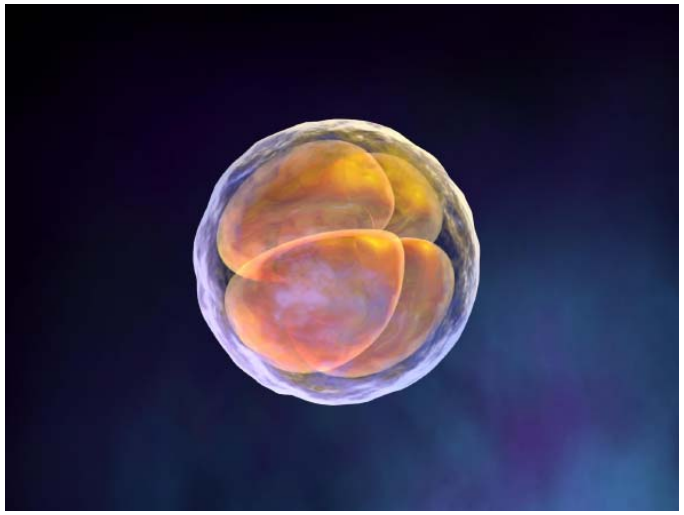
Optimizing Human Embryo Care



Time lapse culture system

Genova 10 maggio 2013

Dott.ssa Valentina Parini

Dipartimento di Ginecologia
UO di Ginecologia e Medicina della riproduzione
Humanitas Clinical and Research Istitute
Direttore, Paolo Emanuele Levi Setti

Time lapse

Tecnica nella quale la frequenza di cattura di ogni fotogramma è molto inferiore a quella di riproduzione

Mediante questa tecnica cinematografica, è infatti possibile documentare eventi non visibili ad occhio nudo o la cui evoluzione nel tempo è poco percettibile dall'occhio umano come il movimento apparente del sole e delle stelle sulla volta celeste, il trascorrere delle stagioni, il movimento delle nuvole o lo sbocciare di un fiore.

Valutazione embrionale statica

Criteri tradizionali

Valutazione dei pronuclei e percentuale di singamia dall'inseminazione

Valutazione morfologica dell'embrione

- dimensione dei blastomeri
- percentuale di frammentazione
- multinucleazione

Controllo fecondazione e divisione al microscopio

- possibile variazione di pH, T, esposizione alla luce

Human Reproduction, Vol.26, No.6 pp. 1270–1283, 2011
Advanced Access publication on April 18, 2011 doi:10.1093/humrep/der037

The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting†

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

Table VI Consensus scoring system for cleavage-stage embryos (in addition to cell number).

Grade	Rating	Description
1	Good	• <10% fragmentation • Stage-specific cell size • No multinucleation
2	Fair	• 10–25% fragmentation • Stage-specific cell size for majority of cells • No evidence of multinucleation
3	Poor	• Severe fragmentation (>25%) • Cell size not stage specific • Evidence of multinucleation

Table IV Timing of observation of fertilized oocytes and embryos, and expected stage of development at each time point.

Type of observation	Timing (hours post-insemination)	Expected stage of development
Fertilization check	17 ± 1	Pronuclear stage
Syngamy check	23 ± 1	Expect 50% to be in syngamy (up to 20% may be at the 2-cell stage)
Early cleavage check	26 ± 1 h post-ICSI 28 ± 1 h post-IVF	2-cell stage
Day-2 embryo assessment	44 ± 1	4-cell stage
Day-3 embryo assessment	68 ± 1	8-cell stage
Day-4 embryo assessment	92 ± 2	Morula
Day-5 embryo assessment	116 ± 2	Blastocyst

ICSI, intracytoplasmic sperm injection.

Table VIII Consensus scoring system for blastocysts.

	Grade	Rating	Description
Stage of development	1		Early
	2		Blastocyst
	3		Expanded
	4		Hatched/hatching
ICM	1	Good	Prominent, easily discernible, with many cells that are compacted and tightly adhered together
	2	Fair	Easily discernible, with many cells that are loosely grouped together
	3	Poor	Difficult to discern, with few cells
TE	1	Good	Many cells forming a cohesive epithelium
	2	Fair	Few cells forming a loose epithelium
	3	Poor	Very few cells

The scoring system for blastocysts is a combination of the stage of development, and of the grade of the ICM and of the TE (e.g. an expanded blastocyst with a good ICM and a fair TE would be scored as 3 1 2). It is a numerical interpretation of the Gardner scale (Gardner and Schoolcraft, 1999a,b).

Valutazione con time lapse

Possibilità di osservazione continua

Breve illuminazione di ciascun embrione con una luce rossa di bassa intensità in 3 giorni di coltura: 635nm 30ms per immagine per un totale medio di illuminazione di 57 secondi vs 167 secondi con osservazione al microscopio

I dati vengono inviati ad una stazione di acquisizione dell'immagine e di rielaborazione dati

Mantenimento costante dei parametri della coltura (temperatura, pH)

Human Reproduction, Vol.27, No.5 pp. 1277–1285, 2012
Advanced Access publication on March 14, 2012 doi:10.1093/humrep/des079

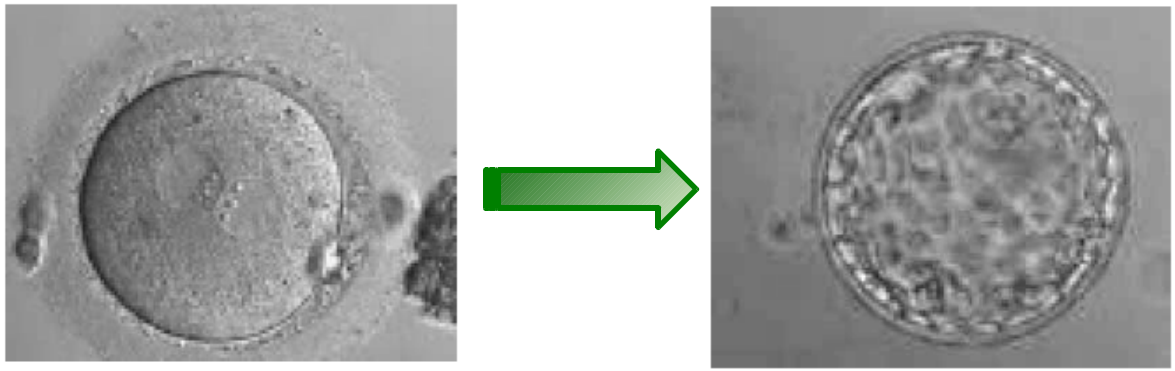
human reproduction

REVIEW *Embryology*

Time-lapse monitoring as a tool for clinical embryo assessment

Kirstine Kirkegaard[1,*], Inge E. Agerholm[2], and Hans Jakob Ingerslev[1]

Review sul time-lapse e sulla valutazione dell'embrione



Partendo dalla fecondazione fino allo sviluppo della blastocisti

Sistemi di rilevamento Time Lapse

Primo Vision Evo
Cryo Innovation

Pribenszky, 2010

Home made system

Payne 1997
Hardarson, 2002
Wong, 2010
Lemmen, 2008

Embryoscope
Unisense Fertilitech

Meseguer, 2010
Cruz, 2012

Home made system

Human Reproduction vol.12 no.3 pp.532–541, 1997

Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography

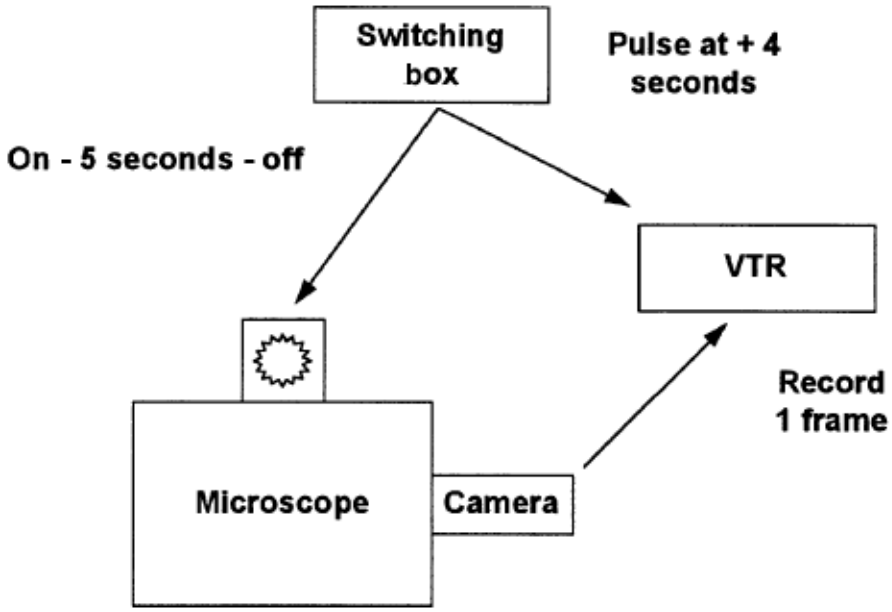
Dianna Payne[1], Sean P.Flaherty, Michael F.Barry and Colin D.Matthews

Time-lapse video recording

A single, randomly selected oocyte was prepared for time-lapse video recording shortly after injection by placing it in a pre-equilibrated 5 µl drop of HTF containing serum under 4 ml mineral oil (Sigma Chemical Co.). Recording commenced within 30 min of injection.

