XXXVIII
SABATO DELL'ANDROLOGIA

COLLOQUI IN PMA
TRA GINECOLOGI,
BIOLOGI E ANDROLOGI

17 FEBBRAIO 2018
PADERNO DUGNANO
Clinica San Carlo - Via Ospedale, 21
(Auditorium del Nuovo Ospedale)

CON IL PATROCINIO DI S.I.R.U.

III SESSIONE - IL BIOLOGO PARLA AI CLINICI

A QUANDO L'EMBRYO TRANSFER?

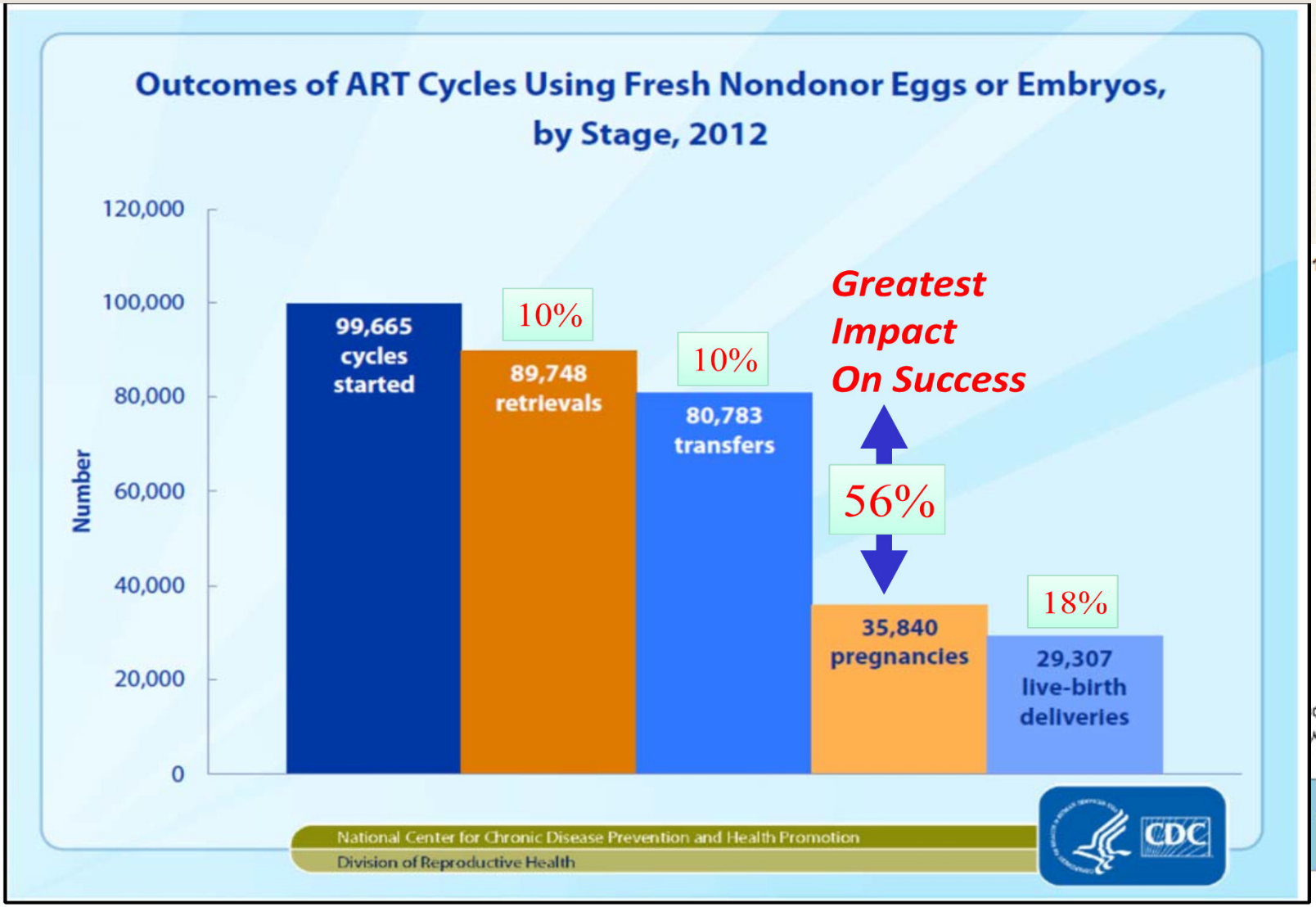
CERVI MARTA

AAS5 "Friuli Occidentale" - Pordenone
S.S.D. di Fisiopatologia della Riproduzione Umana
e Banca del Seme e degli Ovociti
e
Centro di Medicina (CdM)
di San Donà di Piave

Responsabile: Dott. F. TOMEI

Resp Lab PMA: Dott. M. MANNO

ESITI DEI CICLI IVF
DA CICLI OMOLOGHI FRESCHI
– U.S.A. 2012-



15.

