

# NON-INVASIVE PREDICTION OF BLASTOCYST FORMATION BY DAY THREE EMBRYO CULTURE MEDIUM MASS SPECTROMETRY LIPID FINGERPRINTING.

Braga, D.<sup>1</sup>; Setti, A.<sup>1</sup>; Cabral E.<sup>2</sup>; Eberlin M.<sup>2</sup>; Loturco E.<sup>3</sup>; Borges Jr., E.<sup>1</sup>



FERTILITY<sup>01</sup>  
MEDICAL GROUP



# LIPIDÔMICA BLASTOCISTO Introdução:



## International Committee for Monitoring Assisted Reproductive Technologies: World Report on Assisted Reproductive Technologies, 2007

Osamu Ishihara, M.D., Ph.D.,<sup>a</sup> G. David Adamson, M.D.,<sup>b</sup> Silke Dyer, M.D.,<sup>c</sup> Jacques de Mouzon, M.D., M.P.H.,<sup>d</sup>  
Karl G. Nygren, M.D., Ph.D.,<sup>e</sup> Elizabeth A. Sullivan, M.D., M.P.H.,<sup>f</sup> Fernando Zegers-Hochschild, M.D.,<sup>g</sup>  
and Ragaa Mansour, M.D., Ph.D.<sup>h</sup>

Fertility and Sterility® Vol. 103, No. 2, February 2015 0015-0282/\$36.00

>1.251.881 procedimentos

229.442 bebês nascidos

Tx de bebê em casa 20,3%

**Objective:** To analyze information on assisted reproductive technology (ART) performed worldwide, and trends in outcomes over successive years.

**Design:** Cross-sectional survey on access, efficiency, and safety of ART procedures performed in **55 countries** during 2007.

**Setting:** Not applicable.

**Patient(s):** Infertile women and men undergoing ART globally.

**Intervention(s):** Collection and analysis of international ART data.

**Main Outcome Measure(s):** Number of cycles performed, by country and region, including pregnancies, single and multiple birth rates, and perinatal mortality.

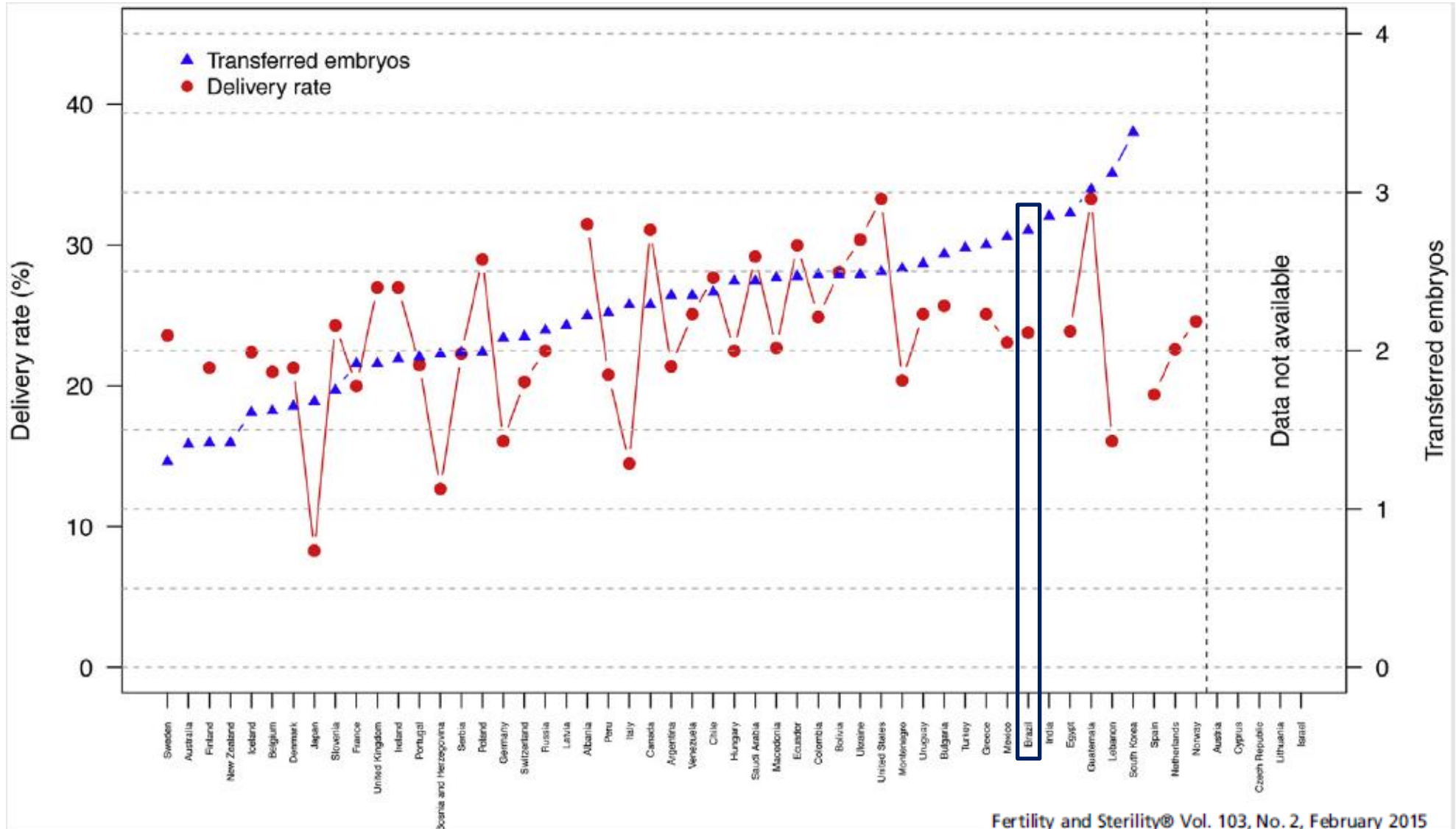
**Result(s):** Overall, **>1,251,881 procedures with ART** were reported, and resulted in **229,442 reported babies born**. The availability of ART varied by country, from 12 to 4,140 treatments per million population. Of all aspiration cycles, 65.2% (400,617 of 614,540) were intracytoplasmic sperm injection. The overall **delivery rate per fresh aspiration was 20.3%**, and for frozen-embryo transfer (FET), 18.4%, with a cumulative delivery rate of 25.8%. With wide regional variations, single-embryo transfer represented 23.4% of fresh transfers, and the proportion of deliveries with twins and triplets from fresh transfers was 22.3% and 1.2%, respectively. The perinatal mortality rate was 19.9 per 1,000 births for fresh in vitro fertilization using intracytoplasmic sperm injection, and 9.6 per 1,000 for FET. The proportion of women aged  $\geq 40$  years increased to 19.8% from 15.5% in 2006.