Standard culture conditions were maintained during time-lapse recording by culturing the oocytes on the stage of an IX-70 inverted microscope (Olympus Optical Co., Tokyo, Japan) equipped with a perspex environmental chamber which was maintained at 37°C and had a humidified atmosphere of 5% CO_2 in air. The chamber was fully covered during the course of recording to exclude extraneous light, and most of the recording occurred during the hours of darkness. Nomarski DIC optics and glass Petri dishes (Glaswerk, Wertheim, Germany) were used to ensure the highest image quality.

A purpose-built switching box turned the microscope lamp on for 5 s every minute (Figure 1). The colour temperature of the lamp stabilized after 4 s, at which time a pulse was sent from the switching box to signal the video recorder (S-VHS time-lapse video cassette recorder, AG-6730; Panasonic, Tokyo, Japan) to capture one frame. The time at which each frame was captured was appended automatically to the recorded image. The microscope light was then switched off. This sequence was repeated every minute for the duration of the recording period (17–20 h). Images were captured using a TK 1280E low-light CCD colour video camera (JVC, Tokyo, Japan), which ran continuously and allowed the recording of a suitable image at a light intensity of only 6 lux. Immediately after the completion of recording, oocytes were returned to conventional culture for an additional 48 h prior to transfer or cryopreservation.



Human Reproduction vol.12 no.3 pp.532–541, 1997

Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography

Dianna Payne[1], Sean P.Flaherty, Michael F.Barry and Colin D.Matthews

38 ovociti fecondati da ICSI

Tempo che intercorre tra le immagini = 1 min permette di avere una descrizione precisa degli eventi dopo la fertilizzazione.

1. Estrusione dei PB
2. Sincronizzazione nella formazione dei PN
3. Allineamento dei nucleoli

→ sono correlati positivamente alla qualità embrionaria in 3° giornata.

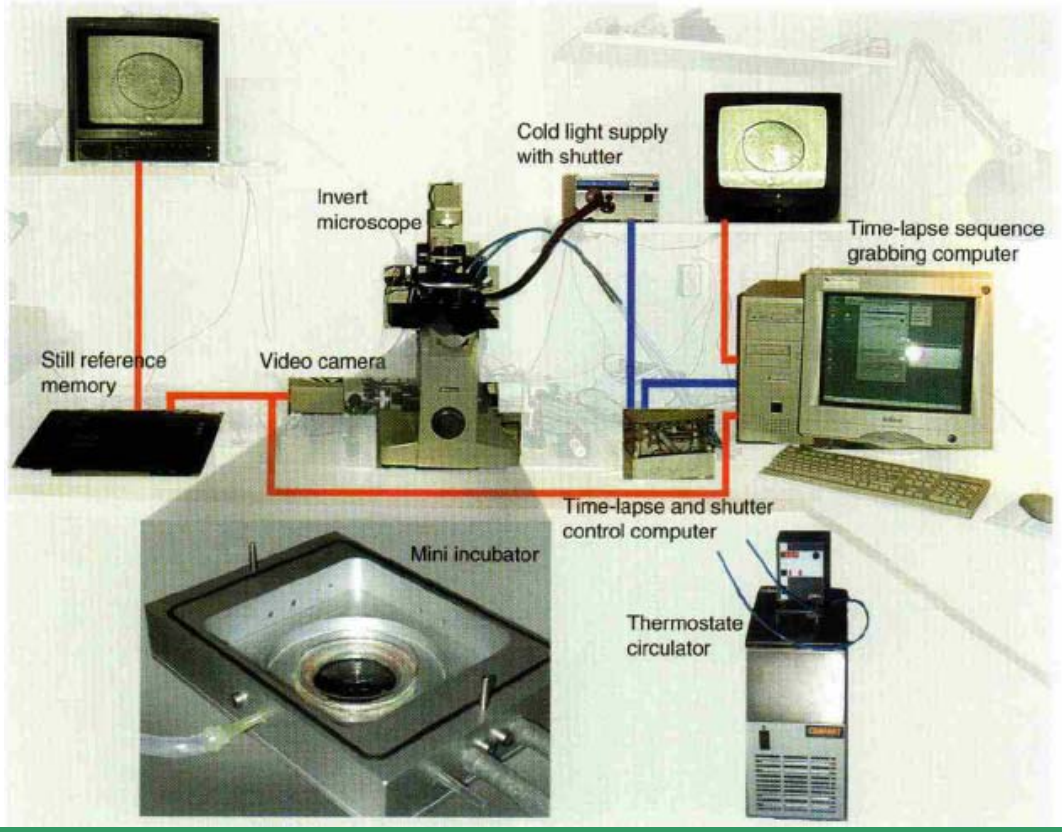
Home made system

RBM online - Vol 5. No 1. 36–38 Reproductive BioMedicine Online; www.rbmonline.com/Article/633 on web 23 April 2002

Articles

Internalization of cellular fragments in a human embryo: time-lapse recordings

Dr Thorir Hardarson

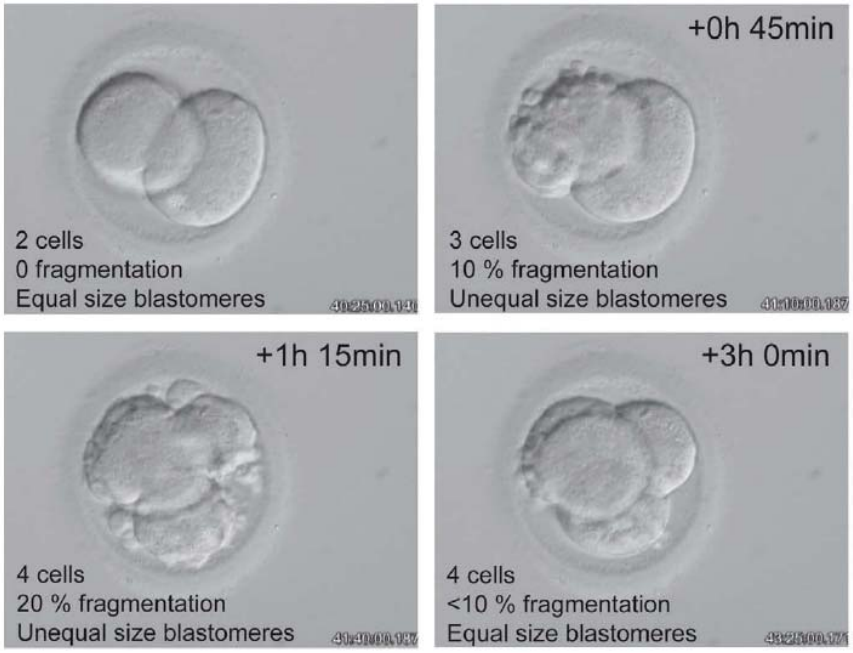


Home made system

RBM Online - Vol 17 No 3. 2008 385-391 Reproductive BioMedicine Online; www.rbmonline.com/Article/3327 on web 30 July 2008

Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes

Dr Josephine Lemmen



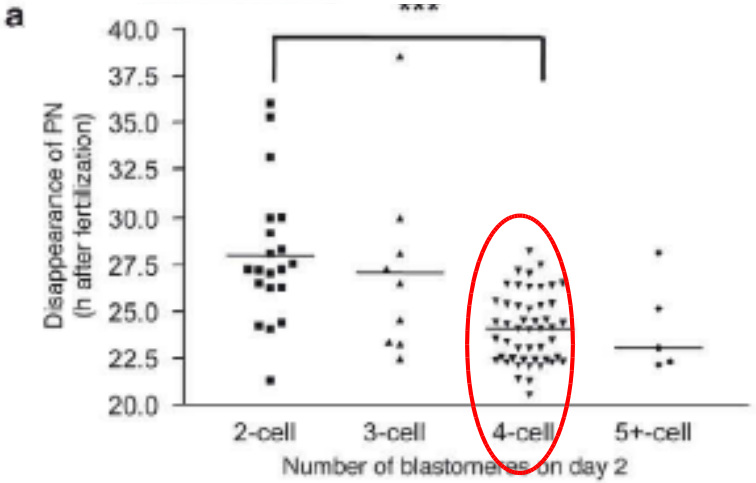
Comparsa e riassorbimento dei frammenti nell'intervallo di tempo in cui normalmente si procede allo scoring degli embrioni in seconda giornata.

