



COMO OTIMIZAR UM CULTIVO DE BLASTOCISTO

Rita Figueira, PhD, TS (ABB)

CONTEXTO MUNDIAL: Onde estamos?

Human Reproduction, Vol.31, No.7 pp. 1588–1609, 2016
Advanced Access publication on May 20, 2016 doi:10.1093/humrep/dew082

human
reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010[†]

S. Dyer^{1,*}, G.M. Chambers², J. de Mouzon³, K.G. Nygren⁴,
F. Zegers-Hochschild⁵, R. Mansour⁶, O. Ishihara⁷, M. Banker⁸,
and G.D. Adamson⁹

>4.461.309 procedimentos

1.144.858 bebês nascidos

Delivery rate ~20,0%

MAIN RESULTS AND THE ROLE OF CHANCE: For the years 2008, 2009 and 2010, >4 461 309 ART cycles were initiated, resulting in an estimated 1 144 858 babies born. The number of aspirations increased by 6.4% between 2008 and 2010, while FET cycles increased by 27.6%. Globally, ART utilization remained relatively constant at 436 cycles/million in 2008 and 474 cycles/million population in 2010, but with a wide country range of 8–4775 cycles/million population. ICSI remained constant at around 66% of non-donor aspiration cycles. The IVF/ICSI combined delivery rate (DR) per fresh aspiration was 19.8% in 2008; 19.7% in 2009 and 20.0% in 2010, with corresponding DRs for FET of 18.8, 19.7 and 20.7%. In fresh non-donor cycles, single embryo transfer increased from 25.7% in 2008 to 30.0% in 2010, while the average number of embryos transferred fell from 2.1 to 1.9, again with wide regional variation. The rates of twin deliveries following fresh non-donor transfers were, in 2008, 2009 and 2010, 21.8, 20.5 and 20.4%, respectively, with a corresponding triplet rate of 1.3, 1.0 and 1.1%. Fresh IVF and ICSI carried a perinatal mortality rate per 1000 births of 22.8 (2008), 19.2 (2009) and 21.0 (2010), compared with 15.1, 12.8 and 14.6/1000 births following FET in the same periods of observation. The proportion of women aged 40 years or older undergoing non-donor ART increased from 20.8 to 23.2% from 2008 to 2010.

CONTEXTO AMÉRICA LATINA: Onde estamos?



Article

Assisted reproduction techniques in Latin America: the Latin American Registry, 2014



Fernando Zegers-Hochschild ^{a,b,c,*}, Juan Enrique Schwarze ^{c,d}, Javier Crosby ^{a,c}, Carolina Musri ^{a,c}, Maria Teresa Urbina ^{c,e} on behalf of the Latin American Network of Assisted Reproduction (REDLARA)

^a Unit of Reproductive Medicine Clínica Las Condes, Lo Fontecilla 441, Santiago, Chile

^b Program of Ethics and Public Policies in Human Reproduction, University Diego Portales, Ejercito 260,

A B S T R A C T

Multinational data on assisted reproduction techniques (IVF and intracytoplasmic sperm injection [ICSI], frozen embryo transfer, oocyte donation, pre-implantation genetic diagnosis and fertility preservation) were collected from **159 institutions in 15 Latin American countries**. A total of 41.34% of IVF-ICSI cycles were conducted in women aged 35–39 years and 23.35% in women aged 40 years and older. After removing freeze-all cases, delivery rate per oocyte retrieval was 25.05% for ICSI and 27.41% for IVF. Multiple births included 20.78% twins and 0.92% triplets and over. In oocyte donation, twins reached 28.93% and triplets 1.07%. Preterm deliveries reached 16.4% in singletons, 55.02% in twins and 76% in triplets. Perinatal mortality in 18,162 births was 23 per 1000 in singletons, 35 per 1000 in twins, and 36 per 1000 in high-order multiples. Elective single embryo transfer represented 2.63% of fresh transfers, with a 32.15% delivery rate per transfer. Elective double embryo transfer represented 23.74% of transfers, with a 41.03% delivery rate per transfer; 11,373 babies (62.6%) were singletons; 6398 (35.2%) twins, and 391 (2.2%), triplets and more. Given the effect of multiple births on prematurity, morbidity and perinatal mortality, reinforcing the existing trend of reducing the number of embryos transferred is mandatory.

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65.534 ciclos iniciados

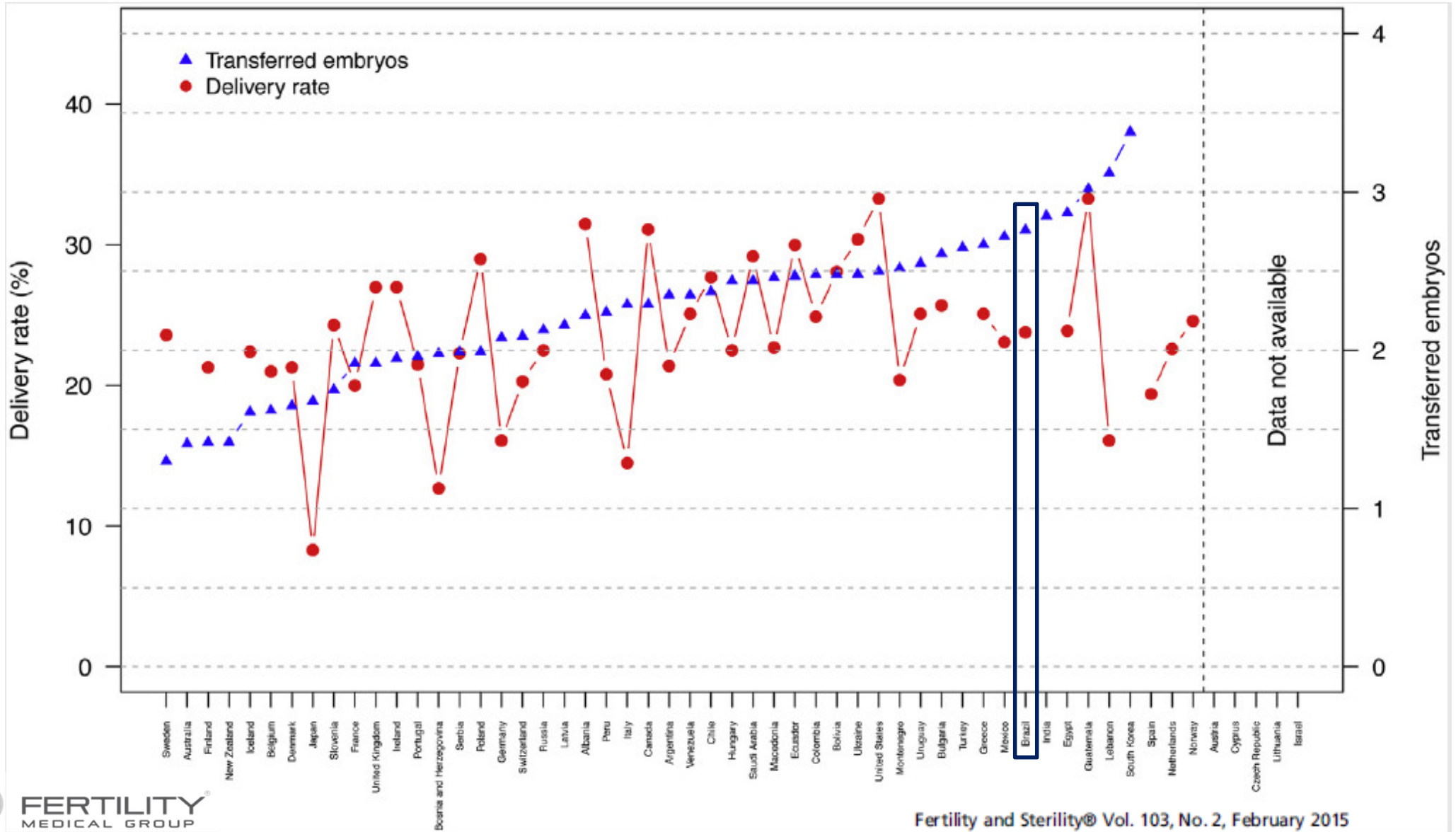
27.269 Brasil

Delivery rate ~25,1%

CONTEXTO MUNDIAL: Onde estamos?

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

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



*CULTIVO
PROLONGADO:
Considerações*

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

OPINION

To blastocyst or not to blastocyst? That is the question

Michael M.Alper^{1,5}, Peter Brinsden², Robert Fischer³ and Matts Wikland⁴

Seleção embrionária

↑ Potencial de implantação

Sincronismo embrião-endométrio

↓ Número de embriões transferidos

↓ Gestação múltipla

↑ *Delivery rate*



CULTIVO PROLONGADO: Diagnóstico genético pré- implantacional

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 7193075, 10 pages
<http://dx.doi.org/10.1155/2016/7193075>



Review Article

The Impact of Biopsy on Human Embryo Developmental Potential during Preimplantation Genetic Diagnosis

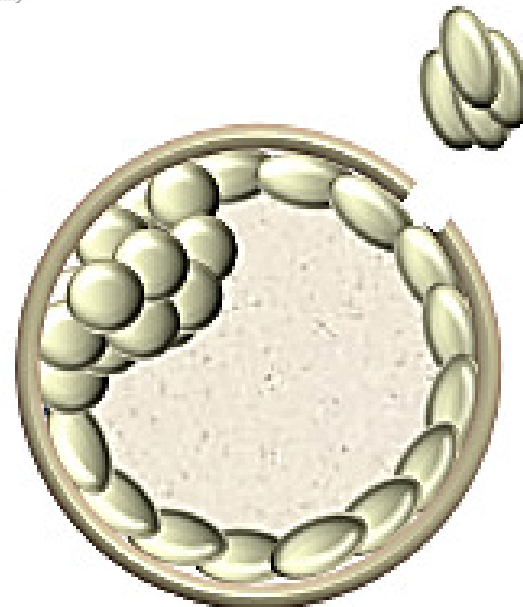
Danilo Cimadomo,^{1,2} Antonio Capalbo,^{1,3} Filippo Maria Ubaldi,^{1,3} Catello Scarica,^{1,2} Antonio Palagiano,⁴ Rita Canipari,² and Laura Rienzi^{1,3}

¹GENERA Centre for Reproductive Medicine, Clinica Valle Giulia, Via G. de Notaris 2/b, 00197 Rome, Italy

²Dipartimento di Scienze Anatomiche, University of Rome "La Sapienza", Istologiche, Medico Legali e dell'Apparato Locomotore, Sezione Istologia ed Embriologia Medica, Via Antonio Scarpa 16, 00161 Rome, Italy

³GENETYX, Molecular Biology Laboratory, Via Fermi 1, 36063 Marostica, Italy

⁴Seconda Università di Napoli, Via Antonio Vivaldi 43, 81100 Caserta, Italy



- ↓ Removal of a low proportion of total blastocyst's cell number
- ↓ Removal of a nonembryonic portion of the blastocyst
- ↓ Accurate, reliable, and reproducible
- ↓ No impact on implantation potential and cryopreservation
- ? No evidence to date of degeneration after biopsy