# LIPIDÔMICA BLASTOCISTO Introdução:



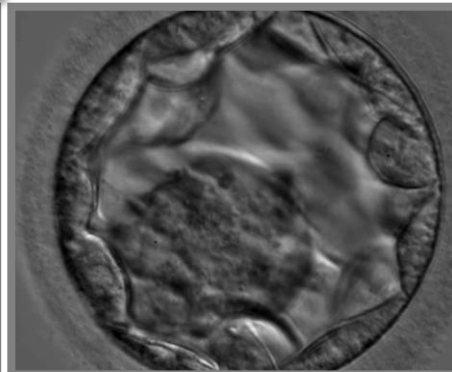
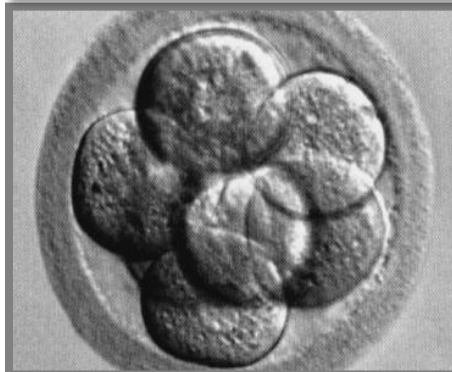
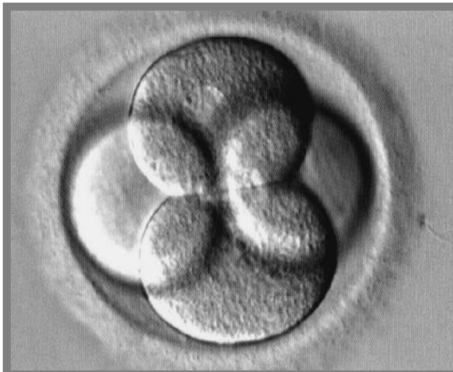
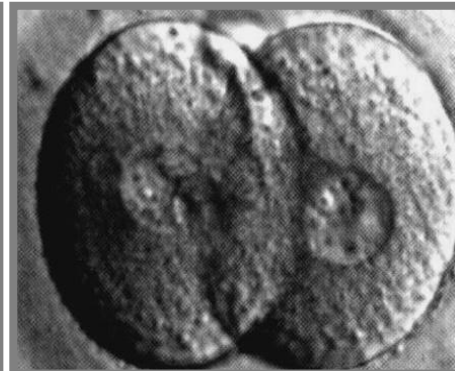
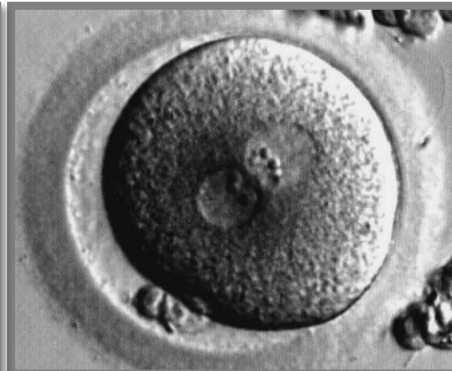
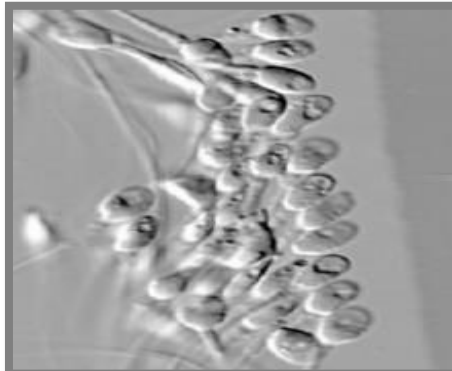
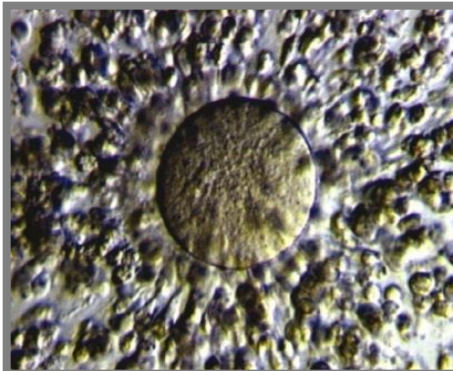
## International Committee for Monitoring Assisted Reproductive Technologies: World Report on Assisted Reproductive Technologies, 2007

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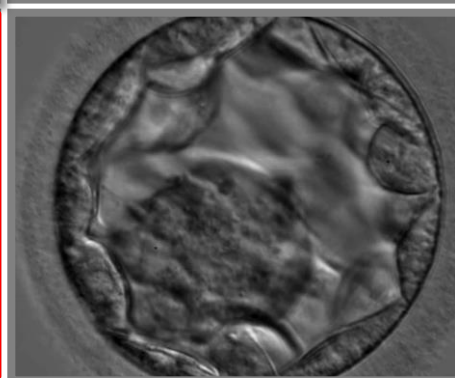
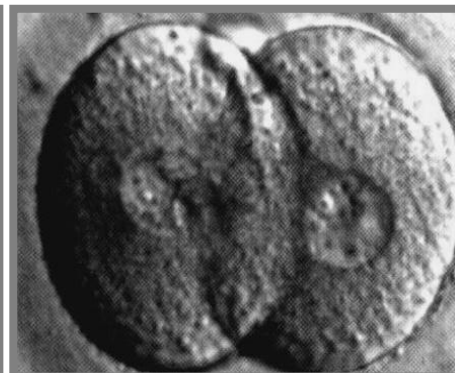
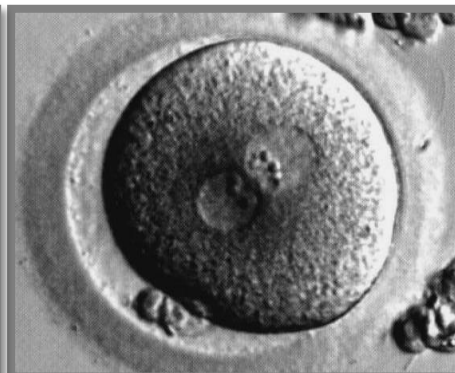
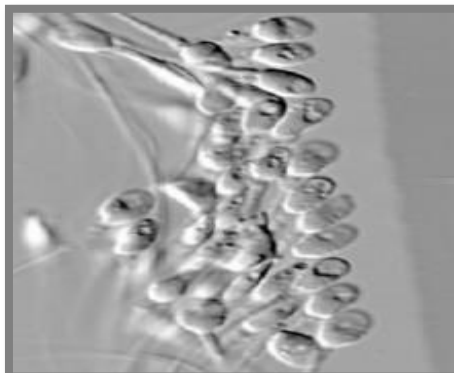
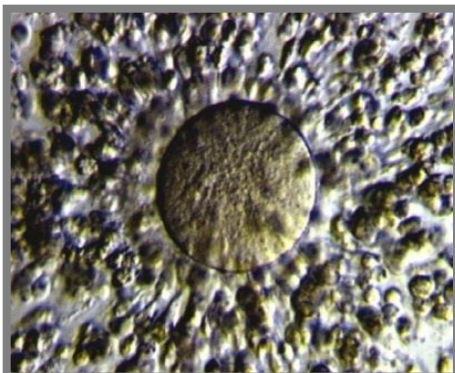
# LIPIDÔMICA BLASTOCISTO

Introdução:



