R. Treff, Ph.D.[a,b,c]

[a] Reproductive Medicine Associates of New Jersey, Morristown; [b] Division of Reproductive Endocrinology, Department of Obstetrics, Gynecology, and Reproductive Sciences, Robert Wood Johnson Medical School, Rutgers University, New Brunswick; and [c] Department of Genetics, Rutgers–State University of New Jersey, Piscataway, New Jersey

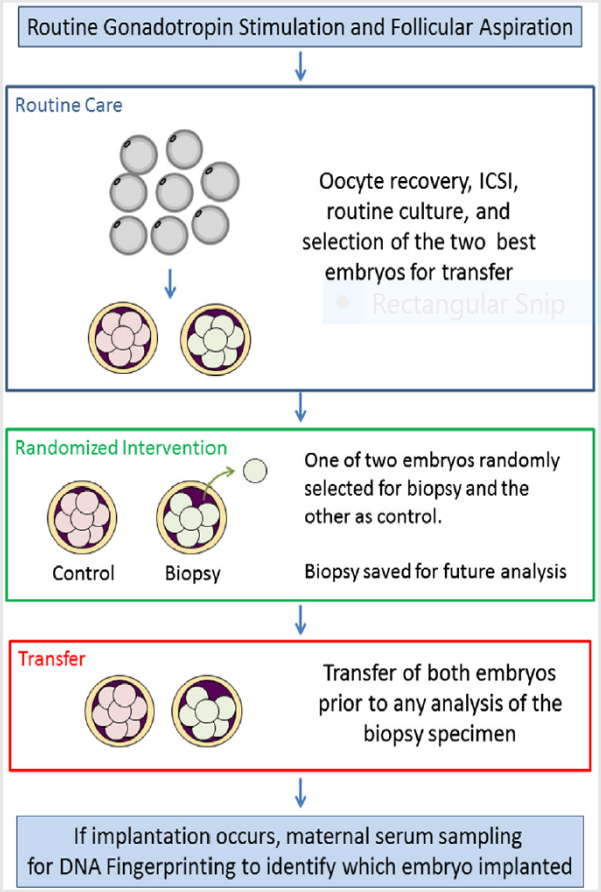
Fertility and Sterility® Vol. 100, No. 3, September 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.04.039



FERTILITY

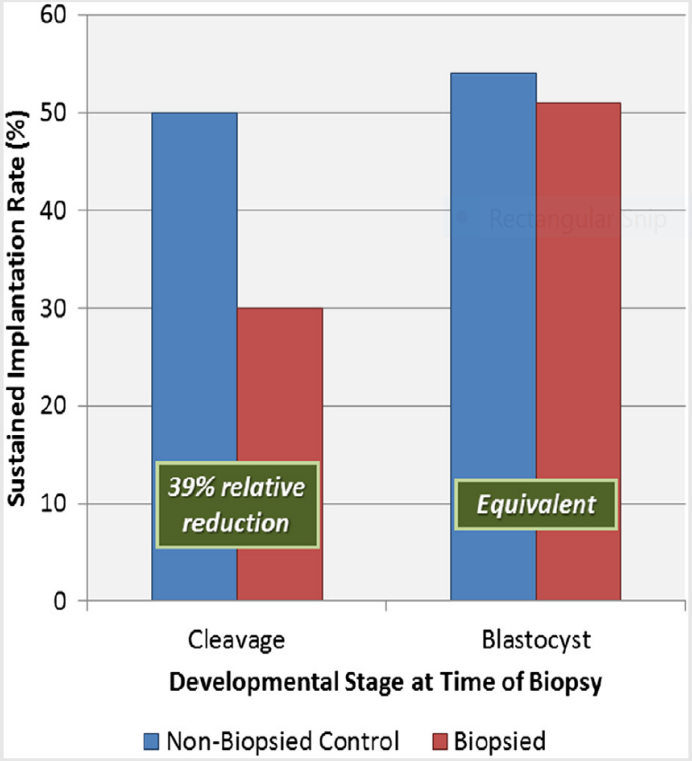
FIGURE 1



Study design to assess the impact of biopsy at the cleavage stage (day 3) on embryonic reproductive potential. The randomized paired experimental design allowed each patient to serve as her own control. An equivalent methodology was used to determine if trophectoderm biopsy at the blastocyst stage affected embryonic potential. ICSI = intracytoplasmic sperm injection.

Scott. Cleavage-stage biopsy is harmful. Fertil Steril 2013.

FIGURE 3



Implantation rates following a randomized paired analysis of the effects of cleavage- and blastocyst-stage biopsies on embryo reproductive potential. Sustained implantation and delivery of the biopsied embryo were significantly reduced compared with its control sibling when biopsy was performed on day 3 at the cleavage stage (McNemar chi-square: $P<.03$). A similar paired analysis demonstrated that the developmental potential of embryos undergoing trophectoderm biopsy at the blastocyst stage was equivalent to the nonbiopsied control siblings.

Scott. Cleavage-stage biopsy is harmful. Fertil Steril 2013.

Journal of Genetic Counseling
December 2016, Volume 25, Issue 6, pp 1327–1337

Preimplantation Genetic Diagnosis (PGD) for Monogenic Disorders: the Value of Concurrent Aneuploidy Screening

Authors | Authors and affiliations

Kara N. Goldman, Taraneh Nazem, Alan Berkeley, Steven Palter, Jamie A. Grifo

We performed a retrospective cohort study of patients who ***underwent PGD with or without 24-chromosome aneuploidy screening*** to explore the value of concurrent screening.

Among the PGD + aneuploidy-screened group (*n* = 355 blastocysts), ***only 25.6 % of embryos were both Single Gene Disorder (SGD)-negative (or carriers) and euploid;*** thus the majority of embryos were ineligible for transfer due to the high prevalence of aneuploidy. ***Despite a young mean age (32.4 ± 5.9y), 49.9 % of Blastocysts were aneuploid***.

The majority of patients ***(53.2 %) had ≥1 blastocyst that was Single Gene Disorder (SGD)-unaffected but aneuploid.*** Despite the transfer of nearly half the number of embryos in the aneuploidy-screened group ***(1.1 ± 0.3 vs. 1.9 ± 0.6, p < 0.0001), the implantation rate was higher (75 % vs. 53.3 %) and miscarriage rate lower (20 % vs. 40 %) (although not statistically significant).***

24-chromosome aneuploidy screening when performed concurrently with PGD provides valuable information for embryo selection, and ***notably improves single embryo transfer rates***.