**CULTIVO
PROLONGADO:
Diagnóstico genético pré-
implantacional**



Blastocysts can be **rebiopsied** for preimplantation genetic diagnosis and s

Reproductive BioMedicine Online (2014) 29, 59–64



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Shuoping Zhar
Yueqiu Tan, Ph
and Ge Lin, M

^a Institute of Rep
Cell Engineering,
Research Center

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www.rbmonline.com



Human Reproduction, Vol.31, No.8 pp. 1653–1661, 2016

Advanced Access publication on June 6, 2016 doi:10.1093/humrep/dew132

ARTICLE

human
reproduction

MINI-REVIEW *Developments in Reproductive Biology and Medicine*

Outcomes



Nuclear and mitochondrial DNA in blastocoele fluid and embryo culture medium: evidence and potential clinical use

Elizabeth R. Hammond¹, Andrew N. Shelling¹, and Lynsey M. Cree^{1,2,*}

*CULTIVO
PROLONGADO:
Considerações*

Fertility and Sterility® Vol. 99, No. 3, March 1, 2013

**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

Laboratório de FIV

Sistema de cultivo / Meio de cultivo

Incubadora / Microambiente

Gases / Baixa tensão de O₂

KPIs / Taxa de blastocisto

KPIs / Criopreservação



QUALIDADE DO AR

Ambiente laboratorial

Reproductive BioMedicine Online (2016) 32, 9-11

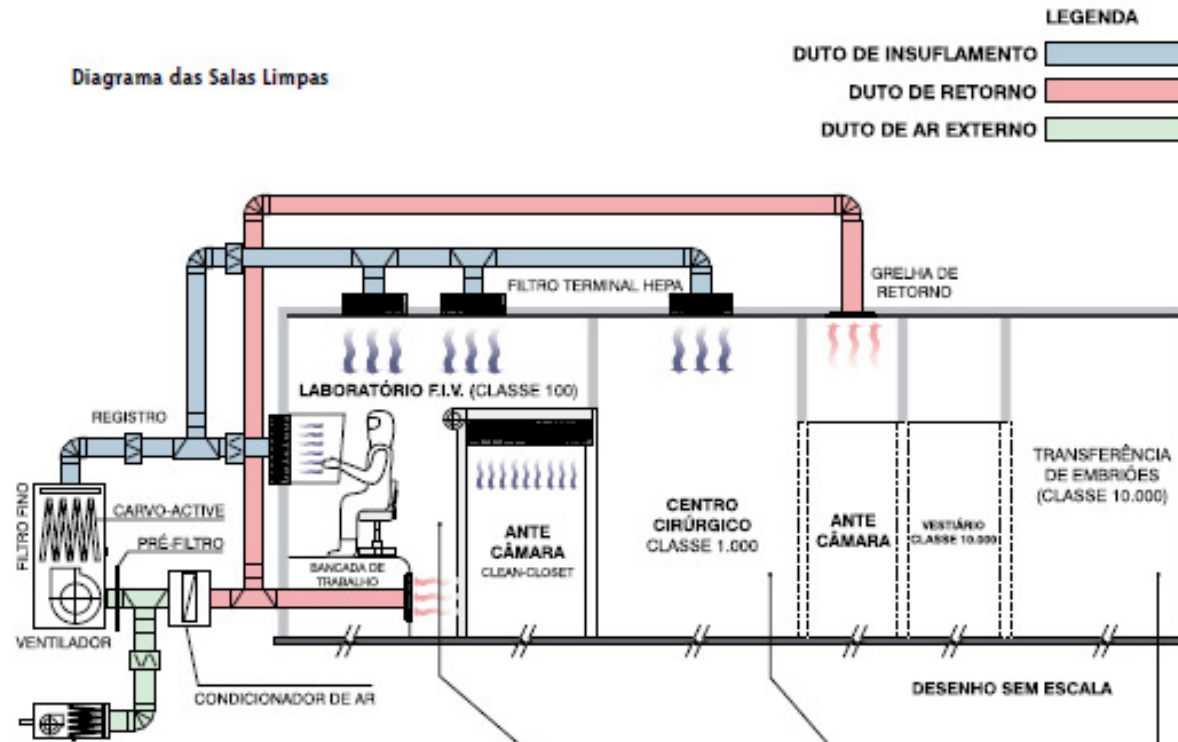


Implementation of cleanroom technology in reproductive laboratories: the question is not why but how

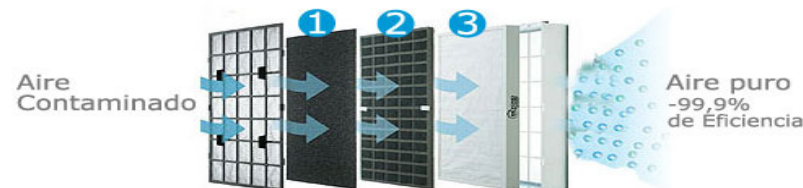
Sandro C Esteves *, Fabiola C Bento



Diagrama das Salas Limpas

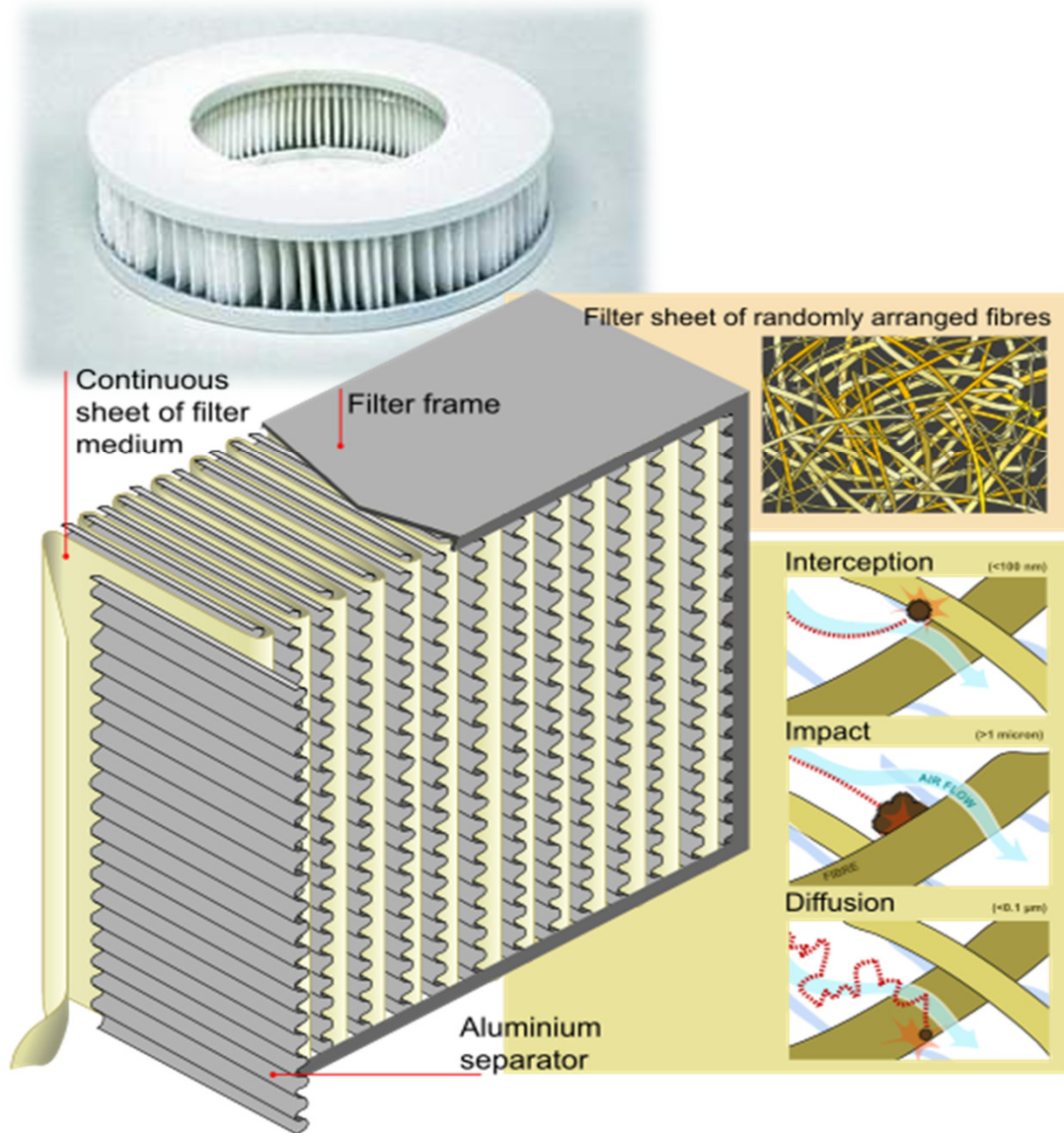


- 1 Prefiltro
 - polvo
 - pelusas
 - polen
- 2 Carbon activado
 - gases
 - olores tóxicos
- 3 Filtro HEPA
 - virus
 - bacterias
 - hongos
 - polen



QUALIDADE DO AR

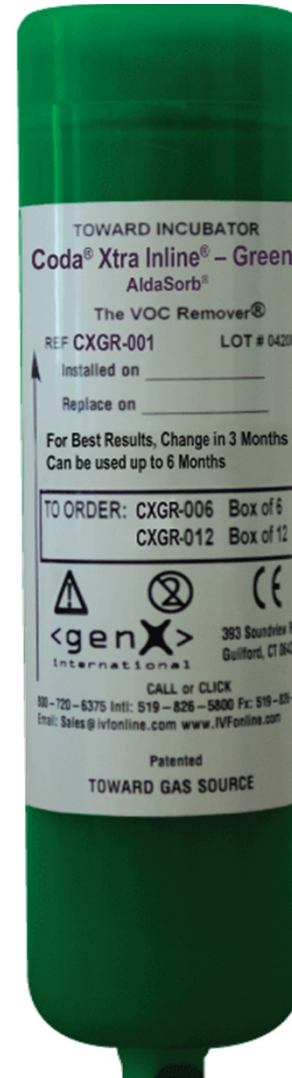
Incubadoras



Effect of air quality on assisted human reproduction[†]

Richard S. Legro^{1,*}, Mark V. Sauer², Gilbert L. Mottla³, Kevin S. Richter³, Xian Li⁴, William C. Dodson¹, and Duanping Liao⁴

¹Department of Obstetrics and Gynecology, Pennsylvania State University College of Medicine, 500 University Drive, H103, Hershey, PA 17033, USA ²Department of Obstetrics and Gynecology, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA ³Shady Grove Fertility, Rockville, MD 20850, USA ⁴Department of Public Health Sciences, Pennsylvania State University College of Medicine, Hershey, PA 17033, USA



TEMPERATURA e GASES

Incubadoras



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SYMPOSIUM: QUALITY MANAGEMENT IN ASSISTED REPRODUCTIVE TECHNOLOGY

Decisions for the IVF laboratory: comparative analysis of embryo culture incubators