# LIPIDÔMICA BLASTOCISTO

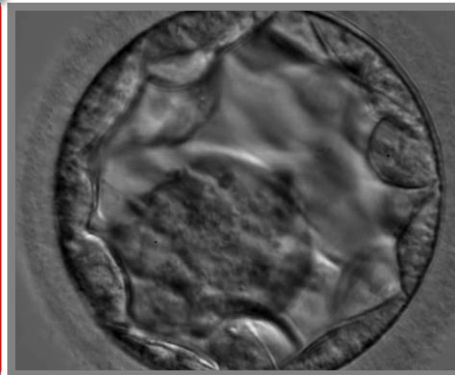
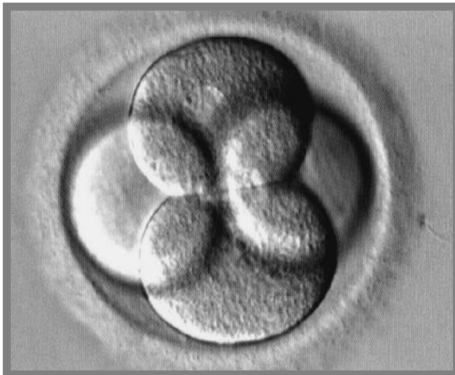
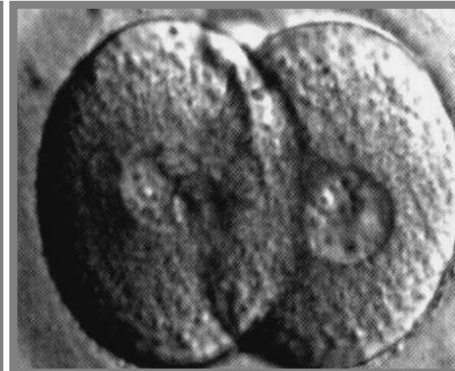
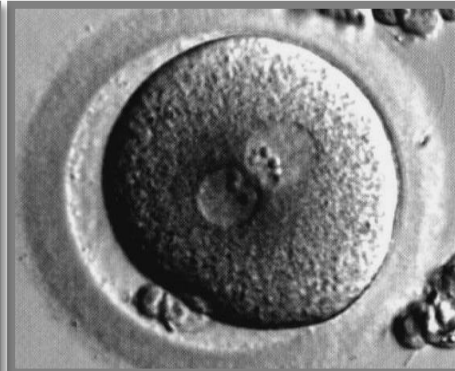
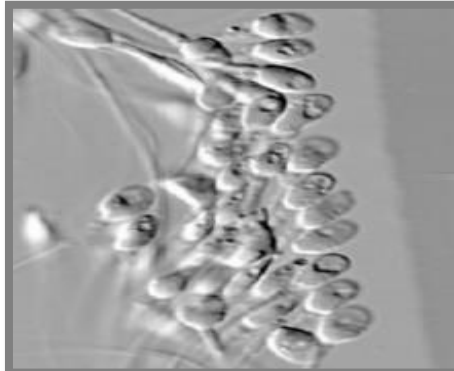
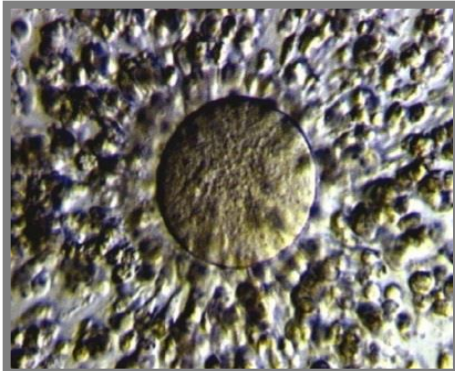
Introdução:



 **20%**

# LIPIDÔMICA BLASTOCISTO

## Introdução:



# LIPIDÔMICA BLASTOCISTO Introdução:

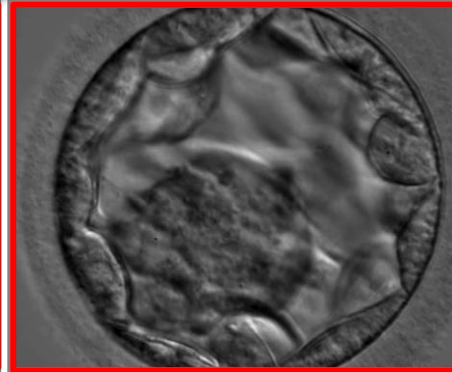
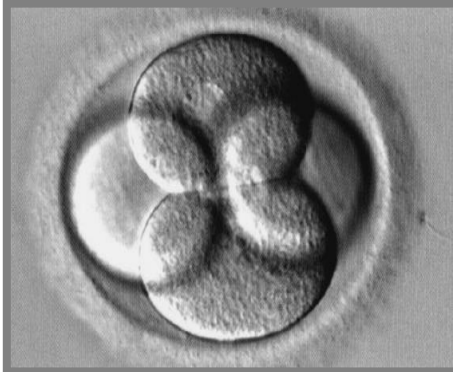
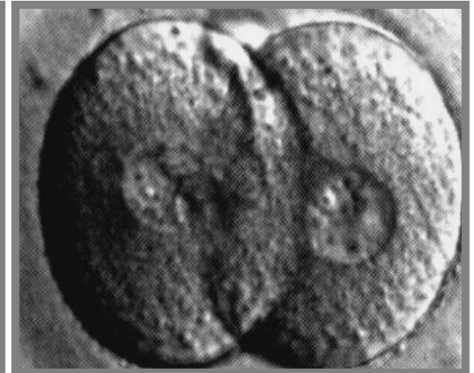
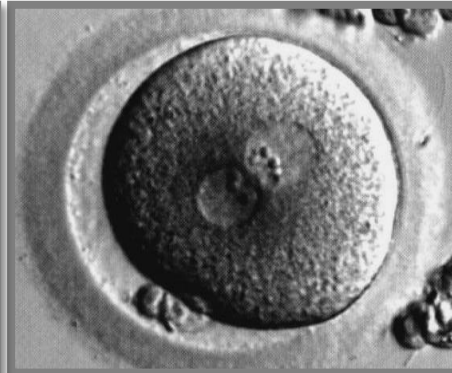
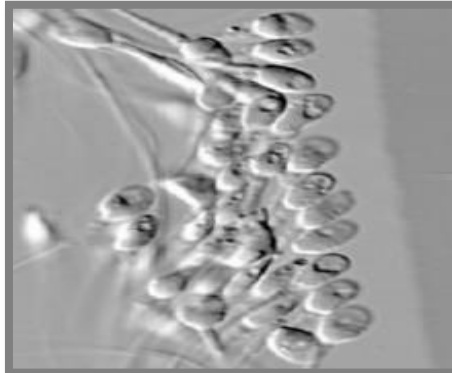
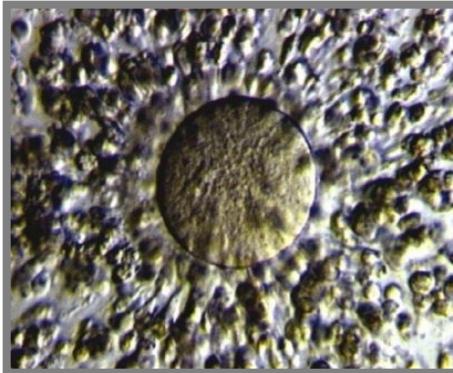


Human Reproduction Vol.16, No.4 pp. 617-619, 2001

OPINION

## To blastocyst or not to blastocyst? That is the question

Michael M.Alper<sup>1,5</sup>, Peter Brinsden<sup>2</sup>, Robert Fischer<sup>3</sup> and Matts Wikland<sup>4</sup>



# LIPIDÔMICA BLASTOCISTO Introdução:



J Assist Reprod Genet  
DOI 10.1007/s10815-014-0326-9

GAMETE BIOLOGY

Fertility and Sterility® Vol. 100, No. 3, September 2013 0015-0282/\$36.00  
Copyright ©2013 American Society for Reproductive Medicine, Published by Elsevier Inc.  
<http://dx.doi.org/10.1016/j.fertnstert.2013.05.021>

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## Influence of oocyte dysmorphisms on

J Assist Reprod Genet

DOI 10.1007/s10815-013-0073-3

Aman  
Livia V  
Assum

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ASSISTED REPRODUCTION TECHNOLOGIES

Dani  
Rita c  
and E

J Assist Reprod Genet (2014) 31:1105–1110

DOI 10.1007/s10815-014-0266-4

Th

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EMBRYO BIOLOGY



Dani  
Rita  
Edso

## The importance of the cleavage stage morphology evaluation for blastocyst transfer in patients with good prognosis

ELSEVIER

COMMENTA

Daniela P. A. F. Braga · Amanda S. Setti ·  
Rita C. S. Figueira · Assumpto Iaconelli Jr. · Edson Borges Jr.