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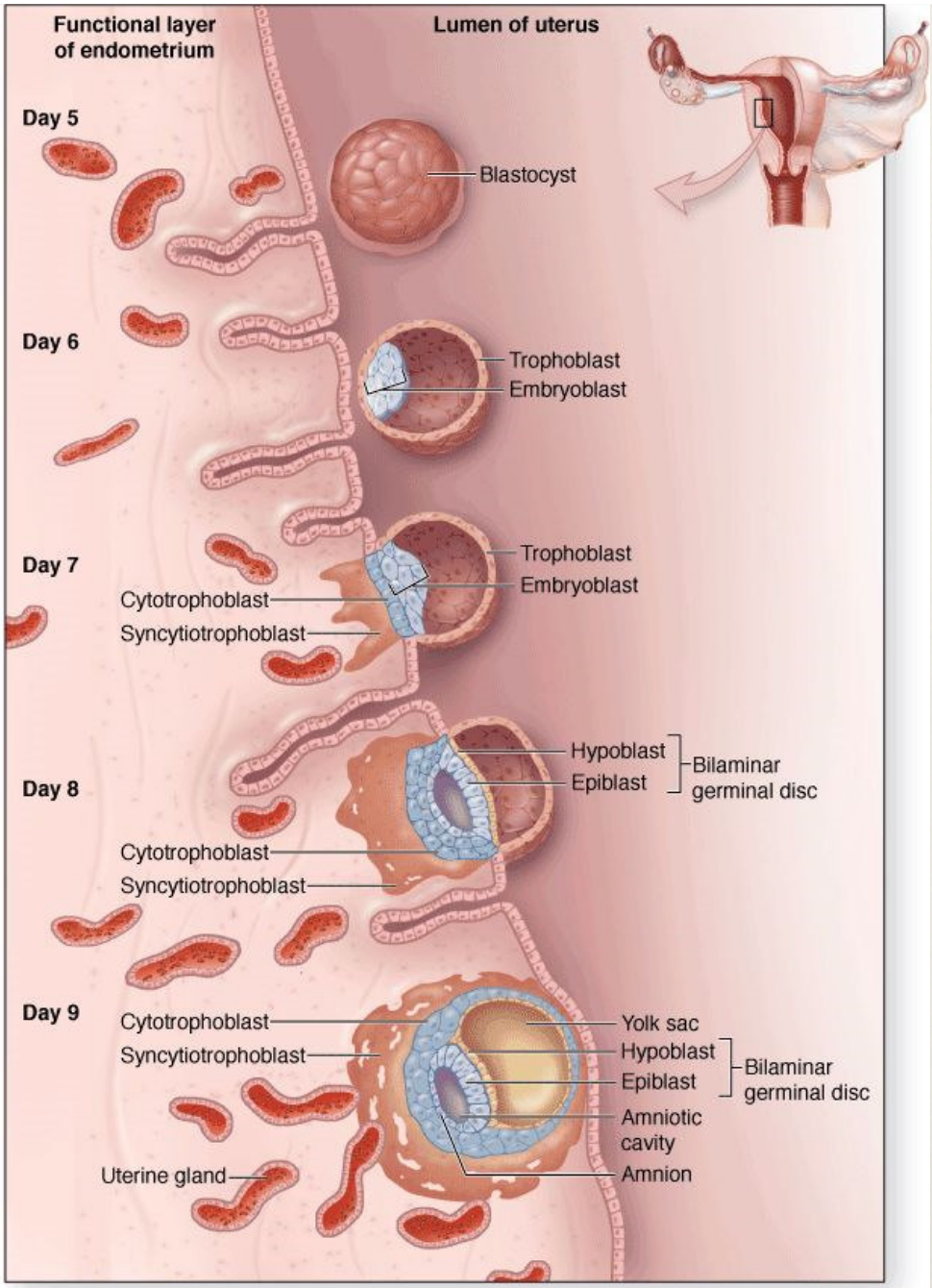
6,482
Live Birth Deliveries
(11,7%)



IMPIANTO OTTIMALE

Richiede SINCRONIA tra 2 PRINCIPALI processi separati:

EMBRIONE EVOLUTIVO (competenza di sviluppo) e

ENDOMETRIO RECETTIVO (funzionale).

È regolato da fattori endocrini, paracrini ed autocrini ad entrambi i livelli.

Allo sviluppo embrionario e dell'endometrio, segue: l' ORIENTAMENTO della Blastocisti (BL) (particolare orientamento), poi APPOSIZIONE, ADESIONE E INVASIONE ENDOMETRALE *(evidenziato nella SCIMMIA RHESUS Enders, 1986).*

The role of the endometrium and embryo in human implantation

Human Reproduction Update, Vol.13, No.4 pp. 365–377, 2007
Advance Access publication June 4, 2007

K. Diedrich[1,4], B.C.J.M. Fauser[2], P. Devroey[3] and G. Griesinger[1] on behalf of the Evian Annual Reproduction (EVAR) Workshop Group

Despite many advances in assisted reproductive technologies (ART) implantation rates are still low. The process of implantation requires a reciprocal interaction between blastocyst and endometrium, culminating in a small window of opportunity during which implantation can occur. This interaction involves the embryo, with its inherent molecular programme of cell growth and differentiation, and the temporal differentiation of endometrial cells to attain uterine receptivity. Implantation itself is governed by an array of endocrine, paracrine and autocrine modulators, of embryonic and maternal origin. Implantation failure is thought to occur as a consequence of impairment of embryo developmental potential and/or impairment of uterine receptivity and the embryo–uterine dialogue.

TIMING DELL'ET:

TEMPORIZZARE L'ET

IN MODO OTTIMALE

LA PRINCIPALE SFIDA IN IVF

SELEZIONARE EMBRIONI EVOLUTIVI:

Morfologia e metodiche invasive e non

ASSICURARE ENDOMETRIO FUNZIONALE:

Adeguata morfologia e composizione cellulare e modulatori dell'impianto

PROGESTERONE ed estrogeni sono i principali

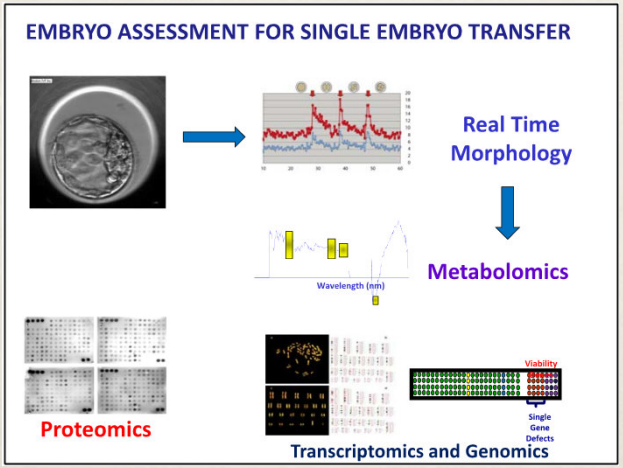
Coltura IN VITRO: il peso dei TERRENI DI COLTURA nel determinare uptake dei nutrienti e secrezione dei metaboliti

STRATEGIE DI SELEZIONE EMBRIONARIA

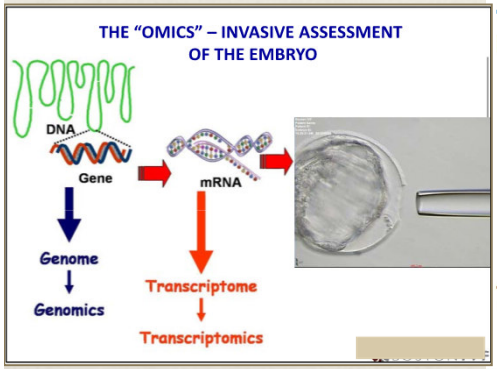
* Fornire un "punteggio" standardizzato e oggettivo della "qualità" per stabilire le potenzialità di impianto di un dato embrione

* Sviluppo di **NUOVI METODI INVASIVI E NON INVASIVI**
(*Rødgaard T1 et al, Reprod Biomed Online. 2015,* ***Non-invasive assessment of in-vitro embryo quality to improve transfer success.)***

* **NESSUN SINGOLO BIOMARKER è stato ancora introdotto nella pratica clinica standard**



STRATEGIE DI SELEZIONE EMBRIONARIA



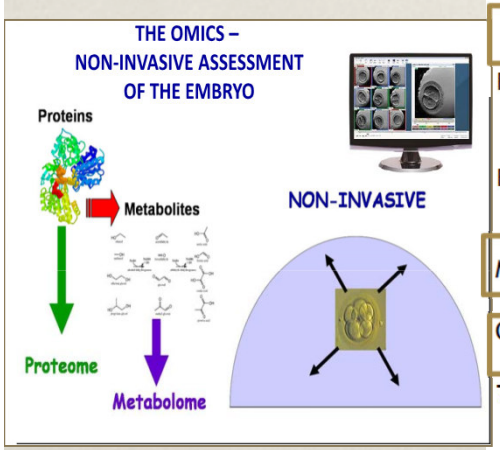


Table 1 Overview of embryo selection strategies.