Jason E Swain



Gas monitoring and recovery



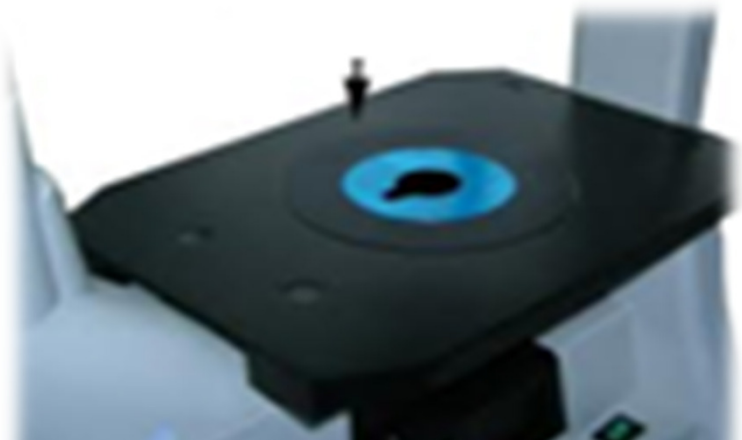
Temperature



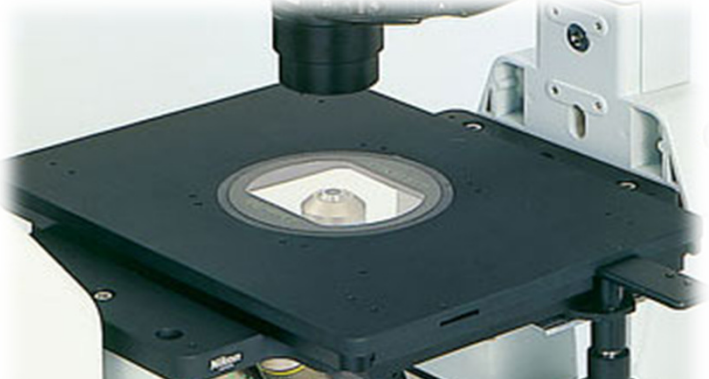
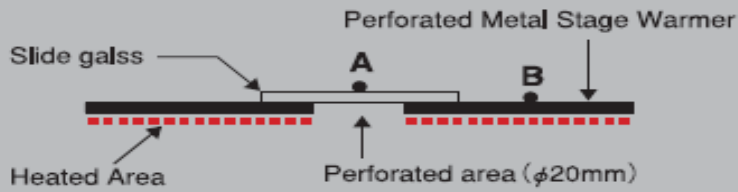
Objective Assessments of Temperature Maintenance Using In Vitro Culture Techniques¹

Simon Cooke,^{2,5} John P. P. Tyler,³ and Geoff Driscoll^{3,4}

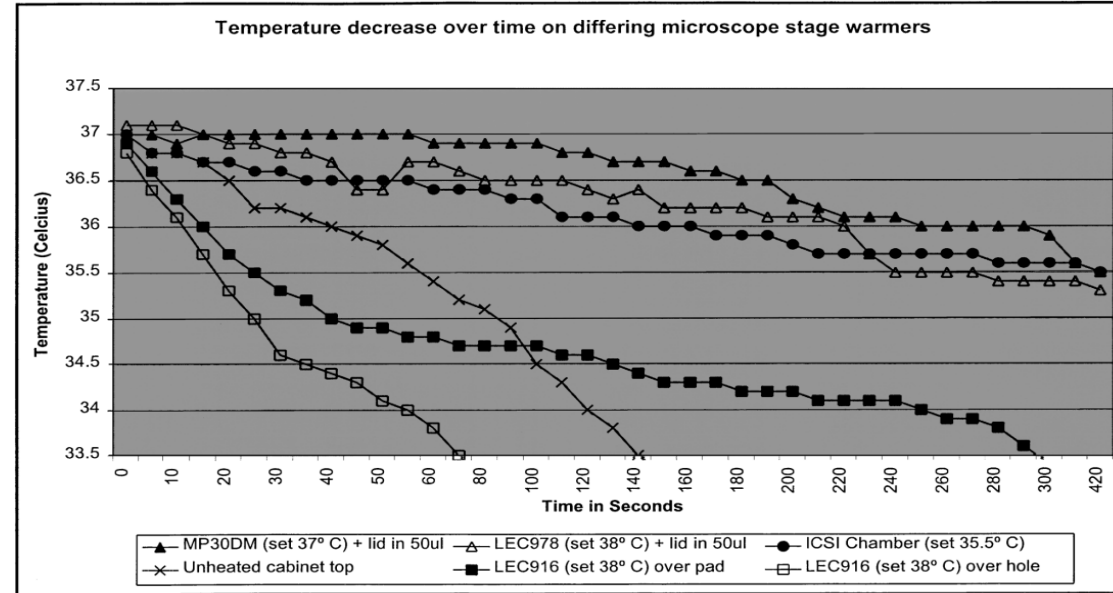
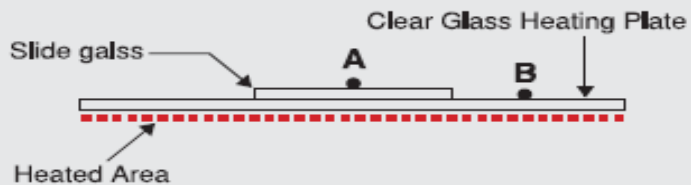
TEMPERATURA Ambiente laboratorial



Point A: 32.9°C
Point B: 37.0°C
B - A = 4.1°C



Point A: 36.8°C
Point B: 37.0°C
B - A = 0.2°C





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REVIEW

Optimizing the culture environment in the IVF laboratory: impact of pH and buffer capacity on gamete and embryo quality

pH Meios de cultivo

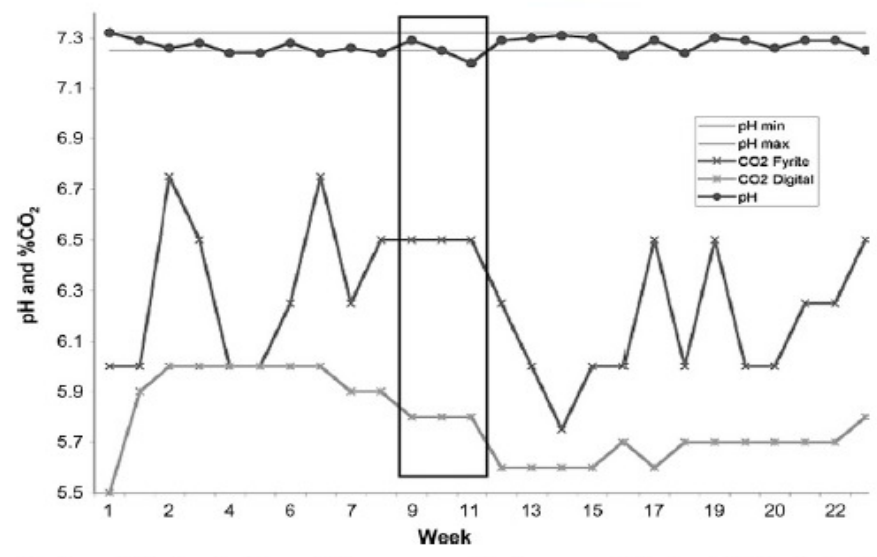


Figure 4 Demonstration of the fluctuation and inaccuracy of fyrite as an indicator of pH (adapted from Pool (2004)).

Table III Recommended pH values for various commercial IVF media (adapted from Swain, 2010).

Company	Medium	Recommended pH
Cook	Sydney IVF Cleavage	7.3–7.5
	Sydney IVF Blastocyst	7.3–7.5
	Sydney IVF Fertilization	7.3–7.5
FertiPro	FertiCult™ IVF	7.2–7.6
	FertiCult™ G3	7.3–7.6
Gynemed	GM501 Basic	7.2–7.45
	GM501 Cult	7.2–7.45
InVitroCare	IVC-ONE™	7.25–7.45
	IVC-TWO™	7.25–7.45
	IVC-THREE™	7.25–7.45
Irvine	PI®	7.27–7.32
	ECM®	7.2–7.25
	SSM™	7.28–7.32
	MultiBlast®	7.3–7.4
	HTF	7.2–7.3
Life Global	global®	7.2–7.4*
	global® for Fertilization	7.2–7.4*
	Blastocyst	7.2–7.4*
	HTF	7.2–7.4*
Origio	HTFextra	7.2–7.4*
	Universal IVF	7.3–7.4
	ISM1™	7.2–7.3
	ISM2™	7.35–7.45
	EmbryoAssist™	7.2–7.3
	BlastAssist®	7.35–7.45
Sage	EmbryoGen®	7.2–7.3
	Quinns Advantage® Fert	7.3 ± 0.1
	Quinns Advantage® Cleavage	7.2 ± 0.1
	Quinns Advantage® Blastocyst	7.3 ± 0.1
Vitrolife	G-IVF™ (G5-Series)	7.35 ± 0.1
	G-1™ (G5 Series)	7.27 ± 0.07
	G-2™ (G5 Series)	7.27 ± 0.07

*Company recommends 7.3.

*CULTIVO
PROLONGADO:
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Fertility and Sterility® Vol. 99, No. 3, March 1, 2013

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American Society for Reproductive Medicine, Birmingham, Alabama

Laboratório de FIV



Sistema de cultivo / Meio de cultivo

Incubadora / Microambiente

Gases / Baixa tensão de O₂

KPIs / Taxa de blastocisto

KPIs / Criopreservação



CULTIVO
PROLONGADO: Sistema
de cultivo

Reproductive BioMedicine Online (2010) 20, 453–469

REVIEW

Embryo culture: can we perform better than nature?