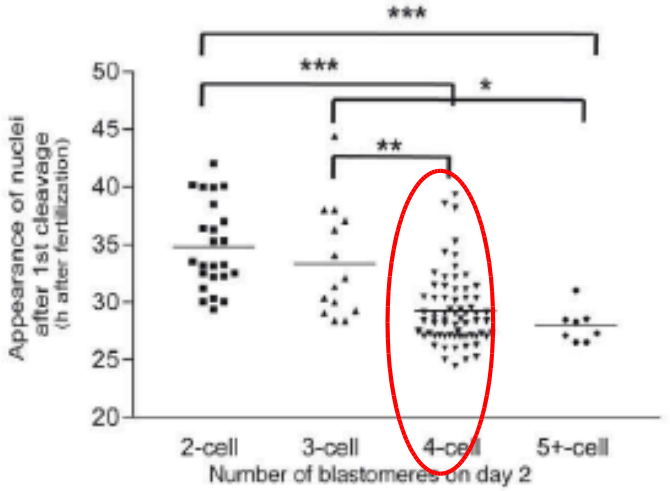
RBM Online - Vol 17 No 3. 2008 385-391 Reproductive BioMedicine Online; www.rbmonline.com/Article/3327 on web 30 July 2008

Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes

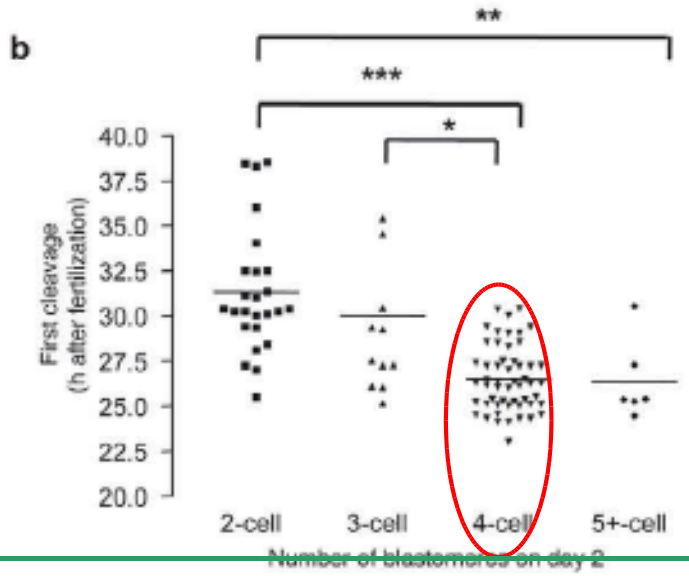
Dr Josephine Lemmen

Riapparizione dei nuclei dopo il primo clivaggio





primo clivaggio



Precoce scomparsa dei PN

Precoce primo clivaggio

Riapparizione simultanea dei nuclei

4cellule in G2 ➔ buona prognosi

Durata della fase a 2 cellule FIV vs ICSI

RBM Online - Vol 17 No 3. 2008 385-391 Reproductive BioMedicine Online; www.rbmonline.com/Article/3327 on web 30 July 2008

Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes

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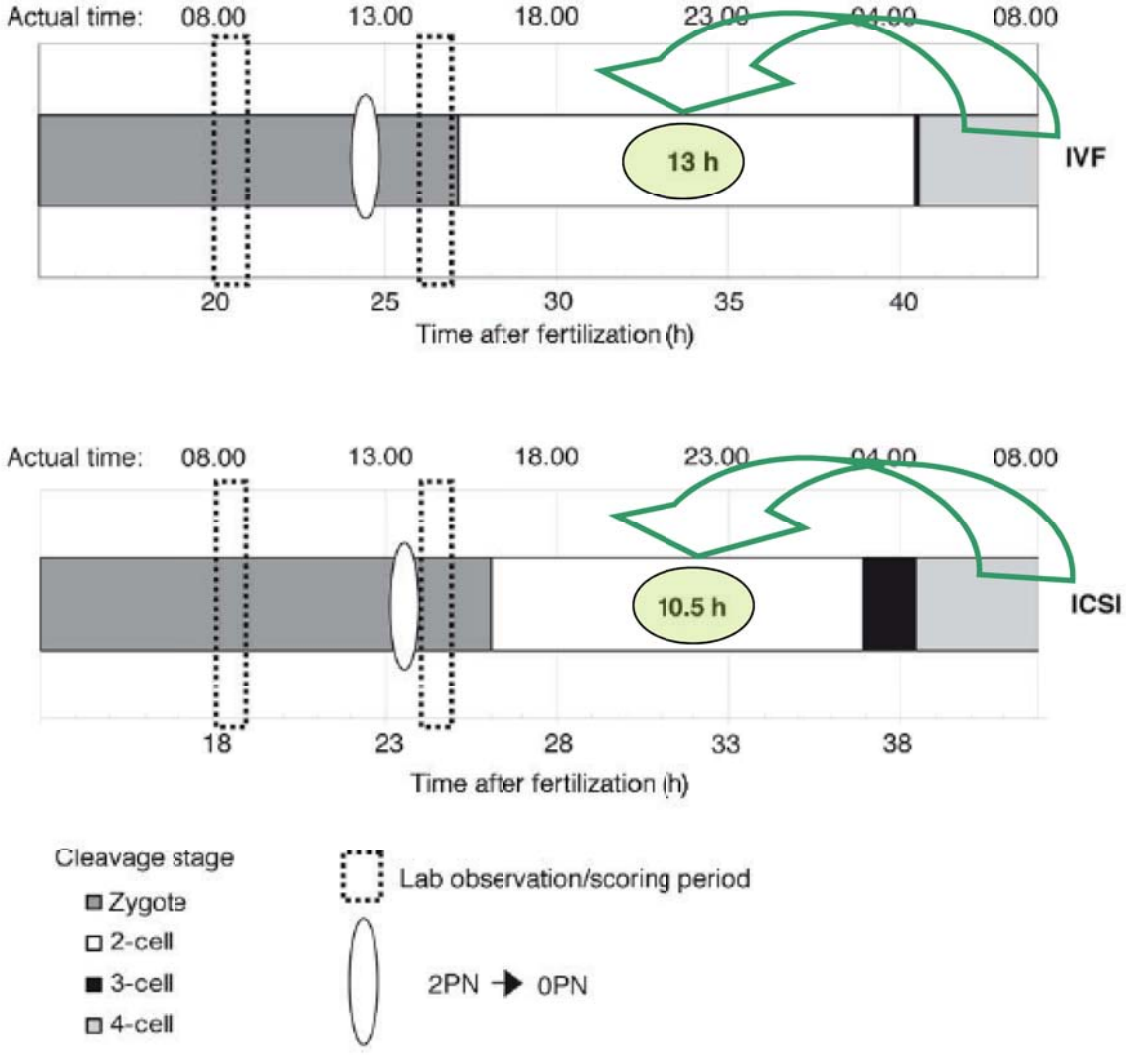


FIV

ICSI

Primo Vision

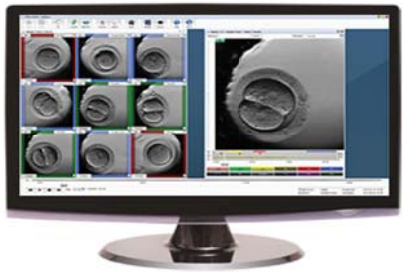
Reproductive BioMedicine Online (2010) 21, 533–536

Pregnancy achieved by transfer of a single blastocyst selected by time-lapse monitoring

Case report

Csaba Pribenszky [a,*], Szabolcs Mátyás [b], Péter Kovács [b], Eszter Losonczi [c], János Zádori [d], Gábor Vajta [e]

Abstract Appropriate selection of a single blastocyst for transfer decreases the risk of multiple gestations. By using a compact time-lapse microscope system placed inside a regular incubator, combined with a microwell embryo culture dish, the development of all the embryos from a patient was continuously monitored by obtaining images at 10 min intervals. The embryos were not moved during the time-lapse observation. The system was switched off completely between image acquisitions in order to avoid exposure to electromagnetic radiation. The analysis of time-lapse records was used to choose a single blastocyst for transfer, which resulted in a singleton pregnancy and birth of a healthy boy on term.