Method	Nature	Applicability	Benefit regarding pregnancy rates	Reference
PGS				
Polar bodies	Invasive			
Blastomeres	Invasive			
Trophectoderm	Invasive			
Transcriptomics				
Blastomeres	Invasive			
Granulosa cells	Noninvasive	Experimental	Not yet proven	(2013)
Cumulus cells	Noninvasive	Experimental	Not yet proven	Hamel et al. (2008)
Embryo morphology	Noninvasive	Standard procedure in embryology	Proven, but with limitations	Assou et al. (2010) Montag et al. (2011)
Blastocyst culture	Noninvasive	Established in most laboratories	Proven, but with limitations	ESHRE (2012) Cruz et al. (2012) Glujovsky et al. (2012)
Metabolomics	Noninvasive	Experimental	No, according to one RCT	Hardarson et al. (2012)
Oxygen measurement	Noninvasive	Experimental	Not yet proven	Tejera et al. (2012)
Time-lapse monitoring	Noninvasive	Clinically applied	Highly promising; but requires confirmation	Meseguer et al. (2012)

ANALISI GENETICA DEL PB, CELLULE DEL TE e DEL TRASCRITTOMA DEL CUMULO/GRANULOSA → A. Paffoni

(MONTAG M et al, Reproductive BioMedicine Online, 2013)

TIME-LAPSE IMAGING

VANTAGGI:

- **IMPERTURBABILITÀ DELLA COLTURA**

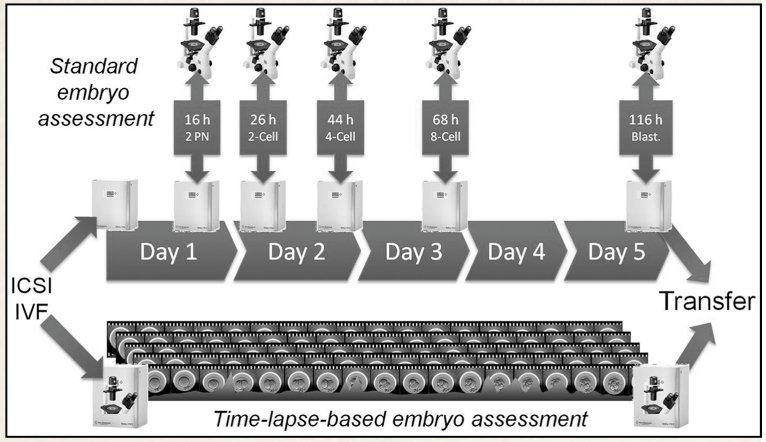
- **CONTINUITÀ DELL' OSSERVAZIONE**
  (direct uneven cleavage , inverse cleavage)

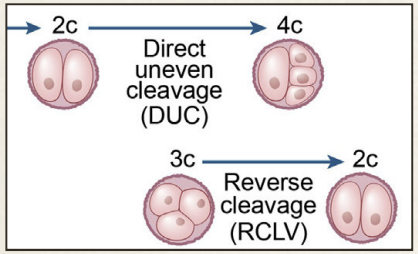
- **RICERCA DI CRITERI DI SELEZIONE E DE-SELEZIONE**

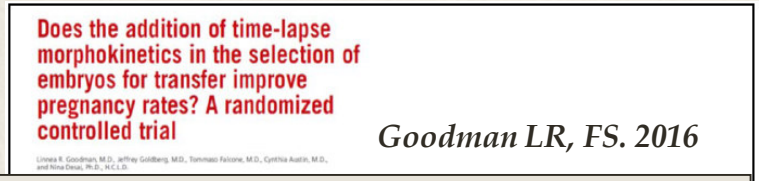
- **COMPARAZIONE rispetto alla MORFOLOGIA TRADIZIONALE** *(Adamson, FS 2016 → studio pilota prospettico controllato)*

- **VALUTAZIONE DELL' IMPORTANZA DELLA SCELTA DEI TERRENI** *(Hardarson, FS 2015: Noninferiority, randomized, controlled trial comparing embryo development using media developed for sequential or undisturbed culture in a time-lapse setup)*

- ***VALUTAZIONE DELL'IMPORTANZA DEL TI… INCUBATOR*** *(Park H, HR 2015: No benefit of culturing embry… system compared with a conventional incubator in terms of number of g… embryos: results from an RCT / Kirkegaard K, JARG 2012 A randomiz… comparing embryo culture in a conventional incubator with a time-laps…*







RCT: The addition of time-lapse morphokinetic data **DID NOT SIGNIFICANTLY IMPROVE CLINICAL REPRODUCTIVE OUTCOMES** in all patients and in those with blastocyst transfers. Absence of multinucleation, timing of blastulation, and morphokinetic score were found to be associated with blastocyst implantation rates.

Reproductive BioMedicine Online
February **2018**

Can time-lapse parameters predict embryo ploidy? A systematic review

Arnaud Reigniera, b, c, Jenna Lammersa, b, Paul Barrierea, b, c, Thomas Freoura, b, c, ,

NESSUN PARAMETRO MORFOCINETICO SINGOLO O COMBINATO è stato costantemente identificato come PREDITTIVO DELLO STATO DI PLOIDIA DELL'EMBRIONE.

F1000Research F1000Research 2017, 6(F1000 Faculty Rev):1616 Last updated: 31 AUG 2017

Check for updates

REVIEW

Recent advances in *in vitro* fertilization [version 1; referees: 2 approved]

Robert Casper [1-3], Jigal Haas[1], Tzu-Bou Hsieh[1], Rawad Bassil[1], Chaula Mehta[1]

Time-lapse algorithms and morphological selection of day-5 embryos for transfer: a preclinical validation study

Fertil Steril® 2018

Ashleigh Storr, Pg.D., Christos Venetis, Ph.D., Simon Cooke, Ph.D., Suha Kilani, Ph.D., and William Ledger, Ph.D.