Gábor Vajta ^{a,*}, Laura Rienzi ^c, Ana Cobo ^d, John Yovich ^{a,b}

- Placas de poliestireno (35-60mm diâmetro)
- Gotas de meios de cultivo (10-50uL)
- Óleo mineral
- Estático
- Ambiente escuro e úmido
- Mistura de gases oxigênio, gás carbônico e nitrogênio
- Temperatura de 37°C

SIMPLES, PRÁTICO e REPRODUTÍVEL



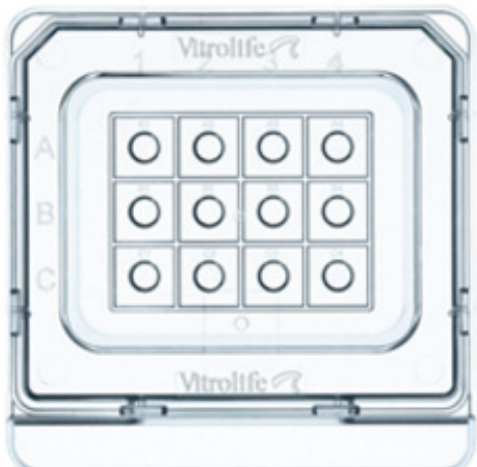
SISTEMA DE CULTIVO:
Placas de cultivo

- Polystyrene may compromise growth of adherent cells (Summer et al. 2012)
 - Alters microenvironment – pH, ROS

Embryo-Specific Dishes

- Rounded bottoms/edges for easy location
 - Rapid identification, control embryo spacing
- Prevent microdrop dispersion or displacement

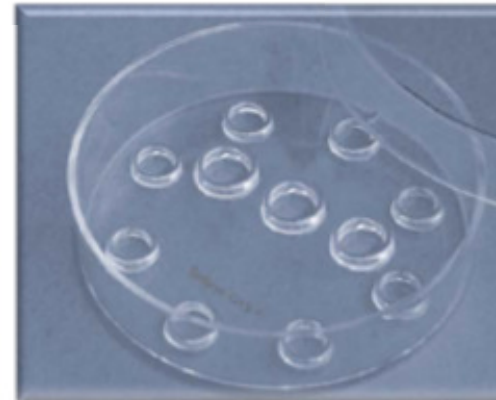
Co - cultivo



Microdroplet Dish



Embryo Corral®

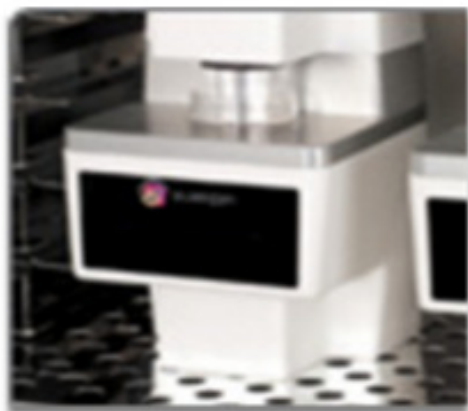


Embryo GPS®



SISTEMA DE CULTIVO:
Placas de cultivo

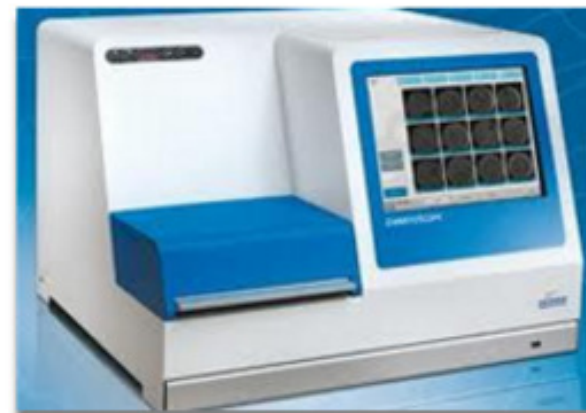
Eeva™



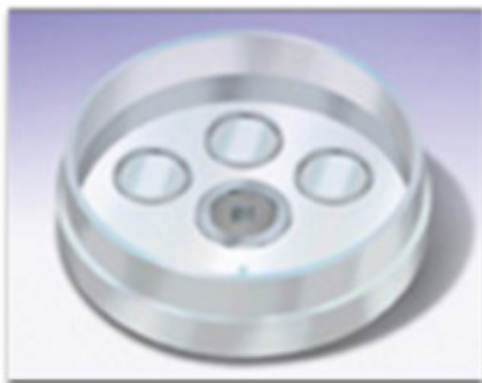
Primo Vision™



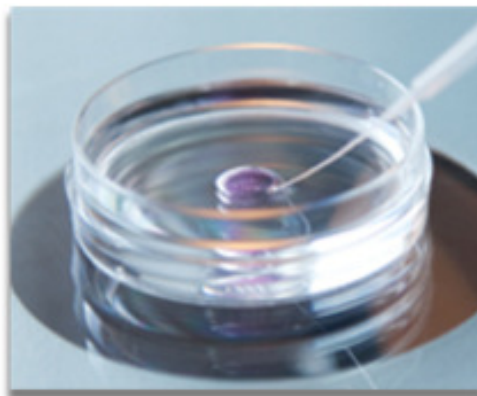
EmbryoScope™



Eeva dish



WOW

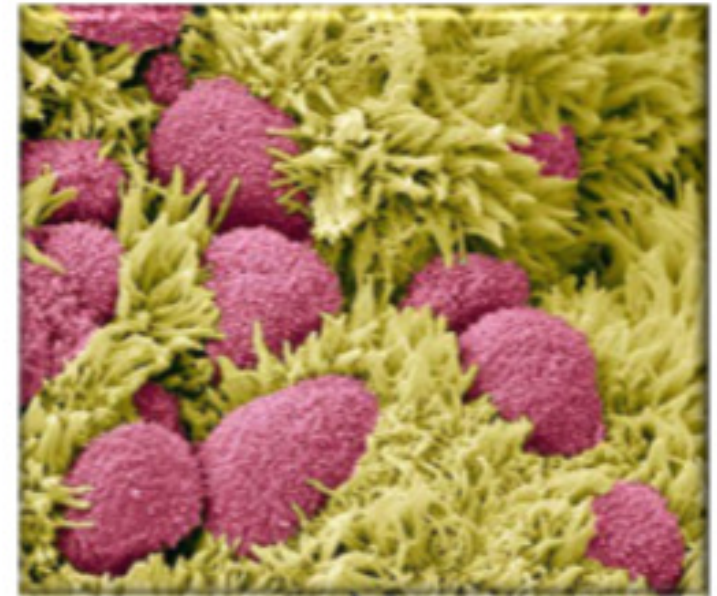
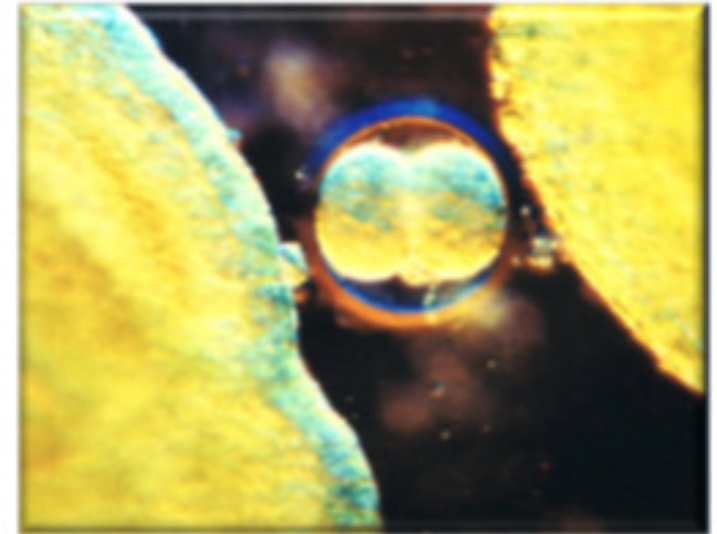


EmbryoSlide™



SISTEMA DE CULTIVO: *Dinâmica do cultivo*

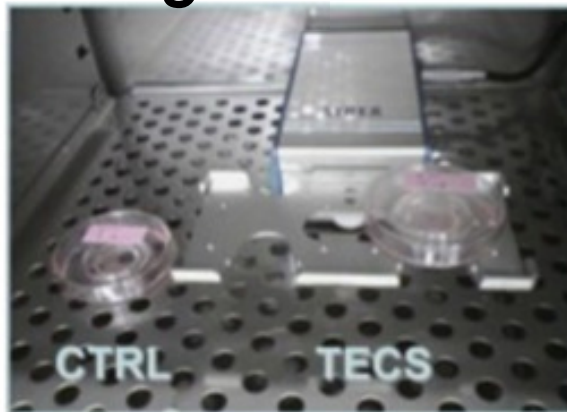
- *In vivo* – batimento ciliar e peristaltismo das contrações musculares
 - Beating frequency of 5-20Hz
(Paltiel et al. 1995, Westrom et al. 1977)
 - Average speed $\sim 0.1\mu\text{m/s}$ (Greenwald 1961)
 - Sheer force $\sim 0-3\text{dyn/mm}^2$



Gentle movement may be “normal” for embryos

SISTEMA DE CULTIVO:
Dinâmica do cultivo

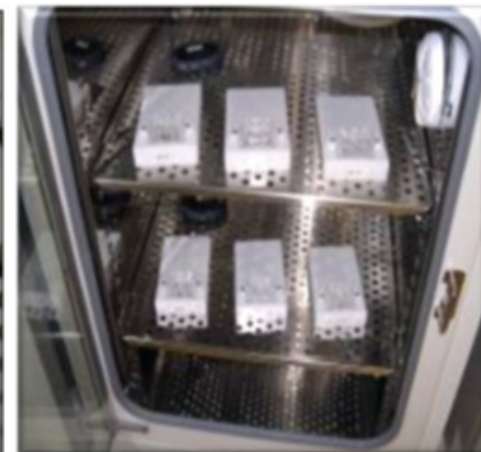
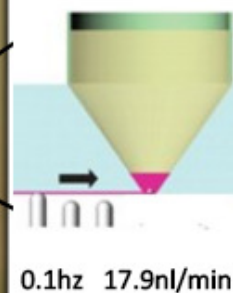
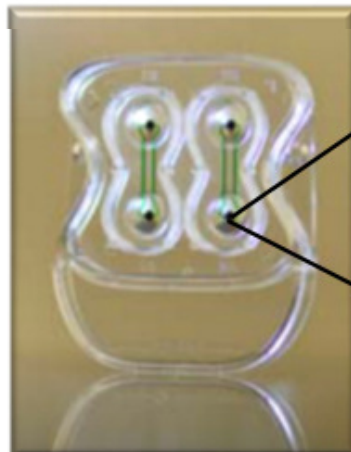
Tilting



Vibration



Pulsatile flow



SISTEMA DE CULTIVO:
Dinâmica do cultivo

Optimizing the culture environment and embryo manipulation to help maintain embryo developmental potential

Jason E. Swain, Ph.D.,^a Doug Carrell, Ph.D.,^b Ana Cobo, Ph.D.,^c Marcos Meseguer, Ph.D.,^c Carmen Rubio, Ph.D.,^d and Gary D. Smith, Ph.D.^e

TABLE 6

Examples of dynamic human embryo culture aimed at replicating growth conditions embryos may experience in vivo.

Approach	Embryo source	Outcome measures	Outcome summary	Reference
Tilting	Frozen day 3 embryos	Blastocyst development High-quality blastocysts Cell no.	Equivalent blastocyst formation Improved cell no.	(151)
	Fresh embryos	Day 5 blastocyst development High-quality blastocysts Positive βhCG	Improved blastocyst development Increased pregnancy	(152)
Vibration	Fresh zygotes	Day 3 embryo quality Blastocyst development Pregnancy rate	Improved day 3 quality Increased blastocyst development Higher pregnancy rate	(153, 154)
	Fresh zygotes	Pregnancy from day 3 transfer Implantation rate	Higher pregnancy rate Higher implantation rate	(155)
Pulsatile Flow	Fresh zygotes	Day 5 blastocyst development Embryo fragmentation Cleavage-stage quality	Greater blastocyst formation Lower fragmentation on day 2 and day 3 Greater number of top-quality embryos	(156)

Swain. *Optimizing IVF laboratory conditions. Fertil Steril* 2016.