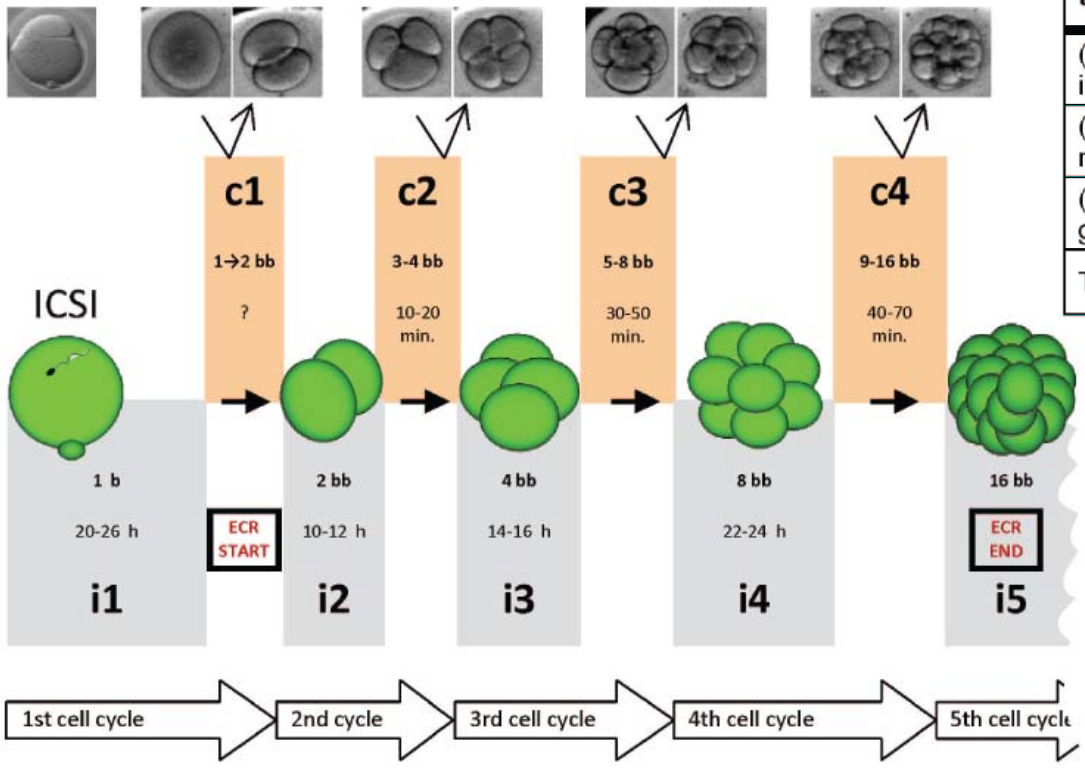


Physiol. Res. 61: 513-525, 2012

Primo Vision

Time-Lapse Cleavage Rating Predicts Human Embryo Viability

D. HLINKA[1], B. KAĽATOVÁ[2], I. UHRINOVÁ[2], S. DOLINSKÁ[2], J. RUTAROVÁ[3], J. ŘEZÁČOVÁ[3], S. LAZAROVSKÁ[1], M. DUDÁŠ[2]



Cleavage timeliness:	Timely (T)	Untimely (U)	Total
(A) Abnormalities in morphology	**14** (7.8 %)	**52** (28.9 %)	66 (36.7 %)
(B) Blastocysts, no pregnancy	**78** (43.3 %)	**8** (4.4 %)	86 (47.7 %)
(BP) Blastocysts giving pregnancy	**28** (15.6 %)	**0** (0 %)	28 (15.6 %)
Total	120 (66.7 %)	60 (33.3 %)	180 (100 %)

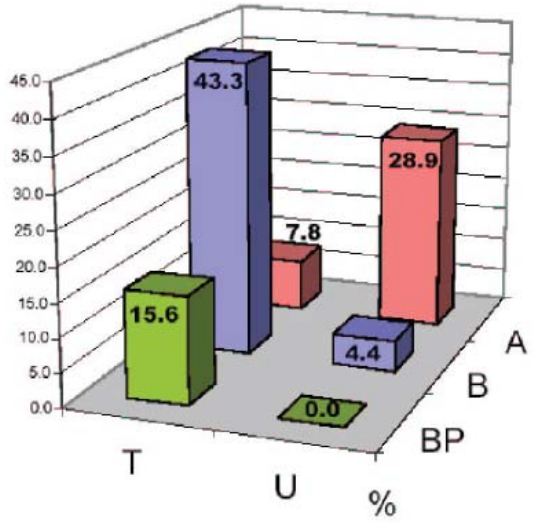


Fig. 4. Simplified correlation of major scoring points in embryo cleavage rating (ECR) with cell cycles and photographs of corresponding developmental stages. Please see Discussion for difficulties with separating i1 from c1. **c1 to c4** – approximated cleavages of the cell cycles 1 to 4; **i1 to i5** – approximated interphases of the cell cycles 1 to 5; **bb** – number of blastomeres.

Embryoscope

8 scatti ogni ora

x

5 fuochi

x

24 h

x

5 gg

=

4800 immagini per embrione

- ✓ 6 piastre
- ✓ 12 embrioni/piastra
- ✓ 12 pozzetti numerati
- ✓ Zone separate per embrione, terreno e olio
- ✓ Non necessitano ambiente umidificato
- ✓ Standard slide format (25 × 75 mm) – spessore 1 mm
- ✓ Più embryoscope per un solo Embryoviewer

Human Reproduction, Vol.26, No.10 pp. 2658–2671, 2011
Advanced Access publication on August 9, 2011 doi:10.1093/humrep/der256

human reproduction ORIGINAL ARTICLE *Embryology*

The use of morphokinetics as a predictor of embryo implantation†

Marcos Meseguer[1,*], Javier Herrero[1], Alberto Tejera[1], Karen Marie Hilligsøe[2], Niels Birger Ramsing[2], and Jose Remohí[1]

285 embryo transfer
522 embrioni trasferiti
247 "fully implanted"/failed implanted (61/186)

Morfologia	***Time lapse***
Assenza di cellule multinucleate	T2
2-5 cellule in G2	T3
6-10 cellule in G3	T4
<15% di frammentazione dell'embrione	T5
Simmetria dei blastomeri	CC2 = T3 - T2
	S2 = T4 - T3

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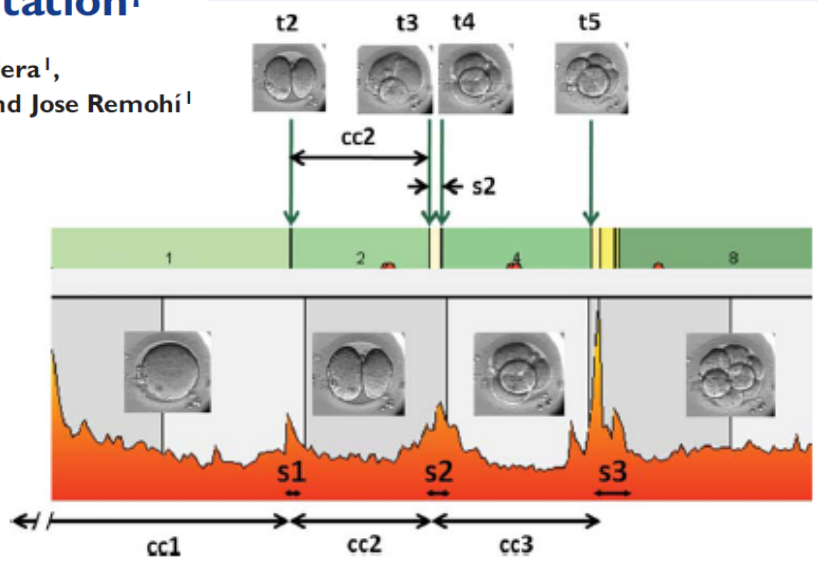

Figure 1 Graphic representation of the considered embryo developmental events t2, t3, t4, t5, cc2 = t3-t2 and s2 = t4-t3. We identified the precise timings and measured them in hours post ICSI microinjection.

Table 1 Exact timing of embryo events analysed from transferred implanted and not implanted embryos.

Parameter	Implanted embryos				Not implanted embryos				Homogeneity of variances
	Mean (h)	SD (h)	*n*	Normal dist.	Mean (h)	SD (h)	*n*	Normal dist.	*P*-value
t2	25.6	2.2	61	Yes	26.7	3.8	186	No	0.022
t3	37.4	2.8	61	Yes	38.4	5.2	185	No	0.002
t4	38.2	3.0	61	Yes	40.0	5.4	182	No	0.004
t5	52.3	4.2	61	Yes	52.6	6.8	167	Yes	<0.001
cc2	11.8	1.2	61	Yes	11.8	3.3	185	No	0.006
s2	0.78	0.73	61	No	1.77	2.83	182	No	0.016

Human Reproduction, Vol.26, No.10 pp. 2658–2671, 2011
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human reproduction

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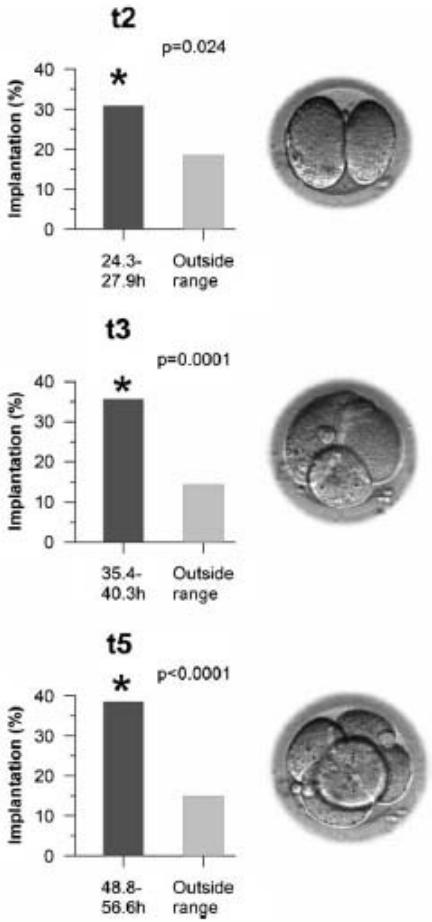