NUMEROSI ALGORITMI di selezione degli embrioni per aumentare il tasso d'impianto.

NON sono CLINICAMENTE APPLICABILI e perde il loro valore diagnostico quando applicato esternamente *(Barrie, F1000Research 2017).*

→ NECESSITÀ DI **UNA CORRETTA CONVALIDA ESTERNA** prima dell'uso clinico.

Attualmente, **LA TECNICA NON INVASIVA PREDOMINANTE** → **MORFOLOGIA**

(VARIABILI DI SCORING MORFOLOGICO ai vari stadi di sviluppo)

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

human reproduction ORIGINAL ARTICLE *ESHRE pages*

2011

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

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

STANDARDIZZAZIONE nella terminologia e pratica di Laboratorio sulla morfologia

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.

Review

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

2017

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 Mayis Mah. 19 Mayis Cad. Nova Baran Center No:4 34360 Sisli, Istanbul, Turkey*

KEY MESSAGE
This proceedings report presents 19 Indicators, including 12 Key Performance Indicators (KPIs), 5 Performance Indicators (PIs), and 2 Reference Indicators (RIs) from an international workshop supported by the European Society of Human Reproduction and Embryology (ESHRE) and Alpha Scientists in Reproduction (Alpha), designed to es-

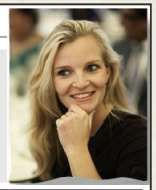
Key performance indicator	Calculation	Competency value	Benchmark value
Cleavage rate	no. cleaved embryos on Day 2 × 100 / no. 2PN/2PB oocytes on Day 1	≥95%	≥99%
Day 2 embryo development rate	no. 4-cell embryos on Day 2 × 100 / no. normally fertilized oocytes [a]	≥50%	≥80%
Day 3 embryo development rate	no. 8-cell embryos on Day 3 × 100 / no. normally fertilized oocytes [a]	≥45%	≥70%
Blastocyst development rate	no. blastocysts Day 5 × 100 / no. normally fertilized oocytes [a]	≥40%	≥60%
Successful biopsy rate	no. biopsies with DNA detected × 100 / no. biopsies performed	≥90%	≥95%
Blastocyst cryosurvival rate	no. blastocysts appearing intact × 100 / no. blastocysts warmed	≥90%	≥99%
Implantation rate (cleavage stage) [b]	no. sacs seen on ultrasound [c] × 100 / no. embryos transferred	≥25%	≥35%
Implantation rate (blastocyst stage) [b]	no. sacs seen on ultrasound [c] × 100 / no. blastocysts transferred	≥35%	≥60%

In conclusion, embryo cleavage rate and embryo development rate are extremely important indicators, while early cleavage rate, rate of good-quality embryos, and embryo fragmentation rate are less important as quality indicators.

Reproductive BioMedicine Online (2014) 29, 222–230

www.sciencedirect.com
www.rbmonline.com

ELSEVIER



ARTICLE

Selection of embryos for transfer in IVF: ranking embryos based on their implantation potential using morphological scoring

Laura van Loendersloot [a,*], Madelon van Wely [a], Fulco van der Veen [a], Patrick Bossuyt [b], Sjoerd Repping [a]

Table 1 Multivariable analysis.

Predictor	Updated model		Embryo score
	Beta (β)	P-value	
Intercept	−1.0579		103
Early cleavage	0.2492	NS	2
No. of blastomeres on day 2 (deviation from 4)[a]	−0.3324	<0.001	−3
No. of blastomeres on day 3 (deviation from 8)[b]	−0.3128	<0.001	−3
Morphological score on day 3[c]	−0.5305	<0.001	−5
Morula on day 3[d]	−1.1940	<0.001	−11

NS = not significant ($P > 0.05$).
[a]No. of blastomeres = absolute value (no. of blastomeres − 4).
[b]No. of blastomeres = absolute value (no. of blastomeres − 8); morula = 0.
[c]Morula = 0.
[d]Presence of morula = 1; no morula = 0.

* Inclusi 6021 ET freschi (01/2004→07/2009)

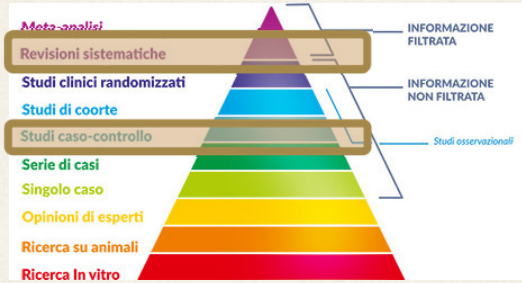
* 9 potenziali fattori predittivi → sviluppo di un **MODELLO PREDITTIVO** (usando la regressione logistica multivariata)

* Solo **5 FATTORI PREDITTIVI** contenuti nel modello finale:
  * **Early Cleavage**
  * **N° Blastomeri D2 e D3**
  * **Score morfologico**
  * **Presenza dello stadio di morula in D3**

* Dimostra una MODERATA CAPACITÀ DISCRIMINANTE (c-statistic 0.70), ben calibrato:

→ è in grado di distinguere Embrioni con MAGGIOR POTENZIALE DI Impianto ongoing.

ET A ZIGOTE



J Assist Reprod Genet (2014) 31:1629–1634
DOI 10.1007/s10815-014-0350-9

ASSISTED REPRODUCTION TECHNOLOGIES

The strategy of group embryo culture based on pronuclear pattern on blastocyst development: a two center analysis

Liliana Restelli · Alessio Paffoni · Laura Corti · Elisa Rabellotti · Alice Mangiarini · Paola Viganò · Edgardo Somigliana · Enrico Papaleo

Abstract

Purpose To compare two embryo grouping strategies.

Methods Retrospective time-course analysis in two different centres. Two culture protocols were used at the zygote stage: "Random Group" in which zygotes were randomly grouped and "Definite Group" in which zygotes were grouped based on pronuclear pattern. Embryo culture was extended to blastocyst stage. Primary and secondary outcomes were respectively the blastulation rate and the cumulative clinical pregnancy and implantation rates.

Result(s) A similar blastulation rate [42 and 41 % day (5+6) blastocysts] was obtained in the two groups. Conversely, after adjusting for baseline and cycle variables, cumulative pregnancy [adjusted Odds Ratio=2.10 (95%CI: 1.08–4.07)] and implantation [adjusted Odds Ratio=1.78 (95%CI: 1.06–2.97)] rates were significantly higher in the "Random Group" compared to the "Definite Group".