MEIO DE CULTIVO: Único ou sequencial?

Noninferiority, randomized, controlled trial comparing embryo development using media developed for sequential or undisturbed culture in a time-lapse setup

Thorir Hardarson, Ph.D.,^a Mona Bungum, Ph.D.,^b Joe Conaghan, Ph.D.,^c Marius Meintjes, Ph.D.,^d Samuel J. Chantilis, M.D.,^d Laszlo Molnar, Ph.D.,^e Kristina Gunnarsson, M.Sc.,^a and Matts Wikland, Ph.D.^a

^a Fertilitetscentrum, Carlanderska Hospital, Gothenburg, Sweden; ^b Reproductive Medicine Centre, Skåne University Hospital, Malmö, Sweden; ^c Pacific Fertility Center, San Francisco, California; ^d Frisco Institute for Reproductive Medicine, Dallas/Austin, Texas; and ^e MediBit Foundation, Budapest, Hungary

Objective: To study whether a culture medium that allows undisturbed culture supports human embryo development to the blastocyst stage equivalently to a well-established sequential media.

Design: Randomized, double-blinded sibling trial.

Setting: Independent in vitro fertilization (IVF) clinics.

Patient(s): One hundred twenty-eight patients, with 1,356 zygotes randomized into two study arms.

Intervention(s): Embryos randomly allocated into two study arms to compare embryo development on a time-lapse system using a single-step medium or sequential media.

Main Outcome Measure(s): Percentage of good-quality blastocysts on day 5.

Result(s): Percentage of day 5 good-quality blastocysts was 21.1% (standard deviation [SD] \pm 21.6%) and 22.2% (SD \pm 22.1%) in the single-step time-lapse medium (G-TL) and the sequential media (G-1/G-2) groups, respectively. The mean difference (-1.2 ; 95% CI, -6.0 ; 3.6) between the two media systems for the primary end point was less than the noninferiority margin of -8% . There was a statistically significantly lower number of good-quality embryos on day 3 in the G-TL group [50.7% (SD \pm 30.6%) vs. 60.8% (SD \pm 30.7%)]. Four out of the 11 measured morphokinetic parameters were statistically significantly different for the two media used. The mean levels of ammonium concentration in the media at the end of the culture period was statistically significantly lower in the G-TL group as compared with the G-2 group.

Conclusion(s): We have shown that a single-step culture medium supports blastocyst development equivalently to established sequential media. The ammonium concentrations were lower in the single-step media, and the measured morphokinetic parameters were modified somewhat.

Clinical Trial Registration Number: NCT01939626. (Fertil Steril® 2015;104:1452–9. ©2015 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Key Words: Blastocyst, sequential media, single-step medium, time-lapse, undisturbed culture

Discuss: You can discuss this article with its authors and with other ASRM members at <http://fertilityforum.com/hardarson-evaluation-time-lapse-medium/>



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*MEIO DE CULTIVO:
Único ou sequencial?*

Sequential versus Monophasic Media Impact Trial (SuMMIT): a paired randomized controlled trial comparing a sequential media system to a monophasic medium

Marie D. Werner, M.D.,^{a,b} Kathleen H. Hong, M.D.,^{a,b} Jason M. Franasiak, M.D.,^b Eric J. Forman, M.D., H.C.L.D.,^{a,b} Christine V. Reda, B.S.N., R.N.,^a Thomas A. Molinaro, M.D., M.S.C.E.,^{a,b} Kathleen M. Upham, B.S.,^a and Richard T. Scott Jr., M.D., H.C.L.D.,^{a,b}

Result(s): A total of 192 patients had their 2PN embryos (N = 2,257) randomized to each culture system. Sequential media had higher blastulation rate than monophasic medium (55.2% vs. 46.9%). No differences were found in the day of blastulation or aneuploidy rate. Of the 168 patients who had euploid blastocysts suitable for transfer, 126 completed a paired ET. **Among the double ETs, there was no difference in implantation between groups.**

Conclusion(s): This is the first randomized controlled trial to examine paired euploid transfers of sibling zygotes cultured in sequential versus monophasic media. This study demonstrates that the usable blastocyst rate is greatest after culture in the sequential media tested in comparison with the monophasic formulation selected for study. However, no difference exists in timing of blastulation, aneuploidy, or SIR. Whether these observations are generalizable to other media systems remains to be determined.

Clinical Trial Registration Number: NCT01917240. (Fertil Steril[®] 2016;105:1215–21. ©2016 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)).

Key Words: Embryo culture, media for IVF, embryo development, in vitro fertilization, culture media

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*MEIO DE CULTIVO:
Cultivo ininterrupto ou
renovação do meio?*

Ammonium accumulation in commercially available embryo culture media and protein supplements during storage at 2–8°C and during incubation at 37°C

Sander H.M. Kleijkers¹, Aafke P.A. van Montfoort¹, Otto Bekers², Edith Coonen¹, Josien G. Derhaag¹, Johannes L.H. Evers¹, and John C.M. Dumoulin^{1,*}

¹Department of Obstetrics and Gynaecology, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands ²Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, The Netherlands

*Correspondence address. E-mail: john.dumoulin@mumc.nl

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WIDER IMPLICATIONS OF THE FINDINGS: Break-down of components into ammonium is more pronounced during incubation at 37°C, however, it is not negligible during storage at 2–8°C. This results in increasing ammonium levels in culture media over time that may affect embryo development. Therefore, it is important that the use of free L-glutamine in human embryo culture media is stopped and that the use of protein supplements is thoroughly evaluated.

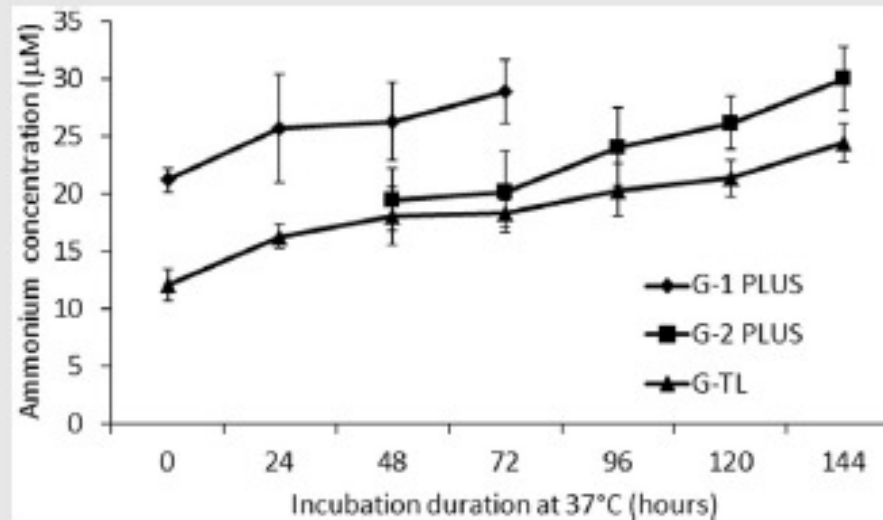
MEIO DE CULTIVO:
*Cultivo ininterrupto ou
renovação do meio?*

Noninferiority, randomized, controlled trial comparing embryo development using media developed for sequential or undisturbed culture in a time-lapse setup

Thorir Hardarson, Ph.D.,^a Mona Bungum, Ph.D.,^b Joe Conaghan, Ph.D.,^c Marius Meintjes, Ph.D.,^d Samuel J. Chantilis, M.D.,^d Laszlo Molnar, Ph.D.,^e Kristina Gunnarsson, M.Sc.,^a and Matts Wikland, Ph.D.^a

^a Fertilitetscentrum, Carlanderska Hospital, Gothenburg, Sweden; ^b Reproductive Medicine Centre, Skåne University Hospital, Malmö, Sweden; ^c Pacific Fertility Center, San Francisco, California; ^d Frisco Institute for Reproductive Medicine, Dallas/Austin, Texas; and ^e MediBit Foundation, Budapest, Hungary

FIGURE 2



Ammonium concentration in G-1, G-2 (sequential), and G-TL (single-step time-lapse) media at 37°C over time.

Hardarson. Evaluation of a time-lapse medium. *Fertil Steril* 2015.

*MEIO DE CULTIVO:
Cultivo ininterrupto ou
renovação do meio?*

Blastocyst development in single medium with or without renewal on day 3: a prospective cohort study on sibling donor oocytes in a time-lapse incubator

Nuno Costa-Borges, Ph.D.,^a Marta Bellés, M.Sc.,^b Marcos Meseguer, Ph.D.,^c Daniela Galliano, M.D.,^b Agustin Ballesteros, M.D.,^b and Gloria Calderón, Ph.D.^a

Conclusion(s): Our findings support the idea that in a TLI with a controlled air purification system, human embryos can be successfully cultured continuously from day 0 onward in single medium with no need to renew it on day-3. This strategy does not affect embryo morphokinetics or development to term and offers more stable culture conditions for embryos as well as practical advantages and reduced costs for the IVF laboratory. (Fertil Steril® 2016;105:707-13. ©2016 by American Society for Reproductive Medicine.)

Key Words: Morphokinetic events, single-step medium, time-lapse incubator, uninterrupted culture

Discuss: You can discuss this article with its authors and with other ASRM members at <http://fertilityforum.com/costaborgesn-uninterrupted-culture-single-medium/>



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MEIOS DE CULTIVO: Composição - considerações

Human Reproduction, Vol.31, No.10 pp. 2174–2182, 2016

Advanced Access publication on August 23, 2016 doi:10.1093/humrep/dew157

human
reproduction

ESHRE PAGES

Time to take human embryo culture seriously[†]

Arne Sunde^{1,*}, Daniel Brison², John Dumoulin³, Joyce Harper⁴,
Kersti Lundin⁵, M. Cristina Magli⁶, Etienne Van den Abbeel⁷,
and Anna Veiga⁸

¹Department of Obstetrics and Gynaecology, St. Olav's University Hospital in Trondheim, Trondheim, Norway ²Department of Reproductive Medicine, St Mary's Hospital, Central Manchester NHS Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, UK ³Department of Obstetrics and Gynaecology, IVF Laboratory, Maastricht University Medical Center, Maastricht, The Netherlands ⁴Embryology, IVF and reproductive genetics group, Institute for Women's Health, University College London, London, UK ⁵Reproductive Medicine, Sahlgrenska University Hospital, Göteborg, Sweden ⁶SISMER, Reproductive Medicine Unit, Bologna, Italy ⁷Reproductive Medicine, Gent University Hospital, Gent, Belgium ⁸Reproductive Medicine Service, Hospital Universitari Dexeus, Barcelona, Spain

*Correspondence address. E-mail: trondheimivf@gmail.com

STUDY QUESTION: Is it important that end-users know the composition of human embryo culture media?

SUMMARY ANSWER: We argue that there is as strong case for full transparency concerning the composition of embryo culture media intended for human use.