## Limitations of a time-lapse blastocyst prediction model: a large multicentre outcome analysis



Kirstine Kirkegaard<sup>a,b,\*</sup>, Alison Campbell<sup>c</sup>, Inge Agerholm<sup>d</sup>,  
Ursula Bentin-Ley<sup>e</sup>, Anette Gabrielsen<sup>f</sup>, John Kirk<sup>g</sup>, Shabana Sayed<sup>h</sup>,  
Hans Jakob Ingerslev<sup>a,b</sup>



# LIPIDÔMICA BLASTOCISTO Introdução:



## OMICS in assisted reproduction: possibilities and pitfalls

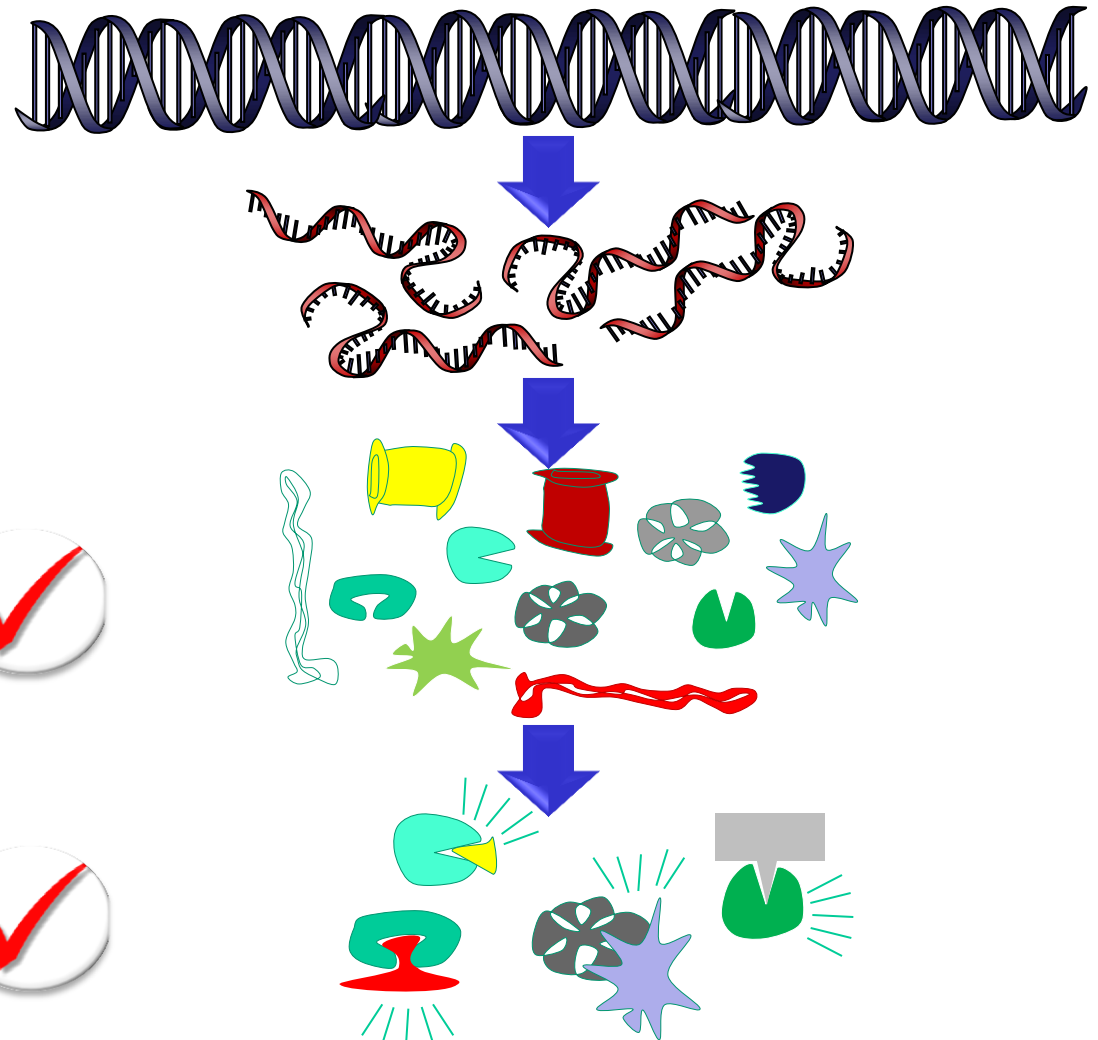
Emre Seli<sup>1</sup>, Claude Robert<sup>2</sup>, and Marc-Andre Sirard<sup>2\*</sup>

**GENOMA**  
~25,000 GENES

**TRANSCRIPTOMA**  
~100,000

**PROTEOMA**  
~1,000,000

**METABOLOMA**  
~2,500



## LIPIDÔMICA BLASTOCISTO Introdução:



**Maroun Bou Khalil, Weimin Hou, Hu Zhou, Fred Elisma, Leigh Anne Swayne, Alexandre P. Blanchard, Zemin Yao, Steffany A.L. Bennett, and Daniel Figeys\***

*Department of Biochemistry, Microbiology and Immunology,  
Faculty of Medicine, University of Ottawa, 451 Smyth Road, Ottawa, ON,  
Canada K1H 8M5*

*Received 16 April 2009; accepted 5 August 2009*

*Published online 7 October 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mas.20294*

## CONSTITUENTES

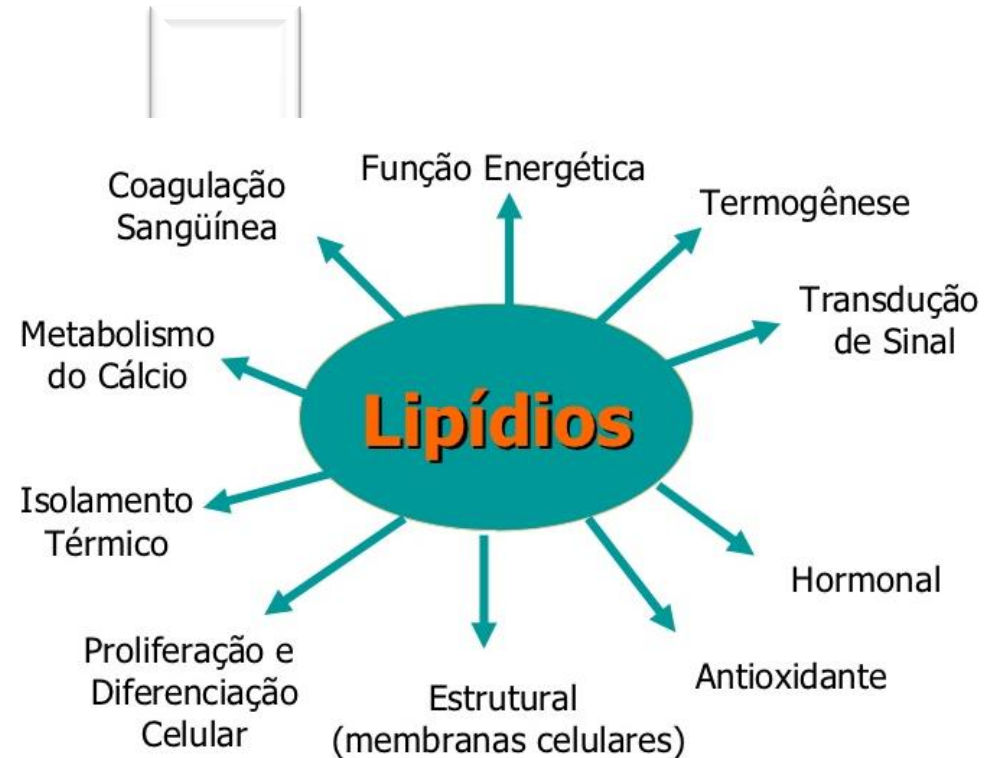
**ALBUMINA**

**LACTATO**

**PIRUVATO**

**GLUTAMATO**

**GLICOSE**



**LIPIDÔMICA  
BLASTOCISTO**  
**Objetivo:**



Identificar marcadores lipídicos associados a capacidade de formação de blastocistos em meio de cultivo de embriões em dia 3 do desenvolvimento.