Conclusion(s) Two strategies of group culture gave similar results in terms of blastulation rate but the random grouping of zygotes improves pregnancy and implantation rates in IVF-cycles.

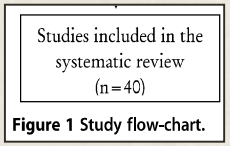


Figure 1 Study flow-chart.

Nicoli *et al. Journal of Ovarian Research* 2013, **6**:64
http://www.ovarianresearch.com/content/6/1/64

JOR JOURNAL OF OVARIAN RESEARCH

REVIEW Open Access

Pronuclear morphology evaluation for fresh in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles: a systematic review

Alessia Nicoli, Stefano Palomba*, Francesco Capodanno, Maria Fini, Angela Falbo and Giovanni Battista La Sala

- NON risultati conclusivi sull'utilità della MORFOLOGIA dello ZIGOTE in ART

→ NON ESISTONO EVIDENZE TRA SCORING AL GIORNO 1 e IR

L'approccio morfo-cinetico sostiene una correlazione tra morfologia e valore prognostico di competenza embrionaria e rischio di aneuploidie

ET allo stadio di Clivaggio

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

human reproduction ORIGINAL ARTICLE *ESHRE pages*

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

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

Assessing cleavage-stage embryos (Days 2 and 3)

J Assist Reprod Genet (2016) 33:1677–1684
DOI 10.1007/s10815-016-0806-1

EMBRYO BIOLOGY

Ultrastructure of cytoplasmic fragments in human cleavage stage embryos

Iman Halvaei[1] · Mohammad Ali Khalili[1] · Navid Esfandiari[2] · Somayyeh Safari[1] ·

- FRAMMENTAZIONE: potrebbe non avere un impatto sul LBR (anche se > 20%), CORRELATO AL BL FORMATION RATE e parametro di valutazione soggettivo
- MULTINUCLEAZIONE: può correlarsi alla coltura ed alla T, ipotizzati differenti meccanismi, ha un impatto sul LBR, ma gestita diversamente dai diversi Lab → coltura prolungata a BL
- TIMING DI DIVISIONE CELLULARE → embrioni troppo lenti o troppo veloci sembrano avere un impatto negativo sull'IR

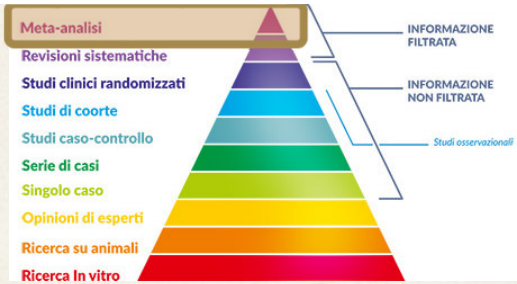
A = top quality
B = good quality (not for elective single embryo transfer)
C = impaired embryo quality
D = do not recommend to transfer (includes all multinucleated embryos).

Cleavage-stage embryos

(1) Proportion of oocyte retrieval cycles that have embryos suitable for freezing.
(2) Proportion of embryos with ≥50% blastomeres intact post cryopreservation.
(3) Proportion of embryos with all blastomeres intact post cryopreservation.

(4) Proportion of post-cryopreservation embryos that cleave during overnight culture.
(5) Implantation rate for post-cryopreservation embryos (women <38 years).

ET in 2 o 3 GIORNATA-parte I



Cochrane Library
Cochrane Database of Systematic Reviews

Day three versus day two embryo transfer following in vitro fertilization or intracytoplasmic sperm injection (Review)

Gunby JL, Daya S, Olive D, Brown J

Cochrane Database of Systematic Reviews 2004, Issue 2. Art. No.: CD004378.
DOI: 10.1002/14651858.CD004378.pub2.

Inclusi 10 studi (2027 pazienti), solo 3 studi riportavano LBR

STADIO ET	LBR	CL.PR	MR
D2	=	<	<
D3	=	> (ICSI)	> (ICSI)
Evidence:	*VERY LOW QUALITY*	*VERY LOW QUALITY*	*VERY LOW QUALITY*

Cochrane Library
Cochrane Database of Systematic Reviews

Day three versus day two embryo transfer following in vitro fertilization or intracytoplasmic sperm injection (Review)

Brown J, Daya S, Matson P

Cochrane Database of Systematic Reviews 2016, Issue 12. Art. No.: CD004378.
DOI: 10.1002/14651858.CD004378.pub3.

Inclusi 15 studi (2894 donne + 969)

STADIO ET	LBR	Cl. PR	Ong.PR	Mult . PR	MR	Ect. PR	Complic.
D2	=	=	=	=	=	=	?
D3	=	=	=	=	=	=	?
Evidence:	*VERY LOW QUALITY*	*VERY LOW QUALITY*	*VERY LOW QUALITY*	*MODERATE QUALITY*	*MODERATE QUALITY*	*MODERATE QUALITY*	

Strettamente finalizzato agli outcome clinici descritti, seppur con una bassa qualità dell' evidenza

MEDESIMA COSA TRASFERIRE IN SECONDA O IN TERZA GIORNATA

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

human reproduction ORIGINAL ARTICLE *ESHRE pages*

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

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

Assessing morulae and blastocysts (Days 4–6)

ET allo stadio di BL

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 3I2). It is a numerical interpretation of the Gardner scale (Gardner and Schoolcraft, 1999a,b).

- Le classificazioni sulla morfologia della Blastocisti

  (Gardner DK et al, Fertil Steril 2000):

  - Regolarità ICM
  - Regolarità TE
  - Grado di espansione → CORRELATO ALLA COMPETENZA EMBRIONARIA

    blastocele e cavità

    - richiedente Energia attraverso le ATPasi Na+/K+ sulla membrana basolaterale del TE
    - Formante le Tight Junction efficaci per formare la barriera del TE

GRADO ESPANSIONE BL

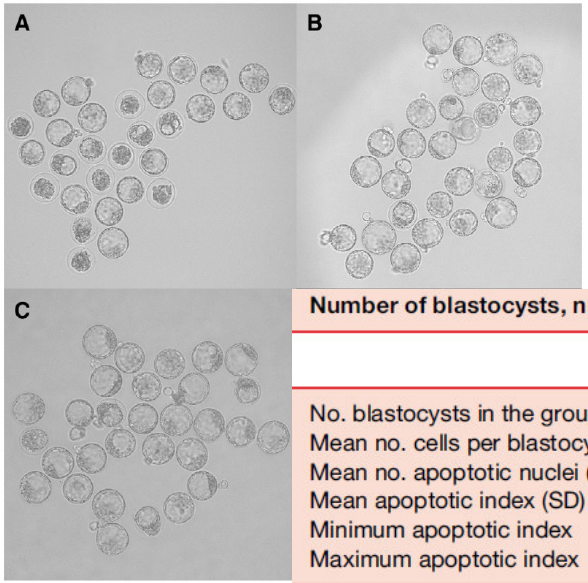
* Danni all'ET della BL TOTALMENTE ESPANSA