WHAT IS KNOWN ALREADY: Published data suggest that the composition of embryo culture media may influence the phenotype of the offspring.

MEIOS DE CULTIVO: Composição - considerações

Composition of single-step media used for human embryo culture

Dean E. Morbeck, Ph.D.,^{a,b} Nikola A. Baumann, Ph.D.,^b and Devin Oglesbee, Ph.D.^b

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<http://dx.doi.org/10.1016/j.fertnstert.2017.01.007>

TABLE 1

Concentrations of glucose and organic acids, and the lactate to pyruvate (L/P) ratio in embryo culture media.

Variable	Medium			
	Global	CSC	G-TL	1-Step
Glucose (mM)	0.18	0.47	0.97	0.19
Citrate (mM)	0	0.02	0.01	0
Octanoate (mM)	0.681	0.324	0.344	0.355
Lactate (mM)	4.9	5.71	10.01	4.35
Pyruvate (mM)	0.24	0.28	0.55	0.22
L/P ratio	20	21	18	20

Morbeck. Composition of continuous culture media. Fertil Steril 2017.

TABLE 2

Amino acid

Lys	176	184	204	204
Lys	182	182	182	182
Met	54	54	54	54
Phe	91	92	92	92
Thr	176	184	204	204
Trp	20	21	23	23
Tyr	75	80	83	83
Val	163	174	200	196
Nonessential (µM)				
Ala	46	48	63	38
Asn	42	46	40	36
Asp	42	43	12	58
Glu	40	41	0	49
Gln	0	0	10	36
Gly	42	44	185	48
Pro	55	60	126	66
Ser	40	42	96	46
Tau	0	0	48	0

Morbeck. Composition of continuous culture media. Fertil Steril 2017.

**The most well kept secret,
embryo culture media: a smart
reveal from an expert**



Marcos Meseguer, Ph.D.
Antonio Pellicer, M.D.
Instituto Valenciano de Infertilidad, Universidad de Valencia,
Valencia, Spain

<http://dx.doi.org/10.1016/j.fertnstert.2017.02.116>
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Fertility and Sterility® Vol. 99, No. 3, March 1, 2013

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Laboratório de FIV



Sistema de cultivo / Meio de cultivo



Incubadora / Microambiente

Gases / Baixa tensão de O₂

KPIs / Taxa de blastocisto

KPIs / Criopreservação



INCUBADORAS: Microambiente - controle



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SYMPOSIUM: QUALITY MANAGEMENT IN ASSISTED REPRODUCTIVE TECHNOLOGY

Decisions for the IVF laboratory: comparative analysis of embryo culture incubators



Jason E Swain

Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI 48108, United States; Fertility Lab Sciences, Englewood, CO 80112, United States
E-mail address: jswain@fertilitylabsciences.com

Table 1 Incubator technology variables that should be considered when evaluating and selecting a unit for the laboratory.

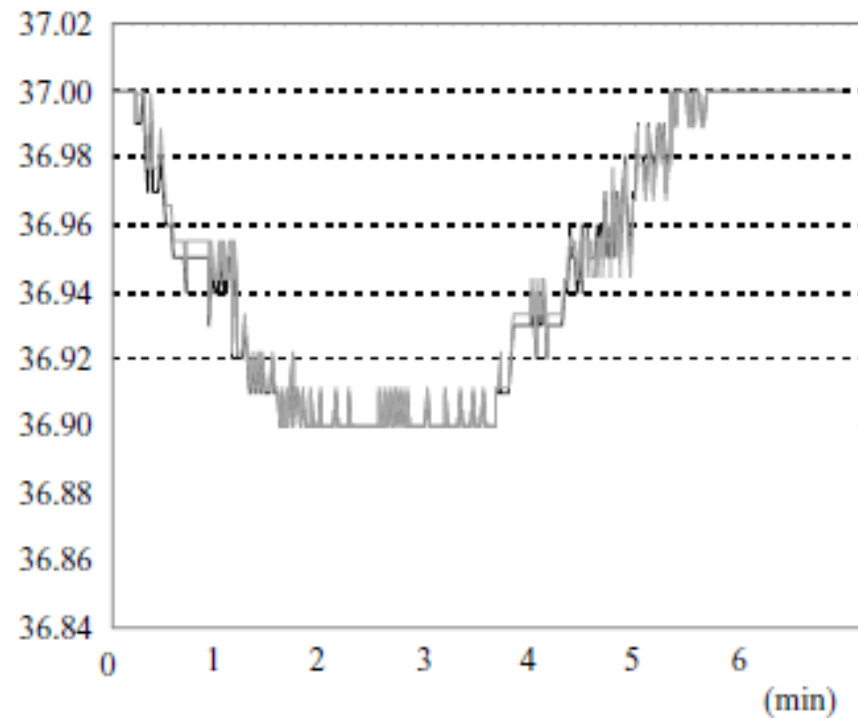
Gas type	CO ₂ sensor	O ₂ sensor	Temperature control ^a	Design ^b	Humidity control	Contamination control ^{a,c}	Other
CO ₂ -only	Infrared	Zirconium	Air jacket	Benchtop	Yes ^d	Heat	Data logging
Low O ₂ – mixer	Thermal conductivity	Galvanic (fuel-cell)	Water jacket	Two-chamber	No	UV	Cost
Low O ₂ – premixed cylinder			Direct heat	Multichamber		H ₂ O ₂	Patient capacity
				Other (i.e. time-lapse imaging)		Copper alloy	Service
				Small box		External HEPA	Technology integration
			Large box				

INCUBADORAS: Microambiente - controle

Benchttop Incubators

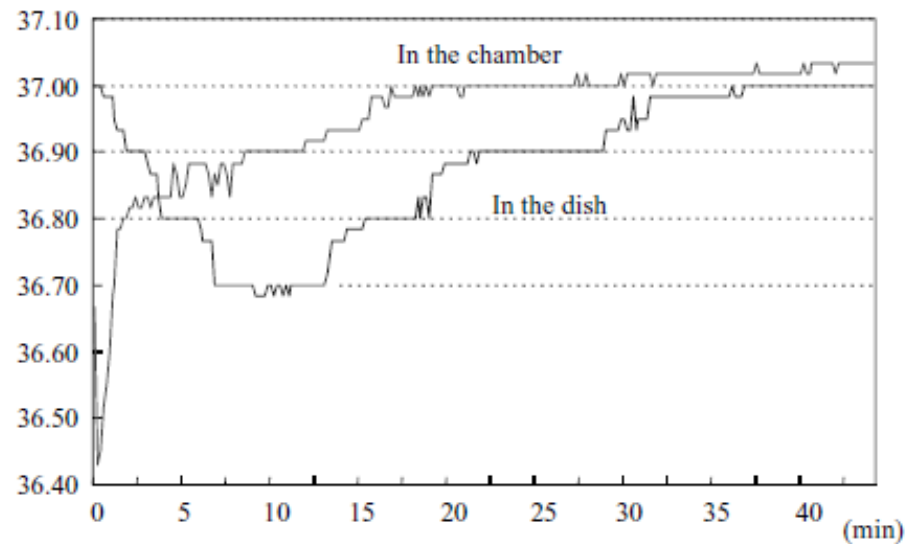


Temperature (°C)



— In the dish
— In the chamber

Temperature (°C)



INCUBADORAS:
Microambiente - controle

Optimizing the culture environment and embryo manipulation to help maintain embryo developmental potential

Jason E. Swain, Ph.D.,^a Doug Carrell, Ph.D.,^b Ana Cobo, Ph.D.,^c Marcos Meseguer, Ph.D.,^c Carmen Rubio, Ph.D.,^d and Gary D. Smith, Ph.D.^e

TABLE 5

Impact of different culture temperature on human embryo development.

Temperature	Meta phase IIs	Fertility rate, %	Day 3 cell no.	Blast rate, %	Usable blast rate, %	Aneuploidy rate, %	Implantation rate, %
36°C	399	86.2	7.0 ± 0.1 ^a	51.6 ^a	41.2 ^a	42.5	67.4
37°C	406	82.0	7.7 ± 0.1 ^a	60.1 ^a	48.4 ^a	46.1	73.3



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SYMPOSIUM: QUALITY MANAGEMENT IN ASSISTED REPRODUCTIVE TECHNOLOGY

Decisions for the IVF laboratory: comparative analysis of embryo culture incubators



Jason E Swain

Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI 48108, United States; Fertility Lab Sciences, Englewood, CO 80112, United States
E-mail address: jswain@fertilitylabsciences.com

Table 1 Incubator technology variables that should be considered when evaluating and selecting a unit for the laboratory.

Gas type	CO ₂ sensor	O ₂ sensor	Temperature control ^a	Design ^b	Humidity control	Contamination control ^{a,c}	Other
CO ₂ -only	Infrared	Zirconium	Air jacket	Benchtop	Yes ^d	Heat	Data logging
Low O ₂ – mixer	Thermal conductivity	Galvanic (fuel-cell)	Water jacket	Two-chamber	No	UV	Cost
Low O ₂ – premixed cylinder			Direct heat	Multichamber		H ₂ O ₂	Patient capacity
				Other (i.e. time-lapse imaging)		Copper alloy	Service
				Small box		External HEPA	Technology integration
				Large box			

Incubator selection is an important decision for the IVF laboratory, as these devices regulate several environmental variables that can impact embryo development. While novel culture approaches may reduce the need for traditional incubators (Blockeel et al., 2009; Hyslop et al., 2012; Itoi et al., 2012; Ranoux and Seibel, 1990; Ranoux et al., 1988; Suzuki et al., 1999; Swain, 2010, 2011; Taymor et al., 1992; Vajta et al., 1997, 2004; Van Blerkom et al., 2013; Varisanga et al., 2000), for the time being they remain a central part of a modern IVF laboratory. Functional aspects of the incubator, such as gas capability and sensor type, as well as temperature control and size/patient capacity need to be considered. **Smaller incubator units, especially benchtop/topload devices, result in faster gas atmosphere and temperature recovery. However, no study has clearly demonstrated a distinct advantage of any specific incubator type in terms of human embryo development or clinical outcomes.** Regardless of the unit, low-O₂ capability should be available and utilized and a IR CO₂ probe is preferable for those units that mix the gases to permit the fastest CO₂ recovery. Practical issues, such as cost and space, must also be weighed. The proper number and type of incubators to

INCUBADORAS: Microambiente - controle



RESEARCH ARTICLE

Does time-lapse imaging have favorable results for embryo incubation and selection compared with conventional methods in clinical in vitro fertilization? A meta-analysis and systematic review of randomized controlled trials

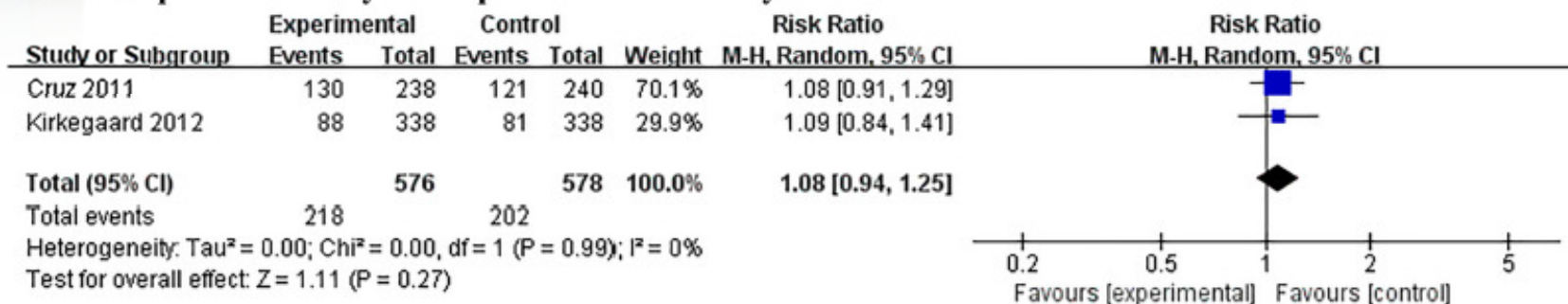
Minghao Chen¹*, Shiyu Wei²*, Junyan Hu³*, Jing Yuan⁴, Fenghua Liu¹*

1 Reproductive Center, Guangdong Women and Children Hospital, Guangzhou, China, **2** Department of Thoracic Surgery, West China Hospital, Sichuan University, Chengdu, China, **3** Department of Emergency, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China, **4** School of Traditional Chinese Medicine, Jinan University, Guangzhou, China

Table 3. Summary of findings of RCTs for the comparison between TLI and conventional methods for incubation and embryo selection in assisted reproduction.