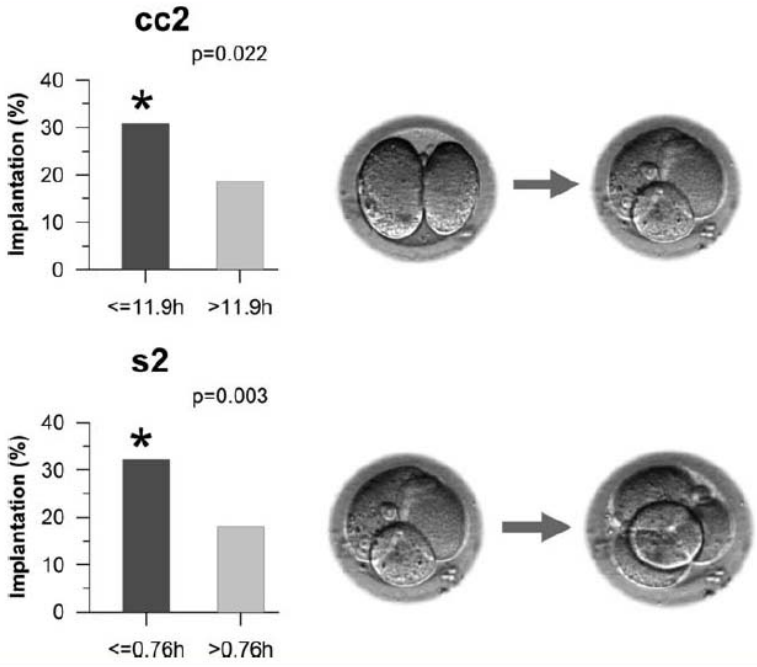

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T2	24.3-27.9h
T3	35.4-40.3h
T5	48.8-56.6h
S2	<0,76
CC2	<11,9

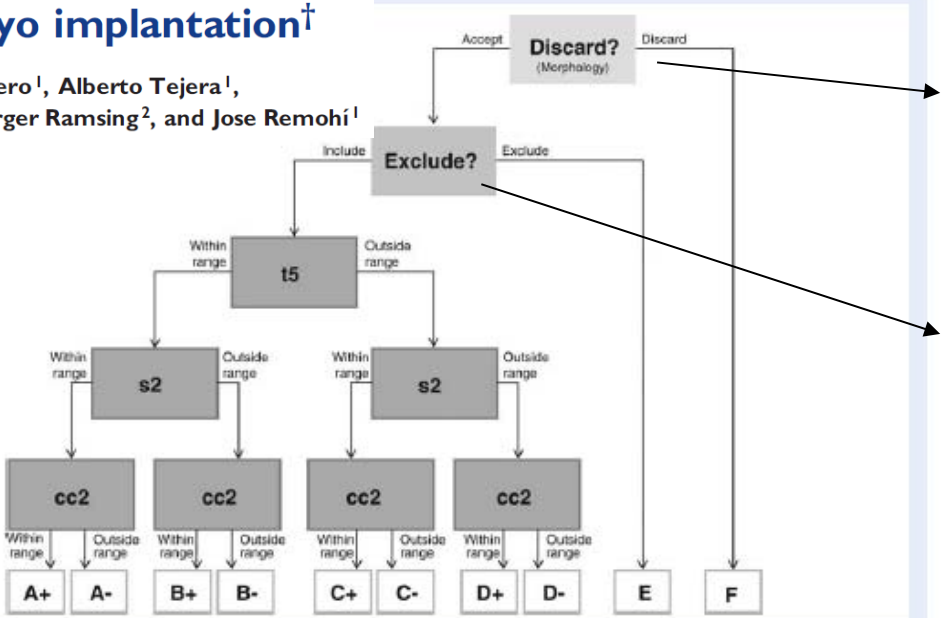


Figure 6 Hierarchical classification of embryos based on: (i) morphological screening; (ii) absence of exclusion criteria; (iii) timing of cell division to 5 cells (t5); (iv) synchrony of divisions from 2 cel to 4 cell stage, s2, i.e. duration of 3 cel stage; (v) duration of second cell cycle, cc2, i.e. the time from division to a two blastomere until divison to a three blastomere embryo. The classification generates 10 categories of embryos with increasing expected implantation potential (right to left) and almost equal number of embryos in each.

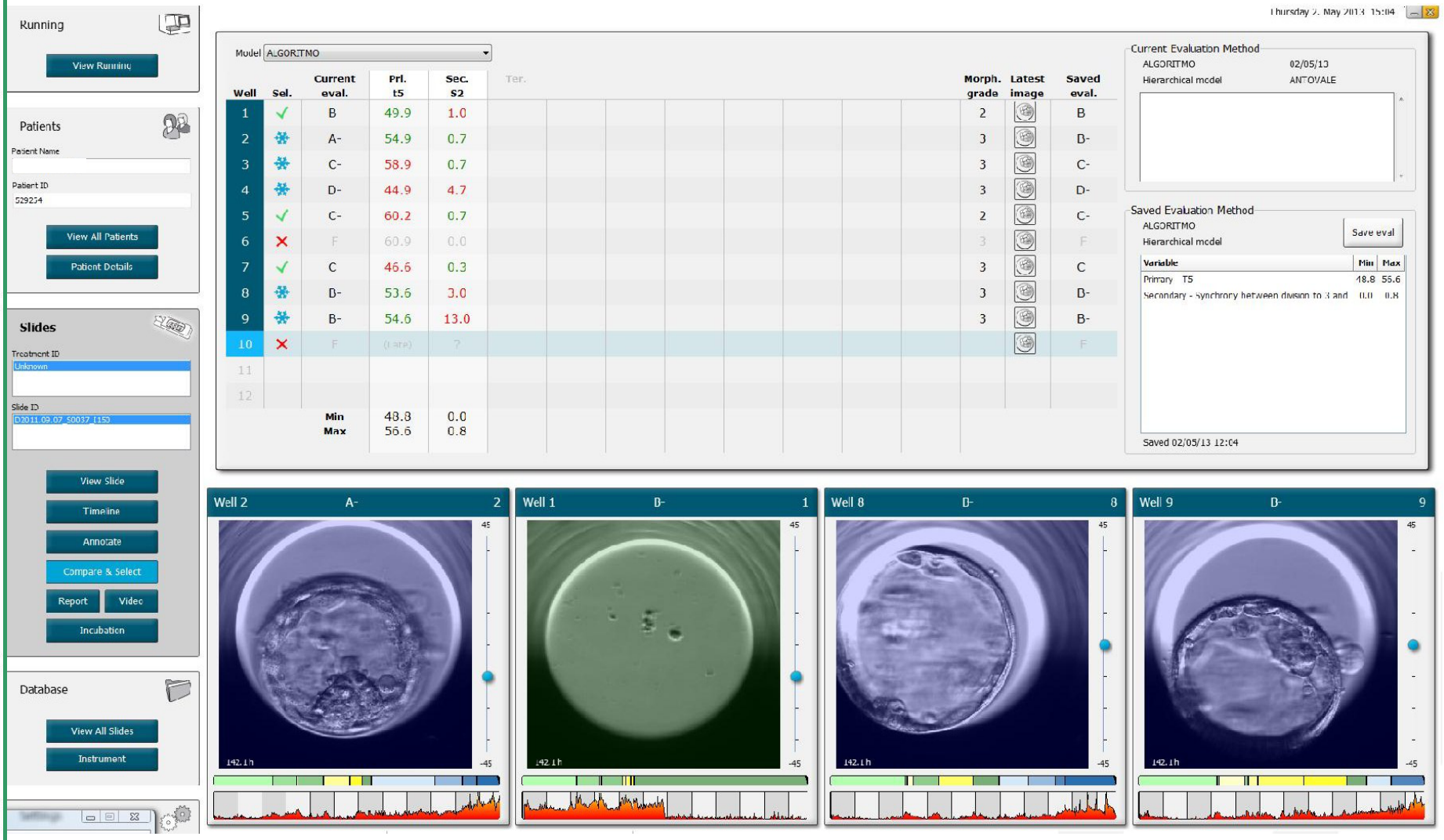
Embrioni atresici, anomali, con arresto nella divisione

Embrioni con irregolarità dei blastomeri, passaggio diretto da 1 a 3 cellule, multinucleazione

Table III Implantation in the embryo categories of the hierarchical classification tree model.

Embryo category	*n* total	*n* implanted	Implantation (%)	Embryo category	Implantation (%)
A+	29	19	66	A	52
A−	25	9	36		
B+	24	7	29	B	27
B−	25	6	24		
C+	32	8	25	C	19
C−	21	2	10		
D+	10	1	10	D	14
D−	33	5	15		
E	48	4	8	E	8

Modello per la selezione … in base alla cinetica



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Marcos Meseguer[1,*], Javier Herrero[1], Alberto Tejera[1], Karen Marie Hilligsøe[2], Niels Birger Ramsing[2], and Jose Remohí[1]

Category 1: The two pronuclei (2PN) embryo consists of 2 cells at 27 h post insemination, 4 cells at Day 2 and 8 cells at Day 3. Even blastomere size at the 2, 4 and 8 cell stage, no multinucleation is observed at any time and the fragmentation is <10%.

Category 2: The 2PN embryo consists of 1–2 cells at 27 h, 3–4 cells at Day 2 and 6–8 cells at Day 3. Only one mismatch is allowed, i.e. either 1 cell at 27 h, 3 cells at Day 2 or 6–7 cells at Day 3. Blastomeres are even sized at the 2, 4 and 8 cell stage; no multinucleation is observed at any time and the fragmentation is <20%.