# LIPIDÔMICA BLASTOCISTO

Método:

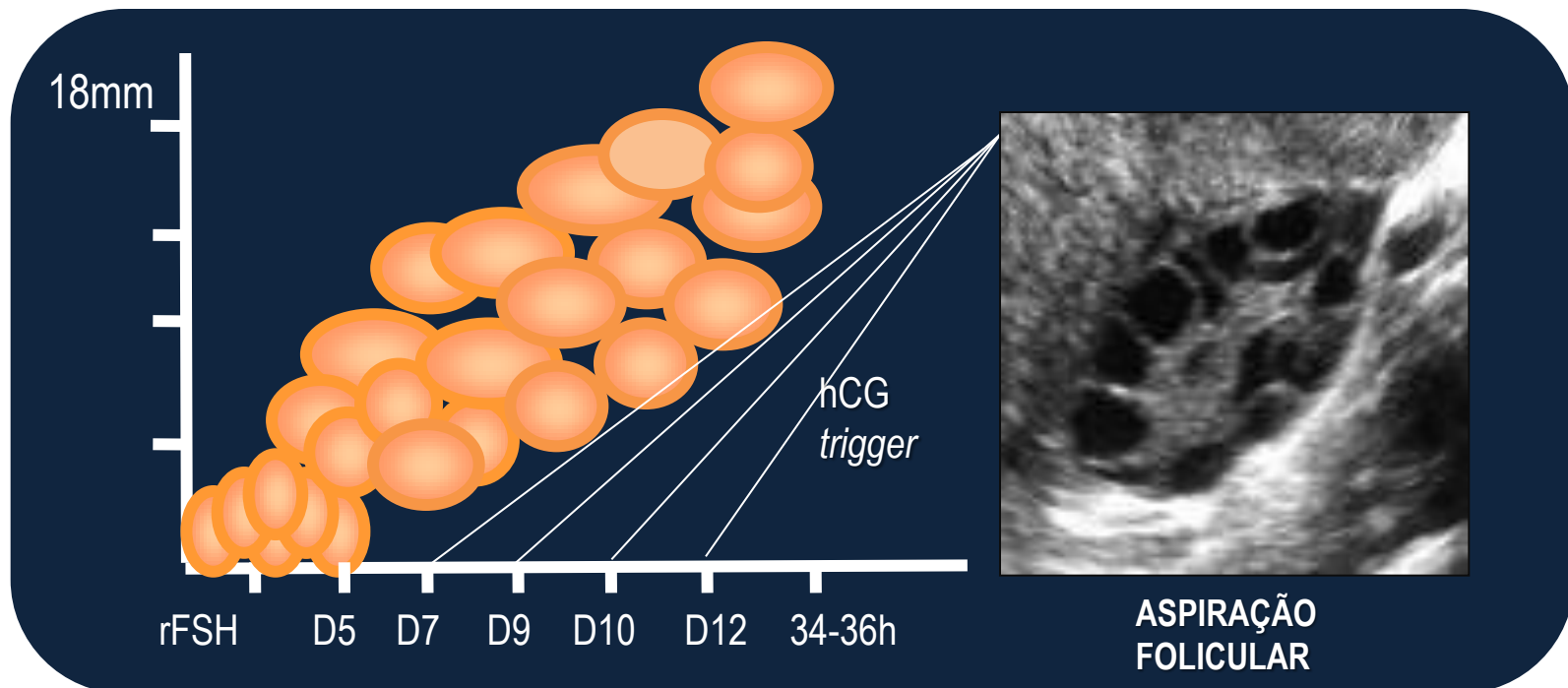


## ■ EOC:

Bloqueio hipofisário: Antagonista (*Cetrotide*®, Merck KGaA)

Estímulo ovariano: r-FSH (*Gonal-F*®, Merck KGaA)

Maturação folicular final: r-hCG (*Ovidrel*®, Merck KGaA)

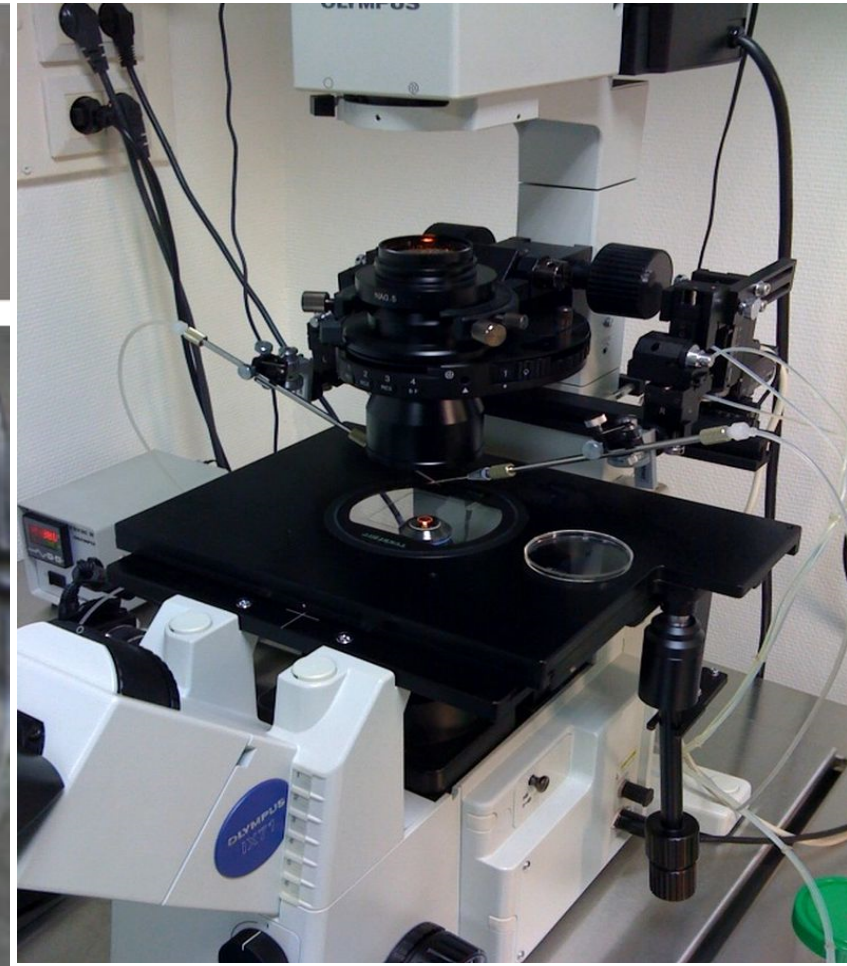
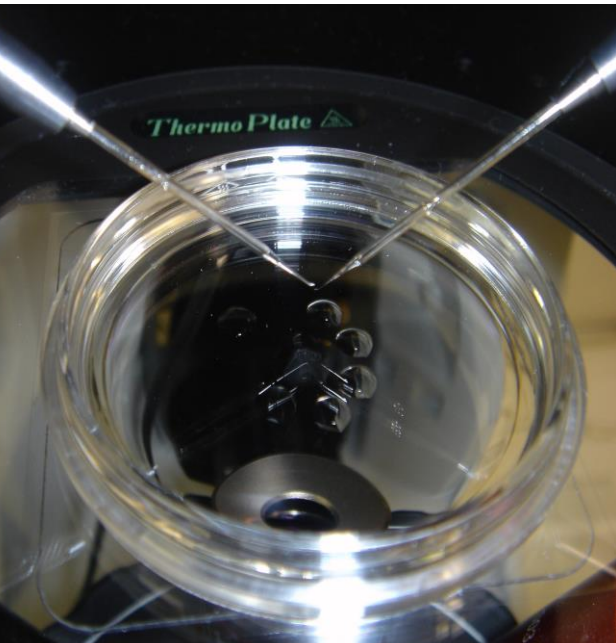
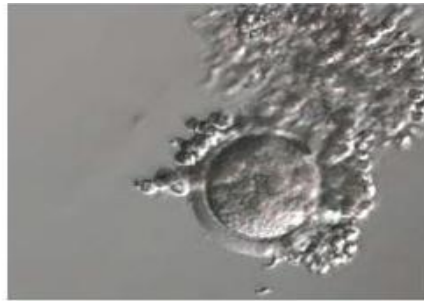


# LIPIDÔMICA BLASTOCISTO

Método:



- Preparo dos oócitos e Injeção de espermatozoides  
Técnica padrão (*Palermo et al., 1992*)



## The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting<sup>†</sup>

Alpha Scientists in Reproductive Medicine and ESHRE Special  
Interest Group of Embryology