Influence of embryo transfer on blastocyst viability

Cezary Grygoruk, M.D., Ph.D.,[a] Piotr Sieczynski, M.Sc., Ph.D.,[b] Jacek A. Modlinski, Ph.D., D.Sc.,[c] Barbara Gajda, Ph.D., D.Sc.,[d] Pawel Greda, M.Sc., Ph.D.,[c] Izabela Grad, M.Sc.,[d] Piotr Pietrewicz, M.Sc.,[a] and Grzegorz Mrugacz, M.D., Ph.D.[a]

Fertility and Sterility® Vol. 95, No. 4, March 15, 2011

Blastocysts 1 hour after embryo transfer (ET). (**A**) Group A. (**B**) Group B. (**C**) Control group C. (**D**) Apoptotic cells in mouse blastocysts stained by TUNEL: (*D1*) blastocyst not exposed to ET (apoptotic index 11%), (*D2*) blastocyst 1 hour after "slow" ET (apoptotic index 21%), (*D3*) morphologically unchanged blastocyst 1 hour after "fast" ET (apoptotic index 86%), (*D4*) collapsed blastocyst 1 hour after "fast" ET (apoptotic index 69%).



Grygoruk. Embryo transfer, part II. Fertil Steril 2011.

The pressure fluctuations during ET can induce morphologic changes and trigger apoptotic processes in the embryos. Because the pressure build-up in the transferred liquid is proportional to the speed of ejection of the transferred load and because the degree of cell damage depends on the pressure amplitude, it is reasonable to recommend transferring embryos at the lowest possible ejection speed.

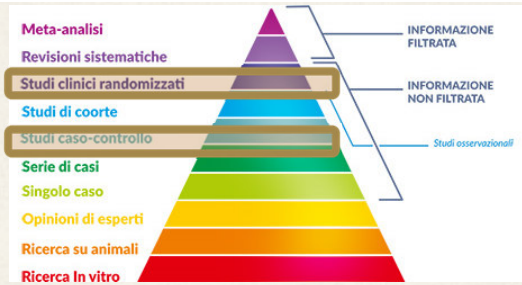
Number of blastocysts, number of cells, and apoptotic index in the groups.

	Group A: fast ET	Group B: slow ET	Group C: no ET
No. blastocysts in the groups	30	30	30
Mean no. cells per blastocyst (SD)	43 (13)	41 (13)	35.4 (14)
Mean no. apoptotic nuclei (SD)	21.6 (9.1)[a]	9.9 (5.5)[a]	4 (2.7)[a]
Mean apoptotic index (SD)	52.2 (19.8)[a]	25.6 (16)[a]	12.8 (10)[a]
Minimum apoptotic index	19	5.4	2.3
Maximum apoptotic index	92.8	67.7	37.9

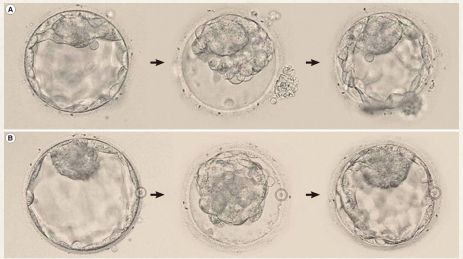
Note: ET = embryo transfer; SD = standard deviation.
[a] $P < .001$.

Grygoruk. Embryo transfer, part II. Fertil Steril 2011.

GRADO ESPANSIONE BL



* Collassamento BL



J Assist Reprod Genet (2016) 33:467–471
DOI 10.1007/s10815-016-0662-z

EMBRYO BIOLOGY

Artificial shrinkage of blastocoel using a laser pulse prior to vitrification improves clinical outcome

Ehab Darwish[1] · Yasmin Magdi[1]

Sopravvivenza → SUPERIORE nel gruppo di studio (115 Controllo vs 309 COLLASSATE) (97.3 and 74.9 %, respectively; p > 0.01).

Cl.PR e IR → Significativamente (p < 0.01) SUPERIORI nel gruppo di studio (67.2 vs. 41.1 %; 39.1 vs. 24.5 %)

LA RIMOZIONE DEL FLUIDO BLASTOCELE MEDIANTE LASER prima della vitrificazione → AUMENTO SOPRAVVIVE

In this study, no significant difference was found between collapsed and non-collapsed blastocysts at the level of implantation but higher survival rates and better post-warm embryo quality were observed when full to expanded blastocysts were collapsed using laser-induced artificial shrinkage before vitrification. However, it should be emphasized that this bene-

Human Reproduction, Vol.30, No.11 pp. 2509–2518, 2015
Advanced Access publication on September 12, 2015 doi:10.1093/humrep/dev218

human reproduction ORIGINAL ARTICLE *Embryology*

A prospective randomized controlled trial investigating the effect of artificial shrinkage (collapse) on the implantation potential of vitrified blastocysts

L. Van Landuyt*, N.P. Polyzos, N. De Munck, C. Blockeel, H. Van de Velde, and G. Verheyen

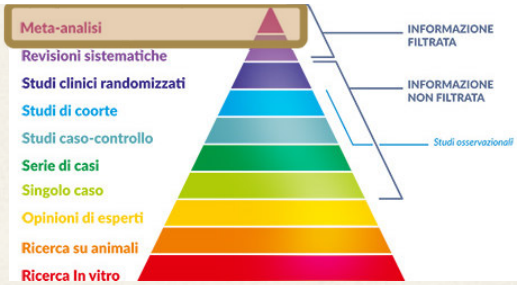
TRA EMBRIONI E BLASTOCISTI

* ET A BL potrebbe sembrare più VANTAGGIOSO perché:
  * Esposizione all'ambiente uterino più simile al ciclo naturale
  * Self-selezione dopo l'attivazione del genoma embrionario dal D3
* ET A BL potrebbe sembrare più SVANTAGGIOSO perché:
  * La coltura *in-vitro* dopo l'attivazione del genoma embrionario potrebbe esser dannosa all'embrione
  * Self-selezione *in-vitro* potrebbe non rispettare la self-selezione naturale

Cochrane Library

The evidence was of low quality for most outcomes.