Category 3: The 2PN embryo consists of 1–2 cells at 27 h, 2–4 cells at Day 2 and 6–8 cells (or morula) at Day 3. The embryo can have asymmetric blastomeres and multinucleation can be observed in maximally one blastomere at each stage. The degree of fragmentation is <20%.

Category 4: The 1PN or 2PN embryo consists of 1–2 cells at 27 h, 2–6 cells at Day 2 and 4 to more than 8 cells or morula at Day 3. The embryo can have asymmetric blastomeres and be multinucleated. The degree of fragmentation is <50%.

Category 5: The embryo consists of any number of cells at 27 h, Day 2 and Day 3. Asymmetric blastomere size, multinucleation and any degree of fragmentation is allowed. Atretic embryos and embryos with arrested development belong to this category.

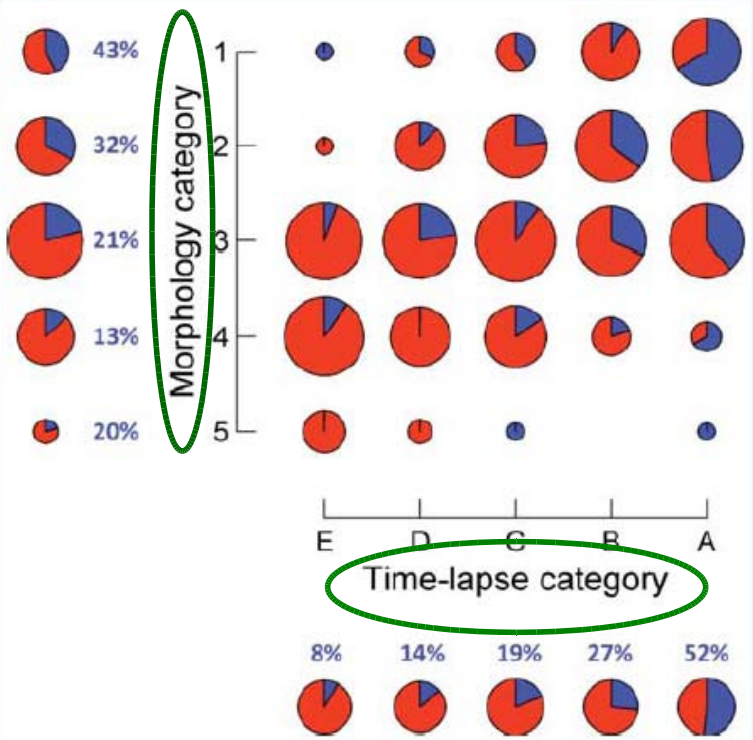

Figure 7 A comparison between the time-lapse categories A–E and the morphology categories 1–5. The areas of the pie charts are proportional to the number of embryos in each of the sub-categories between the two categorization systems. The fraction of implanting embryos in each sub-category is proportional to the blue parts of the pie charts. In the left side (at the bottom) of the figure, a column of pie charts illustrates the overall distribution of embryos in each of the morphology categories (time-lapse categories). The sizes of the time-lapse categories are approximately equal, whereas the sizes of the morphology categories are unequal.

Reproductive BioMedicine Online (2012) 25, 371–381

Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality

María Cruz [a], Nicolás Garrido [b], Javier Herrero [b], Inmaculada Pérez-Cano [a], Manuel Muñoz [a], Marcos Meseguer [b,*]

SCOPO

- ✓ valutare se l'early cleavage è associato con la probabilità di raggiungere lo stadio di blastocisti e con la sua morfologia
- ✓ valutare se il time lapse consente di prevedere lo sviluppo dell'embrione a blastocisti

MATERIALI E METODI

1301 ovociti donati (pazienti tra i 18- 35 anni)

962 fertilizzati (73,94%)

834 time lapse

CRITERI DI ESCLUSIONE:

- ✓ **blastomeri diversi a 2 cellule**
- ✓ **passaggio diretto da 1 a 3 cellule**

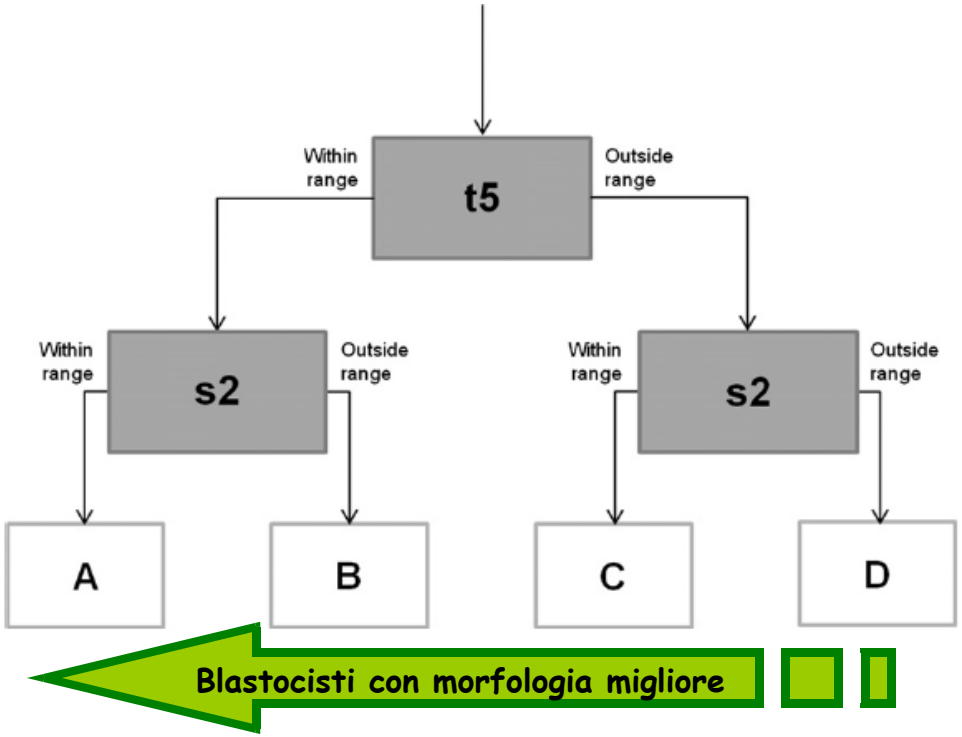

Table 3 Blastocysts and good-morphology blastocysts in the embryo categories of the hierarchical classification tree model.

Embryo category	*Total embryos (n = 804)*	*Blastocysts*	*Total blastocysts (n = 267)*	*GMB*
A	282	77.0 (217)	106	61.3 (65)
B	82	72.0 (59)	36	58.3 (21)
C	356	64.9 (231)	96	45.8 (44)
D	84	53.6 (45)	29	34.5 (10)

EMBRYOSCOPE VS INCUBATORE TRADIZIONALE

J Assist Reprod Genet (2011) 28:569–573
DOI 10.1007/s10815-011-9549-1

ASSISTED REPRODUCTION TECHNOLOGIES

Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging

María Cruz · Blanca Gadea · Nicolás Garrido · Kamilla Søe Pedersen · Mar Martínez · Inma Pérez-Cano · Manuel Muñoz · Marcos Meseguer

478 embrioni → 238 embryoscope
478 embrioni → 240 incubatore standard

NO DIFFERENZE per:

- ✓ qualità embrioni in G3
- ✓ % blastocisti
- ✓ % trasferiti, scartati, crioconservati

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Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study

Marcos Meseguer, Ph.D.,[a] Irene Rubio, Ph.D.,[a] Maria Cruz, Ph.D.,[b] Natalia Basile, Ph.D.,[c] Julian Marcos, Ph.D.,[d] and Antonio Requena, M.D.[c]

10 centri ivf, cicli ICSI

1390 TMS (time lapse system)

5915 SI (standard incubator)

conTMS +20,1 % di aumento PR per pick up
+15,7% embryo transfer

L'AUMENTO ELEVATO DELLA PREGNANCY RATE E' DOVUTO SIA ALLE PIU' STABILI CONDIZIONI DI COLTURA CHE ALLA POSSIBILITA' DI UTILIZZARE PARAMETRI MORFOCINETICI PER LA SCELTA DELL'EMBRIONE DA TRASFERIRE

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Effect of oxygen concentration on human embryo development evaluated by time-lapse monitoring

Kirstine Kirkegaard, M.D., Johnny Juhl Hindkjaer, M.Sc., and Hans Jakob Ingerslev, D.M.Sc.

Objective: To evaluate, using time-lapse monitoring, the temporal influence of culture in 5% O_2 or 20% O_2 on human embryonic development.
Design: Retrospective cohort study.
Setting: University-based fertility clinic.
Patient(s): In vitro fertilized embryos from women aged <38 years with no endometriosis and ≥8 oocytes retrieved.
Intervention(s): Culture in 20% O_2 exclusively (group 1), 20% and 5% O_2 combined (group 2), or 5% O_2 exclusively (group 3).
Main Outcome Measure(s): Developmental rates and timing of developmental stages.
Result(s): The timing of the third cleavage cycle was delayed for embryos cultured in 20% O_2 (group 1) compared with embryos cultured in 5% O_2 (groups 2 and 3). No difference was observed in timing of the early and full blastocyst stages. More embryos in groups 2 and 3 reached the 8-cell, early blastocyst, and full blastocyst stages than in group 1. We found that embryos in group 3 (5% O_2) reached the 8-cell stage faster than embryos in group 2 (5% + 20% O_2), but none of the other parameters (i.e., other time points, cumulative development, and embryo score) differed between the two groups.
Conclusion(s): Culture in 20% O_2 reduces developmental rates and delays completion of the third cell cycle. The delayed development after culture in atmospheric oxygen was seen in the precompaction embryo only and therefore appears to be stage specific.