# LIPIDÔMICA BLASTOCISTO Método:

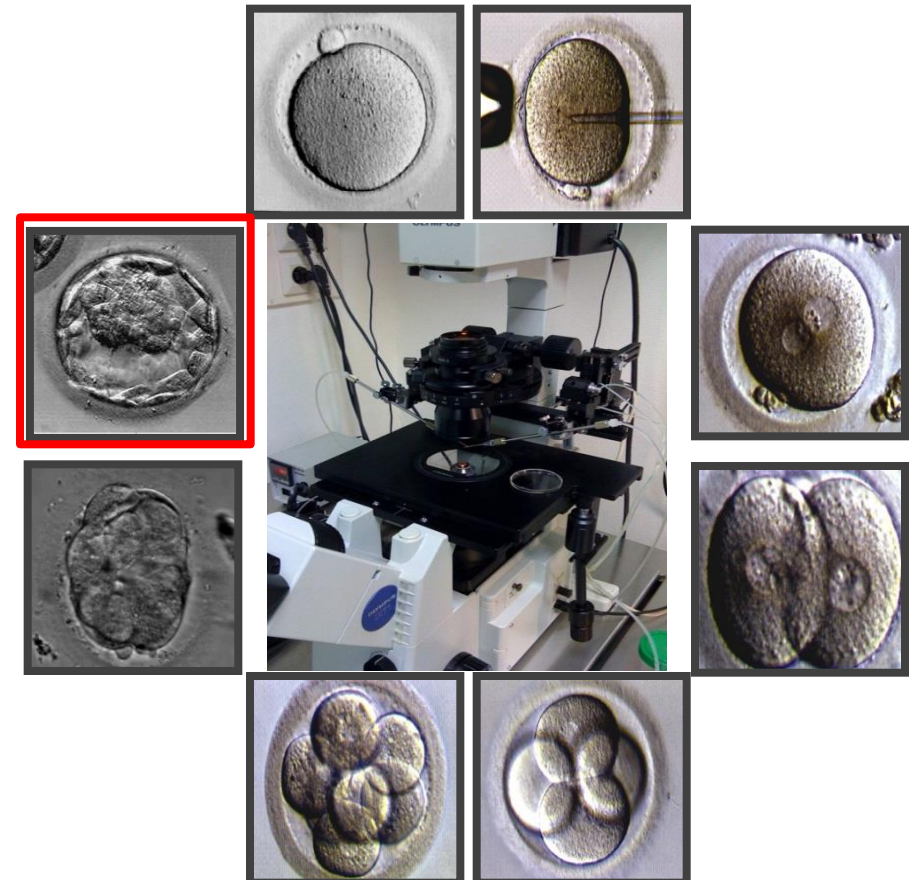


## Classificação embrionária

Type of observation	Timing	Expected stage
Grau 1		Blastocisto jovem, blastocele ocupa menos de 50% do volume do embrião
Grau 2		Blastocele ocupa metade ou mais do volume do embrião
Grau 3		Blastocisto completo, blastocele ocupa todo o volume do embrião
Grau 4		Blastocisto expandido, zona pelúcida de espessura fina
Grau 5		Blastocisto com parte do trofoectoderme eclodindo da zona pelúcida
Grau 6		Blastocisto com eclosão completa da zona pelúcida

Day-5 embryo assessment 110 ± 4

blastocyst

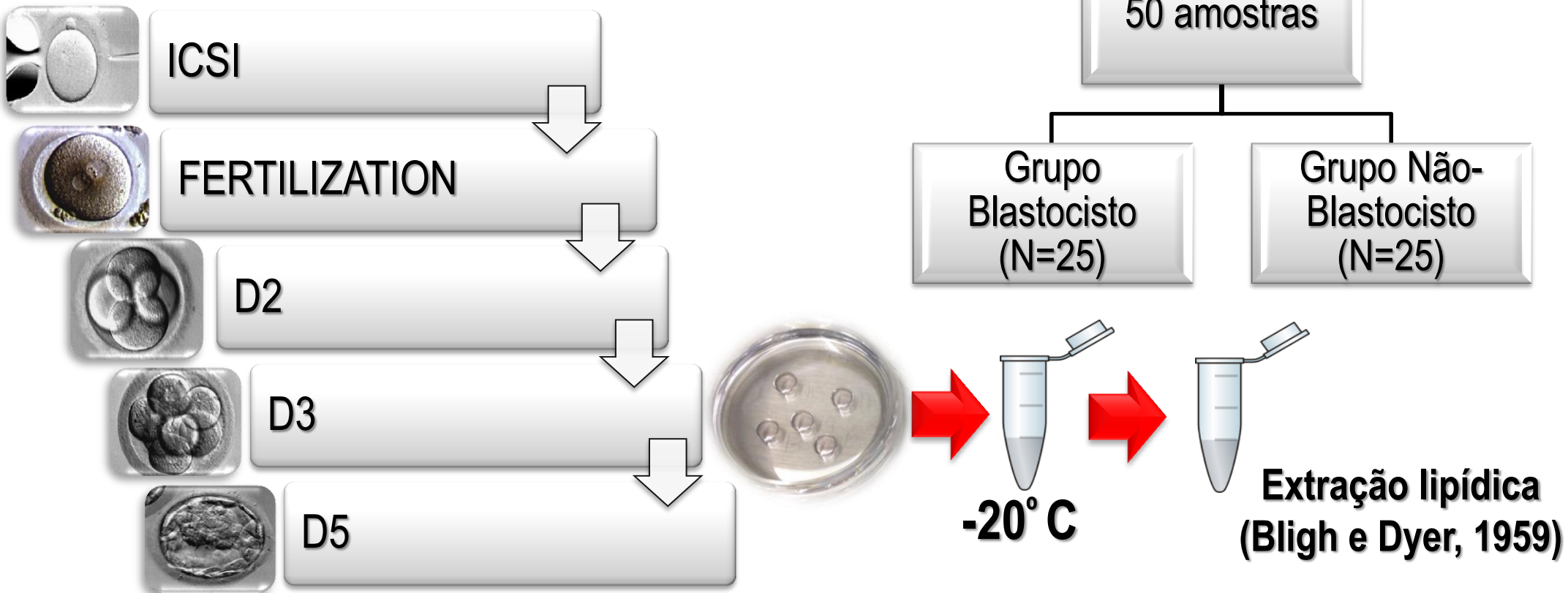


# LIPIDÔMICA BLASTOCISTO

Método:



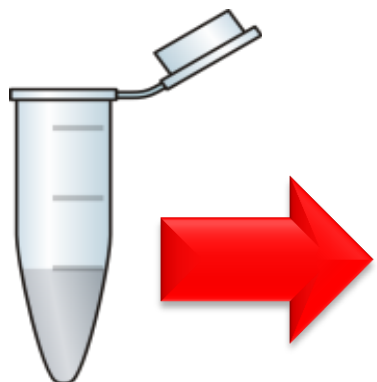
- Desenho experimental e Preparo das amostras



# LIPIDÔMICA BLASTOCISTO Método:



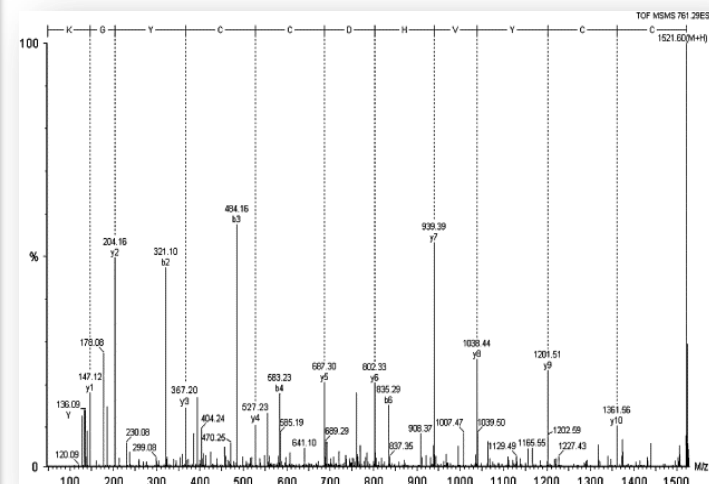
- Espectrometria de massas (ES) e Perfil lipídico (*Fingerprinting*)