	Outcomes	Subgroup ^a	RR (95% CI)	N ^b (studies)	I ²	Interpretation	Quality of the evidence
Oocyte-based review	Balstocyst formation		1.08 (0.94, 1.25)	1154(2)	0%	No difference	Moderate ^c
	Good quality embryo on Day 2/3		0.89 (0.72, 1.11)	720 (3)	42%	No difference	Moderate ^d

A. Forest plot of blastocyst rate per randomized oocyte in the studies



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Gases / Baixa tensão de O₂

KPIs / Taxa de blastocisto

KPIs / Criopreservação



**CULTIVO
PROLONGADO: Baixa
tensão de O₂**

Human Reproduction, Vol.24, No.2 pp. 300–307, 2009

Advanced Access publication on October 16, 2008 doi:10.1093/humrep/den368

human
reproduction

ORIGINAL ARTICLE *Embryology*

A controlled randomized trial evaluating the effect of lowered incubator oxygen tension on live births in a predominantly blastocyst transfer program[†]

Marius Meintjes^{1,3}, Samuel J. Chantilis², James D. Douglas², Alfred J. Rodriguez², Ali R. Guerami², David M. Bookout², Brian D. Barnett², and James D. Madden¹

¹Frisco Institute for Reproductive Medicine, Frisco, TX 75034, USA ²Presbyterian Hospital ARTS Program, Dallas, TX 75231, USA

³Corresponding address. PO Box 703575, Dallas, TX 75370, USA. Fax: +1-972-382-3093. E-mail: mariusmeintjes@aol.com

RESULTS: Embryos cultured in a 5% O₂ environment consistently resulted in higher rates of live birth implantation (106/247, 42.9% versus 82/267, 30.7%; difference of 12.2% with 95% confidence interval (CI) of 3.9–20.3, $P = 0.005$) and live births (66/115, 57.4% versus 49/115, 42.6%; difference of 14.8% with 95% CI of 1.9–27.0, $P = 0.043$) when compared with rates among women whose embryos were cultured in an atmospheric O₂ environment.

CONCLUSIONS: The overall increase in live births demonstrated by this study indicates that the effort and expense to culture embryos in a low-O₂ environment is justified.

The study was registered at clinicaltrials.gov. NCT00708487.

CULTIVO PROLONGADO: Baixa tensão de O₂



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COMMENTARY

The impact of physiological oxygen during culture, and vitrification for cryopreservation, on the outcome of extended culture in human IVF



David K Gardner *

School of BioSciences, University of Melbourne, Australia

* E-mail address: david.gardner@unimelb.edu.au

The consensus (or otherwise) about oxygen concentrations in human IVF laboratories

A recent online survey, in which 265 clinics from 54 different countries participated, revealed that <25% of IVF human embryo culture is performed exclusively under physiological (-5%) oxygen (Christianson et al., 2014). Although this survey represents only a small fraction of the world's IVF clinics, what is notable from the Christianson paper, and from an extensive literature review of the past 10 years, is a clear geographic difference with regard to the use of 5% oxygen, with Australia, New Zealand and Japan representing the only countries to employ, almost exclusively, physiological oxygen for their human embryo culture. The widespread adoption of reduced oxygen in Australian IVF clinics can be readily attributed to several key studies dating back to 1969 from a number of Australian laboratories showing beneficial effects of reduced oxygen on the embryos of many different mammalian species (reviewed by [Wale and Gardner, 2016](#)). In the survey of Christianson and colleagues, 34% of clinics reported the use of 5% oxygen for some aspects of embryo culture while the majority of clinics did not use 5% oxygen at all. Given that even a transient exposure to oxygen has been shown to negatively affect development ([Pabon et al., 1989](#); [Wale and Gardner, 2010](#)), it would appear that most human embryos worldwide experience oxidative stress in the IVF laboratory. So does oxygen concentration really matter and can it affect fetal development?

CULTIVO PROLONGADO: Baixa tensão de O₂

Low versus atmospheric oxygen tension for embryo culture in assisted reproduction: a systematic review and meta-analysis

Carolina O. Nastri, Ph.D., Beatrice N. Nóbrega, M.D., Danielle M. Teixeira, M.D., Jowanka Amorim, M.Sc., Lívia M. M. Diniz, M.D., Marina W. P. Barbosa, M.D., Vanessa S. I. Giorgi, M.Sc., Vicky N. Pileggi, M.Sc., and Wellington P. Martins, Ph.D.

Department of Obstetrics and Gynecology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

TABLE 3

Summary of finding tables: low versus atmospheric oxygen tension for embryo culture in assisted reproduction.

Characteristics	Absolute risk			No. of participants (studies)	I ²	Interpretation	Quality of the evidence
	AtmO ₂	LowO ₂ (95% CI)	RR (95% CI)				
Part A: Clinical outcomes							
Comparison LowO ₂ vs. AtmO ₂ during all embryo culture							
Live birth/ongoing pregnancy	38%	43% (39%–47%)	1.1 (1.0–1.3)	5,401 (8)	25%	LowO ₂ is better	Very low ^{1,2,3}
Clinical pregnancy	42%	47% (44%–50%)	1.1 (1.0–1.2)	5,501 (9)	0	LowO ₂ is better	Very low ^{1,2,3}
Miscarriage	11%	9% (7%–12%)	0.9 (0.6–1.2)	2,371 (8)	26%	No difference	Very low ^{1,2,4}
Comparison LowO ₂ vs. AtmO ₂ after Day 2							
Live birth/ongoing pregnancy	44%	43% (65%–74%)	1.0 (0.9–1.1)	2,389 (1)	–	No difference	Low ⁵
Clinical pregnancy	47%	47% (43%–51%)	1.0 (0.9–1.1)	2,389 (1)	–	No difference	Low ⁵
Miscarriage	7%	9% (6%–14%)	1.2 (0.8–1.8)	1,125 (1)	–	No difference	Very low ^{4,5}
Part B: Laboratory outcomes							
Comparison LowO ₂ vs. AtmO ₂ during all embryo culture							
Fertilization	69%	69% (67%–71%)	1.0 (1.0–1.0)	9,765 (5)	0	No difference	Low ⁵
Cleavage	56%	59% (56%–61%)	1.0 (1.0–1.1)	7,565 (3)	5%	No difference	Low ⁵
High/Top cleavage	22%	26% (24%–28%)	1.2 (1.1–1.3)	9,302 (4)	27%	LowO ₂ is better	Low ⁵
Blastocyst	–	–	–	–	–	–	–
High/Top blastocyst	–	–	–	–	–	–	–
Comparison LowO ₂ vs. AtmO ₂ before Day 3 followed by LowO ₂ in both groups							
Blastocyst	24%	26% (22%–31%)	1.1 (0.9–1.3)	1,737 (1)	–	No difference	Very low ^{4,5}
High/Top blastocyst	9%	11% (9%–15%)	1.3 (1.0–1.8)	1,737 (1)	–	No difference	Very low ^{4,5}
Comparison LowO ₂ vs. AtmO ₂ after Day 2							
Cleavage	31%	68% (45%–100%)	2.2 (1.4–3.3)	126 (1)	–	LowO ₂ is better	Very low ^{4,5}
High/Top cleavage	–	–	–	–	–	–	–
Blastocyst	20%	32% (18%–60%)	1.6 (0.9–3.0)	126 (1)	–	No difference	Very low ^{4,5}
High/Top blastocyst	–	–	–	–	–	–	–
Part C: Pregnancy outcomes							
No study							

Note: AtmO₂ = embryo culture in atmospheric oxygen; CI = confidence interval; LowO₂ = embryo culture in low oxygen; RR = relative risk; 1 = downgraded one level because of the limitations of the included studies; 2 = downgraded one level because of the high risk of publication bias; 3 = downgraded one level because of imprecision: the observed effect is small and the confidence interval almost reaches the no-effect line; 4 = downgraded one level because of imprecision, confidence interval is wide; 5 = downgraded two levels because of serious limitations of the included studies for these outcomes.

Nastri. Low oxygen for embryo culture. *Fertil Steril* 2016.

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Gases / Baixa tensão de O₂



KPIs / Taxa de blastocisto

KPIs / Criopreservação



CULTIVO PROLONGADO: KPIs Taxa de blastocistos

Review

The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators

ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine ^{a,b,*}



^a European Society of Human Reproduction and Embryology, Meerstraat 60, B-1852 Grimbergen, Belgium

^b ALPHA Scientists in Reproductive Medicine, 19 Mayıs Mah. 19 Mayıs Cad. Nova Baran Center No:4 34360 Sisli, Istanbul, Turkey

Table 4 – Key performance indicators.