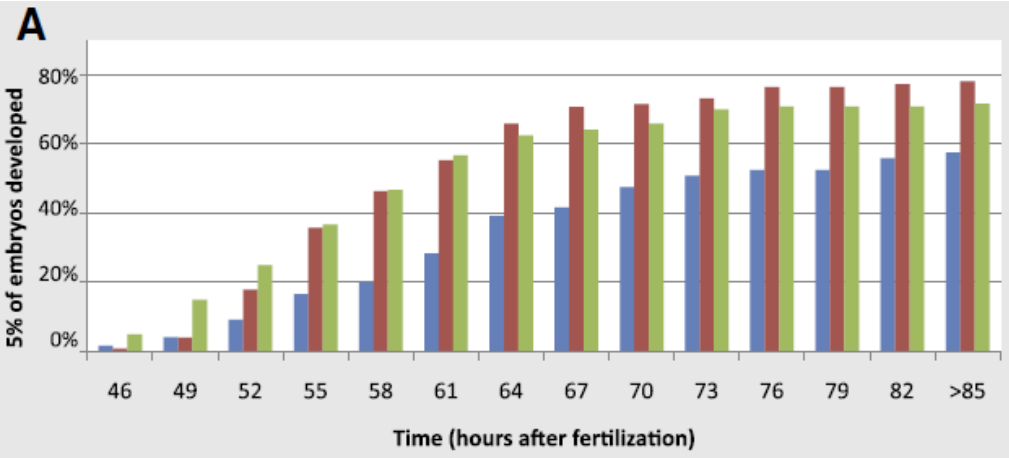
TABLE 2

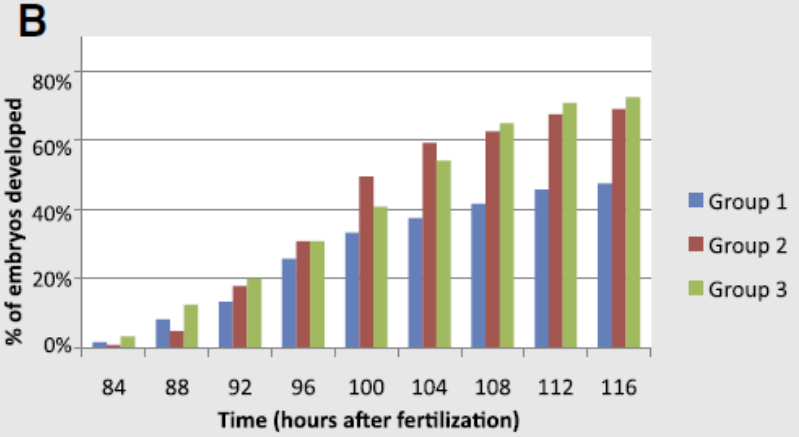
Time points of embryonic stages.

Stage	n	Group 1 (20% O_2)	n	Group 2 (20% O_2 + 5%)	n	Group 3 (5% O_2)	*P* value
2-cell	120	28.0 (27.1–28.9)	123	28.9 (28.0–29.9)	120	27.9 (26.9–28.8)	.24[a]
3-cell	114	38.3 (37.1–39.6)	118	39.0 (37.8–40.2)	118	37.8 (36.6–38.9)	.12[b]
4-cell	110	41.0 (39.7–42.4)	114	41.0 (39.7–42.4)	116	40.5 (39.2–41.9)	.41[b]
5-cell	102	53.0 (51.3–54.9)	110	51.1 (49.4–52.8)	106	50.3 (48.6–52.0)	.09[b]
6-cell	90	55.9 (54.0–57.8)	106	53.4 (51.8–55.1)	102	52.6 (51.0–54.3)	.03[b]
7-cell	69	59.2 (57.2–61.2)	96	55.6 (54.0–57.2)	86	53.7 (52.1–55.4)	<.001[b]
8-cell	55	61.2 (59.0–63.4)	75	57.5 (55.7–59.3)	58	54.5 (52.6–56.5)	<.001[a]
Early blastocyst	57	96.5 (94.5–98.5)	85	96.7 (95.1–98.4)	87	97.2 (95.6–98.8)	.84[a]
Full blastocyst	46	102.2 (100.4–104.0)	69	104.5 (103.0–106.0)	61	104.8 (103.2–106.4)	.08[a]

Note: All data followed a normal distribution after log transformation and are displayed as medians with 95% confidence intervals. *P* value tests the hypothesis of no difference between the groups. The number of 8-cell embryos is smaller than the number of early blastocysts, because some embryos compacted earlier than the 8-cell stage and those embryos were therefore not included in the number of 8-cell embryos.
[a] Analysis of variance.
[b] Kruskal-Wallis test.

Kirkegaard. Temporal effect of O_2 on human embryos. Fertil Steril 2013.





The cumulative development of embryos progressing through (A) third cleavage cycle and (B) early blastocyst stage.

Kirkegaard. Temporal effect of O_2 on human embryos. Fertil Steril 2013.

Rates of embryo development.

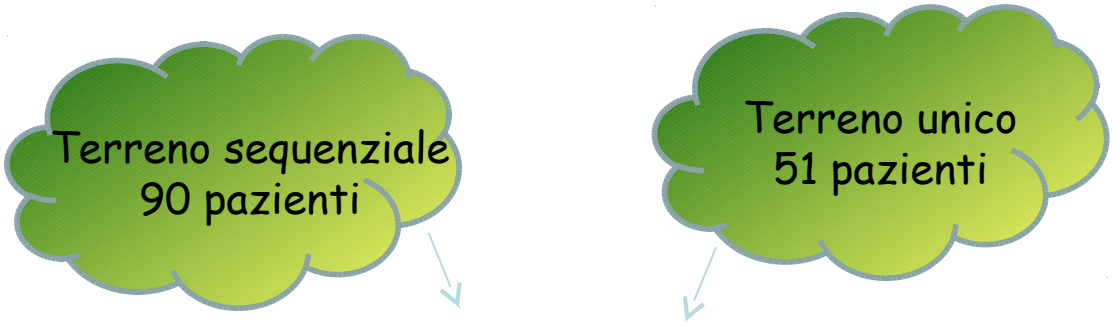
Embryonic stage	Group 1	Group 2	Group 3	*P* value
First cell cycle, n_{start}	120	123	120	
Second cell cycle	110 (92%)	114 (93%)	116 (97%)	.30
Third cell cycle	69 (58%)	96 (78%)	86 (72%)	.004
Early blastocyst	57 (48%)	85 (69%)	87 (73%)	<.001
Full blastocyst	46 (38%)	70 (57%)	61 (51%)	.01

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

LA NOSTRA ESPERIENZA

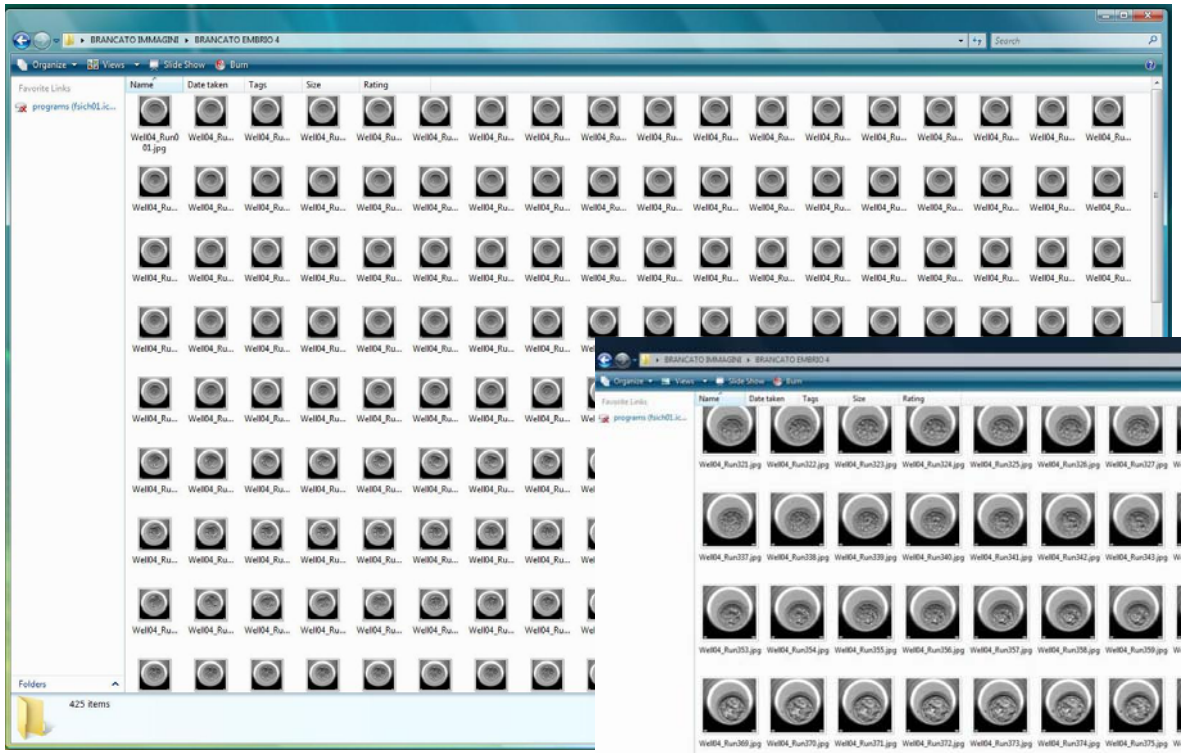
Confronto terreno unico vs sequenziale in un sistema time lapse