2016



BL vs Cleavage ET a fresco

Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology (Review)

Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D

WILEY

* Inclusi 27 RCTs (4031 coppie)

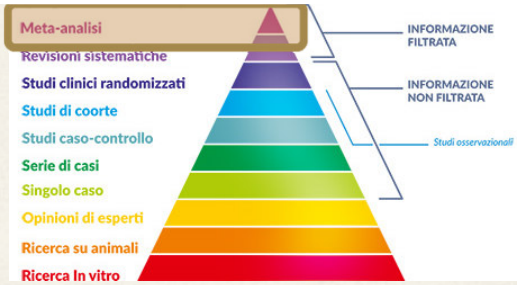
STADIO ET	LBR	Cl. PR	CRIO E SOVRANN.	CANCELL. RATE	MULT.P R & MR
CLEAVAGE	< (29%)	< (36%)	> (60%)	> (1%)	=
BL	> (32-42%)	> (39-46%)	< (37-46%)	< (2-4%)	=
Evidence:	*LOW QUALITY*	*MODERATE QUALITY*	*MODERATE QUALITY*	*MODERATE QUALITY*	*LOW QUALITY*

it remains unclear whether the day of transfer impacts on cumulative rates of live birth and pregnancy. There was no evidence of a difference between the groups in multiple pregnancy and miscarriage rates, but the quality of evidence was low. Future RCTs should

Ultrasound Obstet Gynecol 2017; **49**: 583–591
Published online 10 April 2017 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.17327

Blastocyst *vs* cleavage-stage embryo transfer: systematic review and meta-analysis of reproductive outcomes

W. P. MARTINS[1,2], C. O. NASTRI[1], L. RIENZI[3], S. Z. VAN DER POEL[4,5], C. GRACIA[6] and C. RACOWSKY[7]



BL vs Cleavage ET a fresco

* 33 studi eligibili (12 inclusi)
* 2418 cicli: 1218 stadio di clivaggio (D2-3) + 1200 a BL (D5-6)

STADIO ET	LBR	Cl. PR	Cum. PR	Ong. PR	MR	CRIO E SOVRANN
CLEAVAGE	=	=	=	=	=	>
BL	=	=	=	=	=	<
Evidence:	***LOW QUALITY***	***LOW QUALITY***	***LOW QUALITY***	***LOW QUALITY***	***LOW QUALITY***	***MODERATE QUALITY***

NO SUPERIORITÀ BL VS CLIVAGGIO nella pratica clinica

* Servono RCTs addizionali ben disegnati per dare conclusioni più robuste

Limitations of Embryo Selection Methods

Semin Reprod Med 2014

Kai Mee Wong, MD[1] Sjoerd Repping, PhD[1] Sebastiaan Mastenbroek, PhD[1]

2 PRINCIPALI ASPETTI hanno rivoluzionato la selezione degli embrioni:

* La consapevolezza che la stimolazione ovarica in IVF può avere effetto NEGATIVO SULLA RECETTIVITÀ ENDOMETRIALE e la necessità di sincronizzazione tra Embrione ed Endometrio

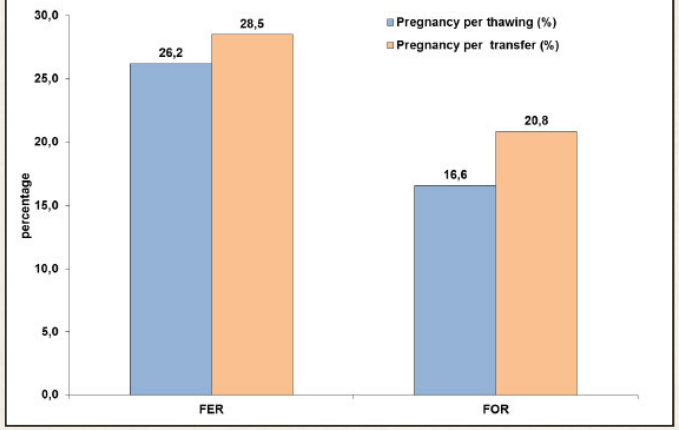
  (D'Angelo A1 et al, Embryo freezing for preventing ovarian hyperstimulation syndrome. Cochrane Database Syst Rev. 2007): → NO differenza tra ET fresco, ET fresco + crio, freeze-all

* Le METODICHE DI CRIOCONSERVAZIONE (introduzione dei protocolli di VITRIFICAZIONE) (AbdelHafez FF ET AL, Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis. RBM Online. 2010)

IARTR – THE ITALIAN ASSISTED REPRODUCTIVE TECHNOLOGY REGISTER

In **Figure 6** the pregnancy rate per thawing and per transfer using FER or FOR are shown. FER rates were significantly higher than FOR ones.

Figure 6: Pregnancy rates per thawing cycle and per transfer using FER and FOR procedures, 2014.



Predicting the chances of a live birth after one or more complete cycles of in vitro fertilisation: population based study of linked cycle data from 113 873 women

David J McLernon,[1] Ewout W Steyerberg,[2] Egbert R te Velde,[2] Amanda J Lee,[1] Siladitya Bhattacharya[3]

the bmj | *BMJ* 2016;355:i5735 | doi: 10.1136/bmj.i5735

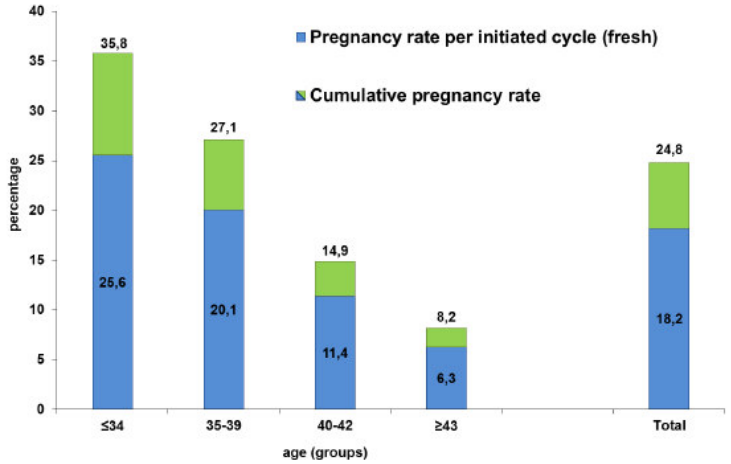
➢ Perché CUMULATIVO?

Generalmente LBR /singolo ET fresco, ma più informativa oggi per l'implementazione dei cicli da embrioni crioconservati

Calcolo della stima delle chance CUMULATIVE di LB su
6 cicli IVF completi (freschi + crio)
prima del trattamento e dopo il primo ET fresco

253.417 donne trattate con IVF OMOLOGA in UK dal 1999 al 2008

Figure 7: Pregnancy rates per initiated cycle for fresh and Cumulative pregnancy rates per initiated cycle, by female patients age groups, 2015.