Key performance indicator	Calculation	Competency value	Benchmark value
ICSI damage rate	$\frac{\text{no. damaged or degenerated}}{\text{all oocytes injected}} \times 100$	≤10%	≤5%
ICSI normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. MII oocytes injected}} \times 100$	≥65%	≥80%
IVF normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. COC inseminated}} \times 100$	≥60%	≥75%
Failed fertilization rate (IVF)	$\frac{\text{no. cycles with no evidence of fert'n}}{\text{no. of stimulated IVF cycles}} \times 100$		<5%
Cleavage rate	$\frac{\text{no. cleaved embryos on Day 2}}{\text{no. 2PN/2PB oocytes on Day 1}} \times 100$	≥95%	≥99%
Day 2 embryo development rate	$\frac{\text{no. 4-cell embryos on Day 2}}{\text{no. normally fertilized oocytes}^a} \times 100$	≥50%	≥80%
Day 3 embryo development rate	$\frac{\text{no. 8-cell embryos on Day 3}}{\text{no. normally fertilized oocytes}^a} \times 100$	≥45%	≥70%
Blastocyst development rate	$\frac{\text{no. blastocysts Day 5}}{\text{no. normally fertilized oocytes}^a} \times 100$	≥40%	≥60%
Successful biopsy rate	$\frac{\text{no. biopsies with DNA detected}}{\text{no. biopsies performed}} \times 100$	≥90%	≥95%
Blastocyst cryosurvival rate	$\frac{\text{no. blastocysts appearing intact}}{\text{no. blastocysts warmed}} \times 100$	≥90%	≥99%
Implantation rate (cleavage stage) ^b	$\frac{\text{no. sacs seen on ultrasound}^c}{\text{no. embryos transferred}} \times 100$	≥25%	≥35%
Implantation rate (blastocyst stage) ^b	$\frac{\text{no. sacs seen on ultrasound}^c}{\text{no. blastocysts transferred}} \times 100$	≥35%	≥60%

**CULTIVO
PROLONGADO:
KPIs Taxa de blastocistos**

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^a European Society of Human Reproduction and Embryology, Meerstraat 60, B-1852 Grimbergen, Belgium

^b ALPHA Scientists in Reproductive Medicine, 19 Mayıs Mah. 19 Mayıs Cad. Nova Baran Center No:4 34360 Sisli, Istanbul, Turkey

Table 3 – Performance Indicators.

Performance Indicator	Calculation	Competency value	Benchmark value
Sperm motility post-preparation (for IVF and IUI)	$\frac{\text{progressively motile sperm}}{\text{all sperm counted}} \times 100$	90%	≥95%
IVF polyspermy rate	$\frac{\text{no. fertilized oocytes with } > 2\text{PN}}{\text{no. COC inseminated}} \times 100$		<6%
1PN rate (IVF)	$\frac{\text{no. 1PN oocytes}}{\text{no. COC inseminated}} \times 100$		<5%
1PN rate (ICSI)	$\frac{\text{no. 1PN oocytes}}{\text{no. MII oocytes injected}} \times 100$		<3%
Good blastocyst development rate	$\frac{\text{no. good quality blastocysts on Day 5}}{\text{no. 2PN/2PB oocytes on Day 1}} \times 100$	≥30%	≥40%

COC = cumulus-oocyte complexes; ICSI = intracytoplasmic sperm injection; IUI = intrauterine insemination; PB = polar body; PN = pronucleus.

*CULTIVO
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Considerações*

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KPIs / Criopreservação



**CULTIVO
PROLONGADO:
KPIs Taxa de sobrevivida**

Review

The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators

ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine ^{a,b,*}



^a European Society of Human Reproduction and Embryology, Meerstraat 60, B-1852 Grimbergen, Belgium

^b ALPHA Scientists in Reproductive Medicine, 19 Mayıs Mah. 19 Mayıs Cad. Nova Baran Center No:4 34360 Sisli, Istanbul, Turkey

Table 4 – Key performance indicators.

Key performance indicator	Calculation	Competency value	Benchmark value
ICSI damage rate	$\frac{\text{no. damaged or degenerated}}{\text{all oocytes injected}} \times 100$	≤10%	≤5%
ICSI normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. MII oocytes injected}} \times 100$	≥65%	≥80%
IVF normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. COC inseminated}} \times 100$	≥60%	≥75%
Failed fertilization rate (IVF)	$\frac{\text{no. cycles with no evidence of fert'n}}{\text{no. of stimulated IVF cycles}} \times 100$		<5%
Cleavage rate	$\frac{\text{no. cleaved embryos on Day 2}}{\text{no. 2PN/2PB oocytes on Day 1}} \times 100$	≥95%	≥99%
Day 2 embryo development rate	$\frac{\text{no. 4-cell embryos on Day 2}}{\text{no. normally fertilized oocytes}^a} \times 100$	≥50%	≥80%
Day 3 embryo development rate	$\frac{\text{no. 8-cell embryos on Day 3}}{\text{no. normally fertilized oocytes}^a} \times 100$	≥45%	≥70%
Blastocyst development rate	$\frac{\text{no. blastocysts Day 5}}{\text{no. normally fertilized oocytes}^a} \times 100$	≥40%	≥60%
Successful biopsy rate	$\frac{\text{no. biopsies with DNA detected}}{\text{no. biopsies performed}} \times 100$	≥90%	≥95%
Blastocyst cryosurvival rate	$\frac{\text{no. blastocysts appearing intact}}{\text{no. blastocysts warmed}} \times 100$	≥90%	≥99%
Implantation rate (cleavage stage) ^b	$\frac{\text{no. sacs seen on ultrasound}^f}{\text{no. embryos transferred}} \times 100$	≥25%	≥35%
Implantation rate (blastocyst stage) ^b	$\frac{\text{no. sacs seen on ultrasound}^f}{\text{no. blastocysts transferred}} \times 100$	≥35%	≥60%

*CULTIVO
PROLONGADO:
Considerações*

Fertility and Sterility® Vol. 99, No. 3, March 1, 2013

**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

Laboratório de FIV



Sistema de cultivo / Meio de cultivo



Incubadora / Microambiente



Gases / Baixa tensão de O₂



KPIs / Taxa de blastocisto



KPIs / Criopreservação



*CULTIVO
PROLONGADO:
Sempre?*

Cleavage-stage or blastocyst transfer: what are the benefits and harms?

Demían Glujovsky, M.D., M.Sc.^a and Cynthia Farquhar, M.D., M.P.H.^b

ET is a critical step in an assisted reproduction cycle. Over the past decade there has been an increasing trend to extending culture from cleavage-stage to blastocyst transfer. There has also been a trend to single ET and reporting the success of an assisted reproductive cycle as a cumulative live-birth rate after using both fresh and frozen embryos. There is low evidence that fresh blastocyst transfer is associated with improved live-birth rates compared with fresh cleavage-stage embryos. However, in the few studies that report cumulative pregnancy rates after fresh and frozen transfers, no significant difference was found. Cleavage-stage transfer is associated with greater numbers of embryos available for freezing, and blastocyst transfer is associated with increased number of cycles with no embryos to transfer. Further well-designed studies are warranted to evaluate the outcomes for blastocyst transfer including cumulative live-birth rate after fresh and frozen transfers, time to live birth, costs of the different transfer strategies, and perinatal mortality and severe perinatal morbidity. (Fertil Steril® 2016;106:244–50. ©2016 by American Society for Reproductive Medicine.)

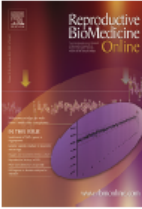
Key Words: Embryo transfer, cleavage, blastocyst

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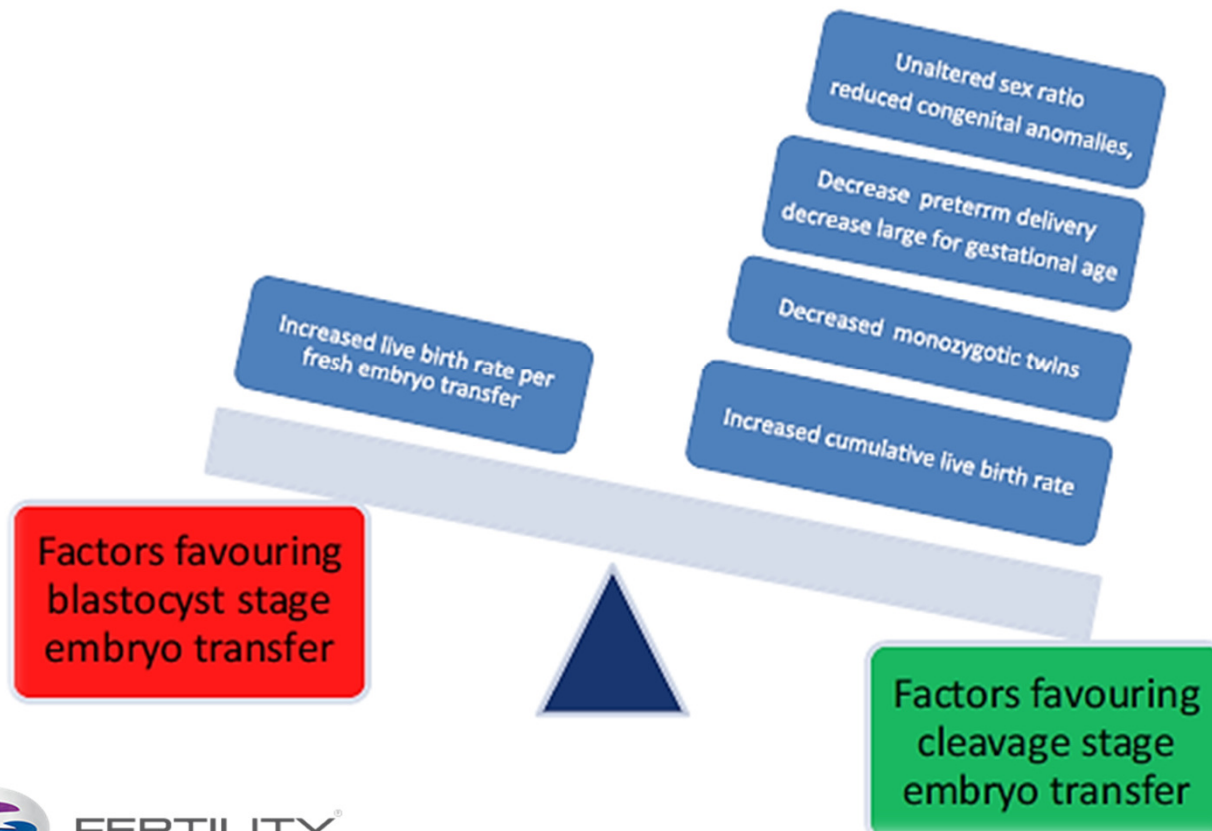


COMMENTARY

Should we be promoting embryo transfer at blastocyst stage?



Abha Maheshwari ^{a,*}, Mark Hamilton ^a, Siladitya Bhattacharya ^b



CULTIVO PROLONGADO: Quando?

Critérios clínicos

Risco de
cancelamento de
transferência

Individualização

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

ASSISTED REPRODUCTION TECHNOLOGIES

Patient selection criteria for blastocyst transfers in extended embryo culture programs

Daniela Paes Almeida Ferreira Braga · Amanda S Setti ·
Rita de Cássia S. Figueira · Rogério Bonassi Machado ·
Assumpto Iaconelli Jr. · Edson Borges Jr.

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

Different perspectives of patients and health care professionals on the potential benefits and risks of blastocyst culture and multiple embryo transfer

G.M.Hartshorne^{1,3} and R.J.Lilford²



OBRIGADA!

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Is there a new future for poor-quality embryos?



rita@fertility.com.br