Nanomate



LC-MS Q-TOF



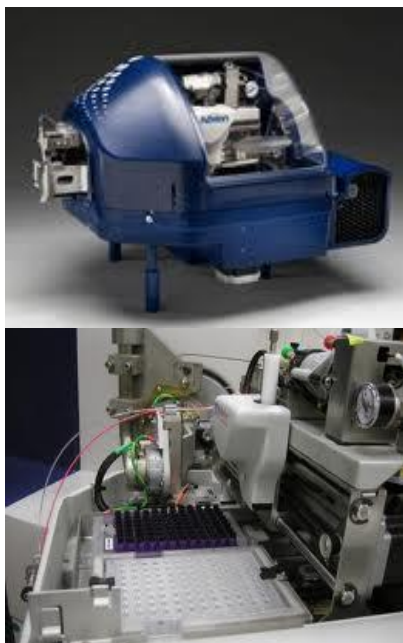
Aquisição de dados



# LIPIDÔMICA BLASTOCISTO Método:



- Análise estatística dos dados



**Nanomate**



**LC-MS Q-TOF**

Normalização dos  
espectros



PCA / Pls Da



VIP scores

**LIPIDÔMICA  
BLASTOCISTO**  
**Resultados:**



1657 íons



Análise univariável  
(teste *t*)



165 íons

$\geq 4x$ ,  $p < 0.001$

LIPIDÔMICA  
BLASTOCISTO  
Resultados:



1657 íons

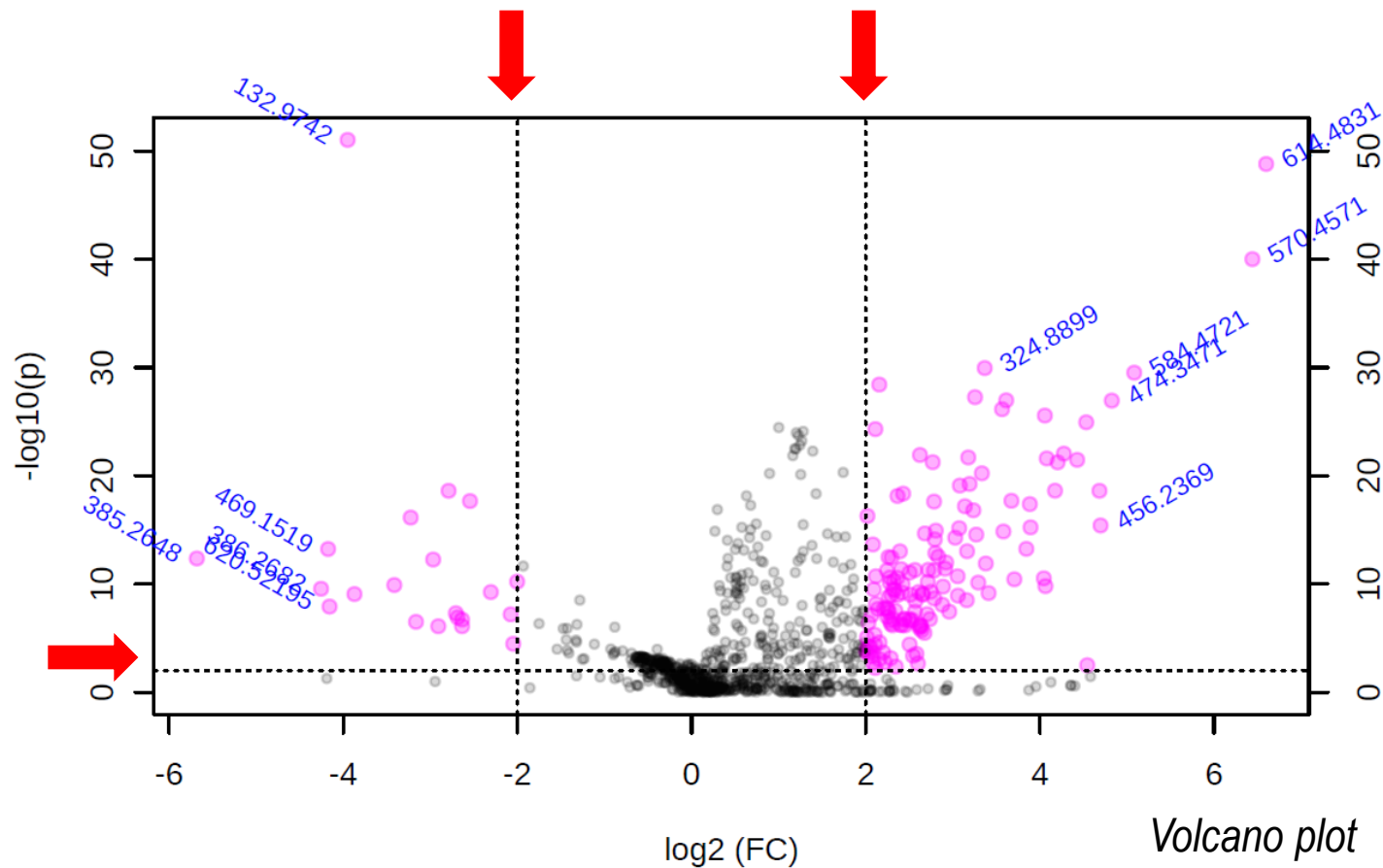


Análise univariável  
(teste *t*)