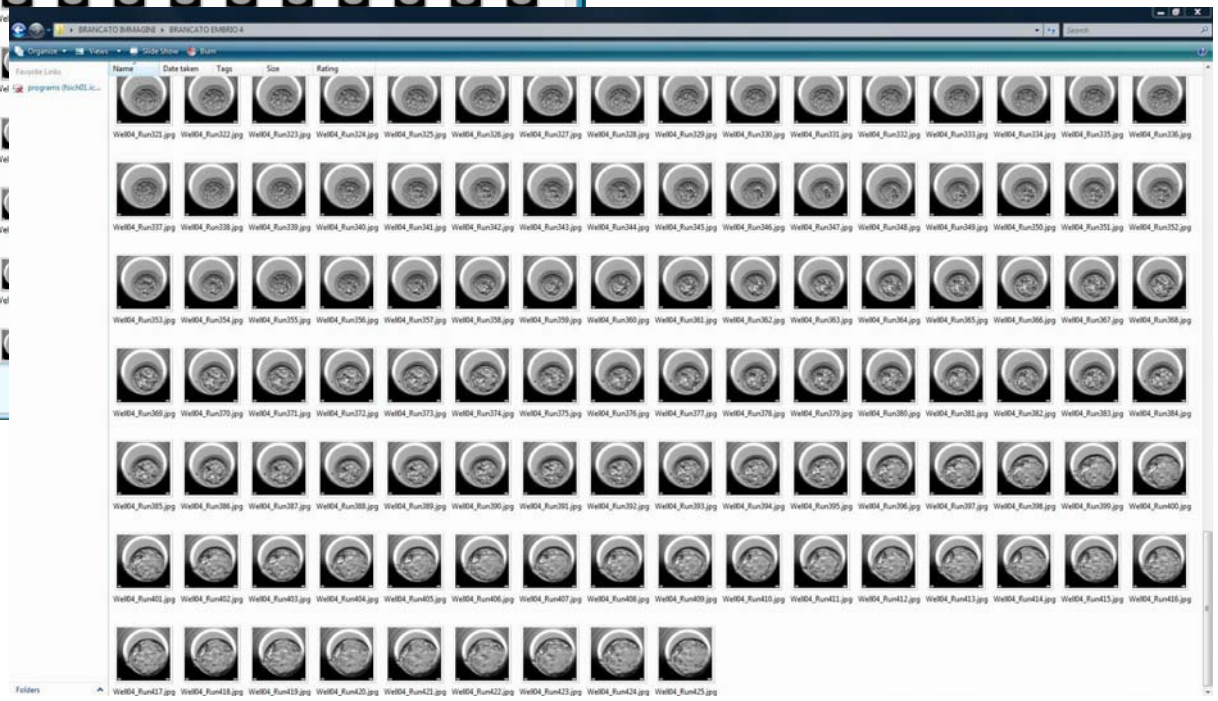
Giugno 2012 – Dicembre 2012



	GRUPPO A	GRUPPO B
Ovociti recuperati	1217	618
Ovociti inietatti	722	383
Ovociti fecondati	511	269
Embrioni trasferiti	201	120
Pregnancy rate	34,4%	47%
% blastocisti sovrannumerarie	25,5%	33,3%

PER CONCLUDERE



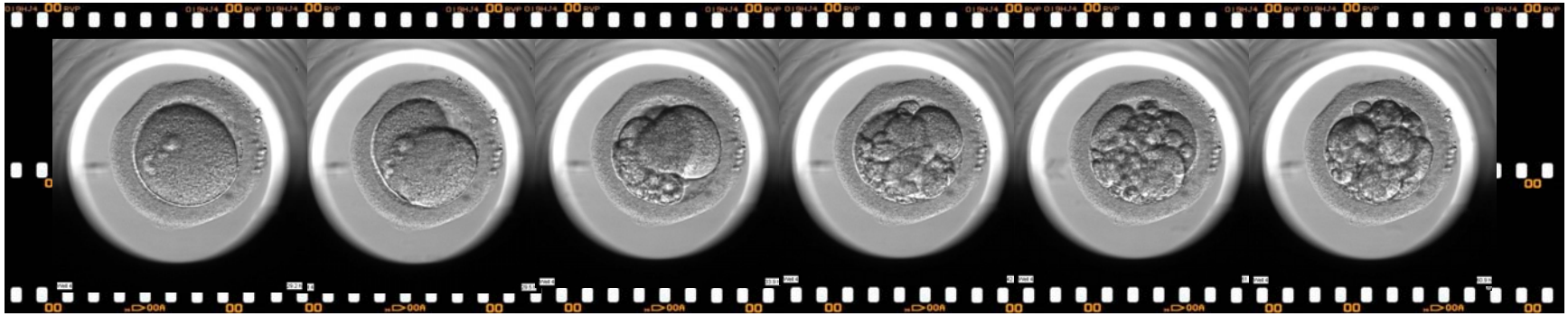


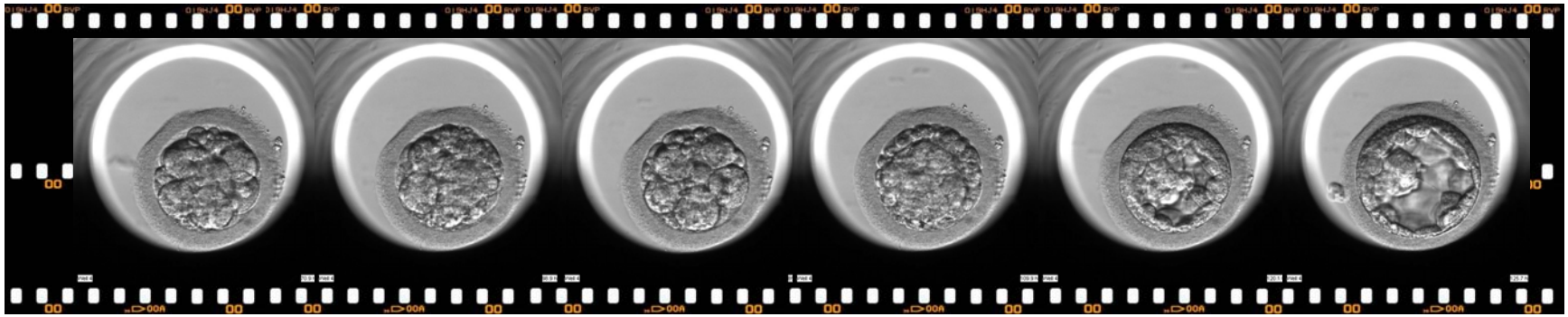
... immagini...
... immagini...
... immagini.....

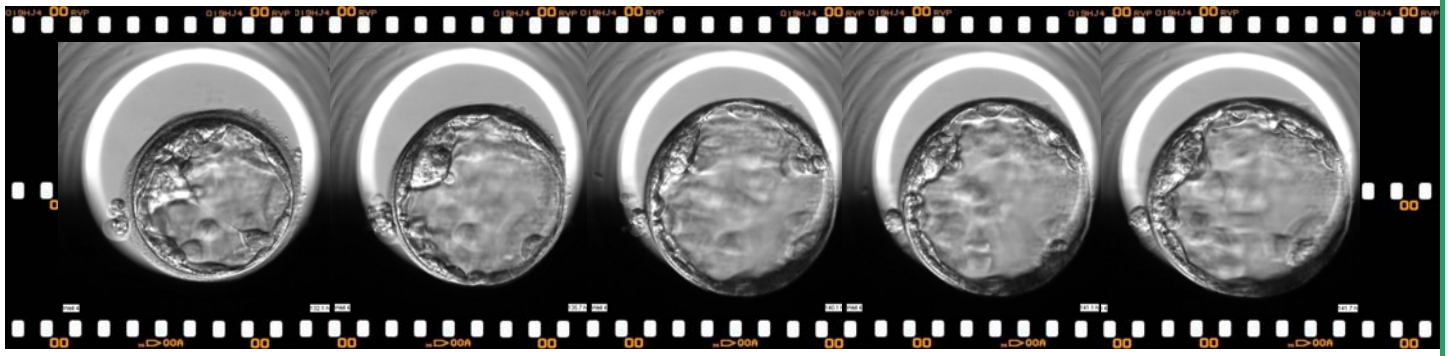
... dalla fecondazione alla blastocisti...

... passo dopo passo ...

PER CONCLUDERE








Grazie