165 íons

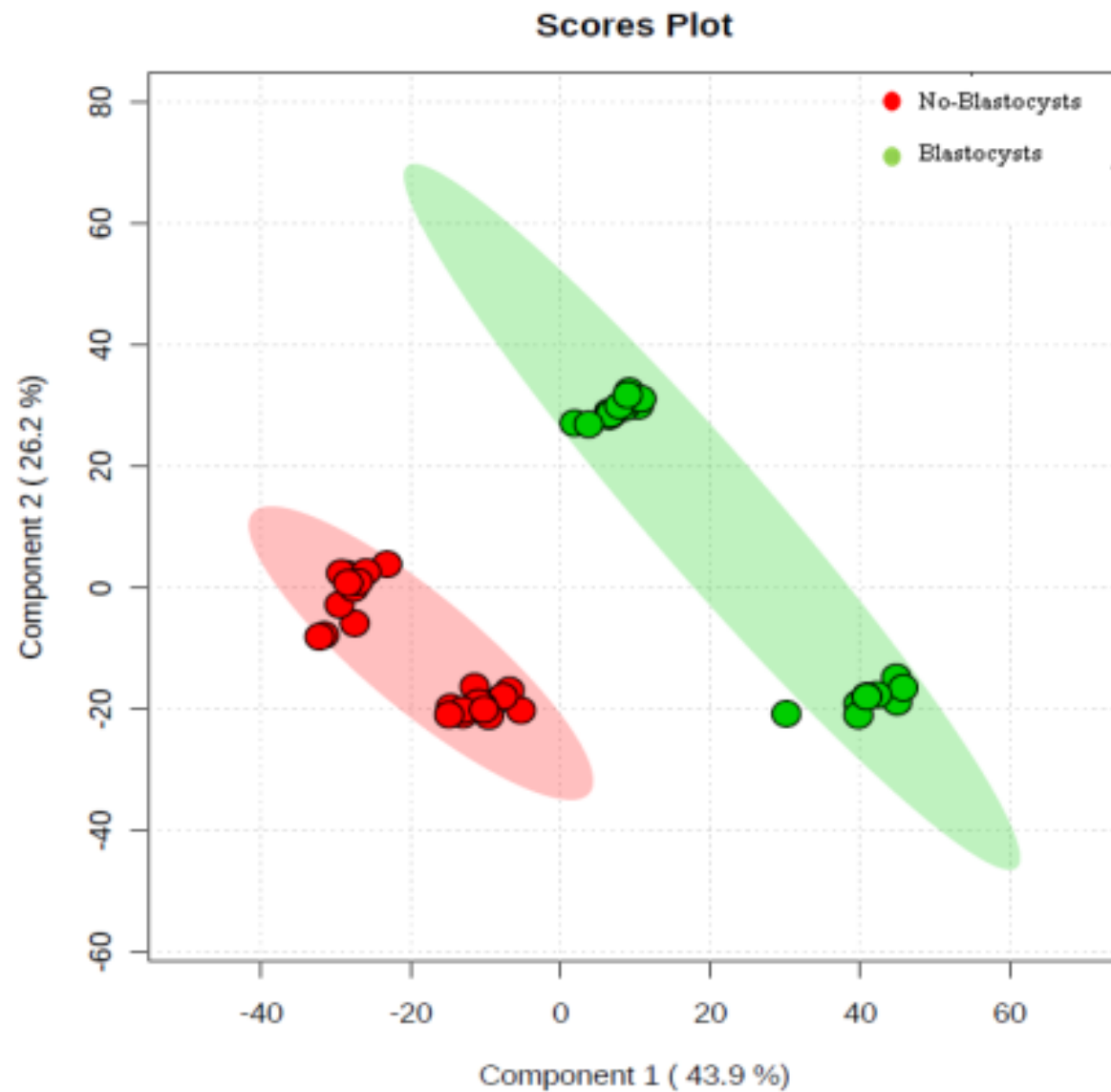
$\geq 4x$ ,  $p < 0.001$



LIPIDÔMICA  
BLASTOCISTO  
Resultados:



PLS-DA



PLS-DA plot

# LIPIDÔMICA BLASTOCISTO Resultados:

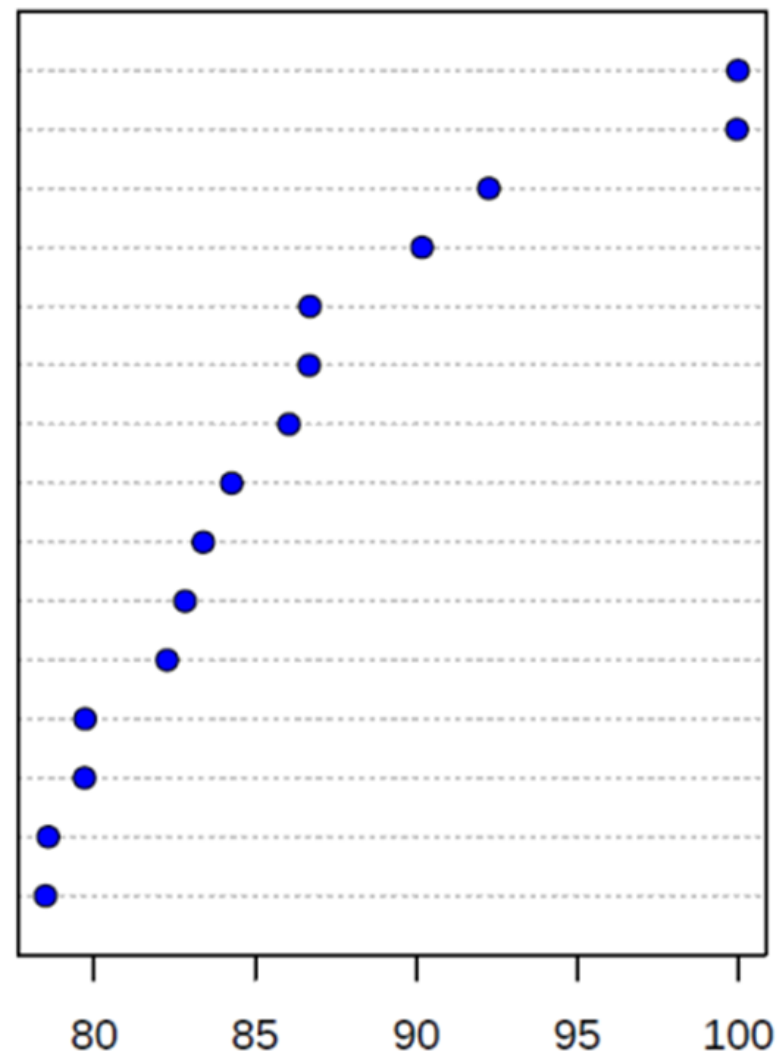


VIP

13 Grupo  
Blastocisto

2 Grupo Não-  
Blastocisto

614.4831  
570.4571  
584.4721  
385.2648  
413.3228  
474.3471  
457.3493  
628.4988  
444.3351  
672.525  
488.3626  
620.4395  
487.3606  
132.9742  
698.4966



No-Blast  
Blast



High  
Low

Coefficients

# LIPIDÔMICA BLASTOCISTO Discussão:



## Blastocyst culture and transfer in clinical-assisted reproduction: a committee opinion

The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology

American Society for Reproductive Medicine, Birmingham, Alabama

Fertility and Sterility®

### What do fertilizat

Bradley J. Van Voorhis, M.D.,<sup>a</sup> Eric Sills, M.D.,<sup>b</sup> and Robert M. Marks, Ph.D.<sup>a</sup>

<sup>a</sup> Department of Obstetrics and Gynecology, University of Iowa Carver College of Medicine, Iowa City, Iowa; <sup>b</sup> Dallas-Fort Worth Fertility Associates, Dallas, Texas; and <sup>c</sup> Colorado Center for Reproductive Medicine, Denver, Colorado

**TABLE 4**

**Intangibles mentioned by the committee as factors leading to success.**

Experience (physicians with a mean of 18 years experience after fellows training, 12–21 y)

Consistency of approach and attention to detail

Good communication between laboratory and clinic (cycle planning and cycle review)

Avoiding possible exposure of embryos to toxins (stringent quality control, powder storage, no fluorescent light, no perfumes or makeup, no personnel, limited time for embryos on the table, no back transfers)

Van Voorhis. High-quality blastocyst culture and transfer. 2010.

# LIPIDÔMICA BLASTOCISTO Discussão:



Human Reproduction Vol.17, No.3 pp. 1023–1030, 2002

Different  
profession  
blastocyst

G.M.Hartshor

J Assist Reprod Genet (2012) 29:1357–1362  
DOI 10.1007/s10815-012-9875-y

ASSISTED REPRODUCTION TECHNOLOGIES

Patient selec  
embryo cult

Daniela Paes Almeida  
Rita de Cássia S. Fig  
Assumpto Iaconelli J

Yang *et al. Molecular Cytogenetics* 2012, 5:24  
<http://www.molecularcytogenetics.org/content/5/1/24>



MOLECULAR  
CYTOGENETICS

METHODOLOGY

Open Access

Selection of single blastocysts for fresh transfer  
via stand  
with arra  
results fr

## Freeze-all policy: fresh vs. frozen-thawed embryo transfer

Fertility and Sterility® Vol. 103, No. 5, May 2015

Zhihong Yang<sup>1</sup>, Jae  
E Scott Sills<sup>1\*</sup> and R

Matheus Roque, M.D.,<sup>a</sup> Marcello Valle, M.D.,<sup>a</sup> Fernando Guimarães, B.S.,<sup>a</sup> Marcos Sampaio, M.D., Ph.D.,<sup>b</sup>  
and Selmo Geber, M.D., Ph.D.<sup>b,c</sup>

<sup>a</sup> ORIGEN, Center for Reproductive Medicine, Rio de Janeiro; <sup>b</sup> ORIGEN, Center for Reproductive Medicine, Belo Horizonte;  
and <sup>c</sup> Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

**LIPIDÔMICA  
BLASTOCISTO**  
Conclusão:



EM de alta resolução → Viabilidade técnica.

Primeiro relato da determinação do perfil lipídico em meio de cultivo de embriões humanos.

Perfil lipídico por EM → Ferramenta não invasiva para predição da formação de blastocistos.



**Obrigada!**



**FERTILITY**<sup>®</sup>  
MEDICAL GROUP



**[rita@fertility.com.br](mailto:rita@fertility.com.br)**