Hanno stimato le CHANCES INDIVIDUALI DI AVERE 1 NATO VIVO:

★Età PAZIENTE (DAI 30 ANNI) → IL principale INDICATORE PREDITTIVO DI LB

★N° OVOCITI RECUPERATI e LA CRIOCONSERVAZIONE EMBRIONARIA
→ I SUCCESSIVI PRINCIPALI INDICATI

J Assist Reprod Genet
DOI 10.1007/s10815-017-1040-1

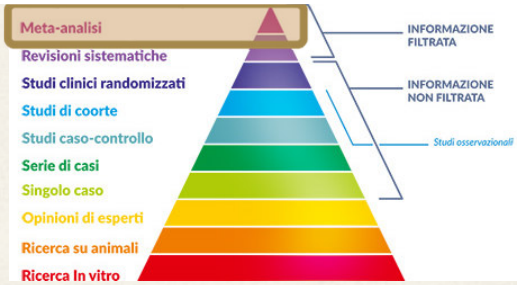
CrossMark

ASSISTED REPRODUCTION TECHNOLOGIES

Comparison of pregnancy outcomes after vitrification at the cleavage and blastocyst stage: a meta-analysis

MeiFang Zeng[1] · SuQin Su[1] · LiuMing Li[1]

Published online: 22 September 2017



ET da Embrioni Crioconservati

* 8 studi (7 retrospettivi osservaz. + 1 prospettico)
* 6590 cicli: 4594 stadio di clivaggio + 1996 a BL

STADIO ET VITRIF	IR (I)	Cl. PR (I)	LBR	Ong. PR	Mult. PR	MR
CLEAVAGE	<	=	=	=	=	<
BL	>	=	=	–	–	>
Evidence:	*LOW QUALITY*	*LOW QUALITY*	*LOW QUALITY*			

Conclusion In summary, this meta-analysis shows that vitrification at any stage has no detrimental effect on clinical outcome. Blastocyst transfer will still remain a favorable and promising option in ART. Due to the small sample evaluated in the pool of included studies, large-scale, prospective, and randomized controlled trials are required to determine if these small effects are clinically relevant.

Cochrane Library

Cochrane Database of Systematic Reviews

Fresh versus frozen embryo transfers in assisted reproduction (Review)

Wong KM, van Wely M, Mol F, Repping S, Mastenbroek S

Fresh versus frozen embryo transfers in assisted reproduction (Review)

WILEY

Strategia del "FREEZE-ALL"

serious risk of bias and (for some outcomes) serious imprecision

PRESUPPOSTO: LE 2 STRATEGIE PER L'ET da IVF o ICSI:

I. un singolo transfer a fresco + differenti FETs.

II. L'alternativa è la strategia del « freeze-all» (solo FETs).

Entrambe con differenti strategie di stadi di sviluppo e di tecniche di congelamento

STADIO ET	LBR	Cum. LBR	PREV. OHSS	MR	Complic.	Mult. PR al I ET
FRESCO + CRIO	=	= (56%)	< (7%)	<	>	=
FREEZE ALL	=	= (56-65%)	> (1-3%)	>	<	=
Evidence:	*MODERATE QUALITY*	*LOW QUALITY*	*LOW QUALITY*	*LOW QUALITY*	*LOW QUALITY*	*LOW QUALITY*

Authors' conclusions

We found moderate-quality evidence showing that one strategy is not superior to the other in terms of cumulative live birth rates. Time to pregnancy was not reported, but it can be assumed to be shorter using a conventional IVF/ICSI strategy in the case of similar cumulative live birth rates, as embryo transfer is delayed in a freeze-all strategy. Low-quality evidence suggests that not performing a fresh transfer lowers the OHSS risk for women at risk of OHSS.

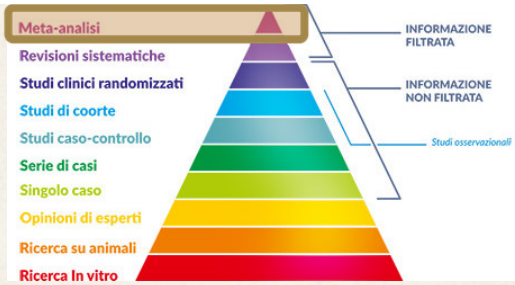
Wang et al. Reproductive Biology and Endocrinology (2017) 15:36
DOI 10.1186/s12958-017-0255-4

Reproductive Biology and Endocrinology

REVIEW Open Access

Comparative neonatal outcomes in singleton births from blastocyst transfers or cleavage-stage embryo transfers: a systematic review and meta-analysis

Xingling Wang*†, Mingze Du†, Yichun Guan, Bijun Wang, Junwei Zhang and Zihua Liu



ET da Embrioni Freschi e Crioconservati

* *12 STUDI INCLUSI con SET (molti fattori confondenti → necessità di solidi RCTs))*

STADIO ET A FRESCO	PREMAT. (<32)	LARGE PREMAT. (<37)	LOW W. (< 2500G)	LARGE FOR GEST. AGE	SMALL FOR GEST. AGE
CLEAVAGE	<	<	> NEI CICLI	<	>
BL	>	>	A FRESCO	>	<

* No differenze: su cicli da crioconservato

Conclusions: The risks of preterm and very preterm births increased after fresh blastocyst transfers versus the risks after fresh cleavage-stage embryo transfers. However, in frozen embryo transfers, there were no differences. Blastocyst embryo transfers resulted in high risks of infants who were large for gestational age, and cleavage-stage embryo transfers resulted in high risks of infants who were small for gestational age.

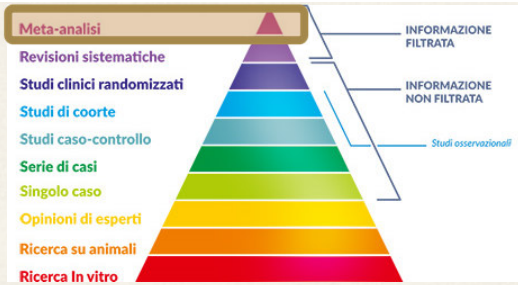
Human Reproduction Update, Vol.24, No.1 pp. 35–50, 2018
Advanced Access publication on November 13, 2017 doi:10.1093/humupd/dmx031

human reproduction update

Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer?

Abha Maheshwari [1,*], Shilpi Pandey[2], Edwin Amalraj Raja[3], Ashalatha Shetty[1], Mark Hamilton[1], and Siladitya Bhattacharya[3]

ET da Embrioni Crioconservati

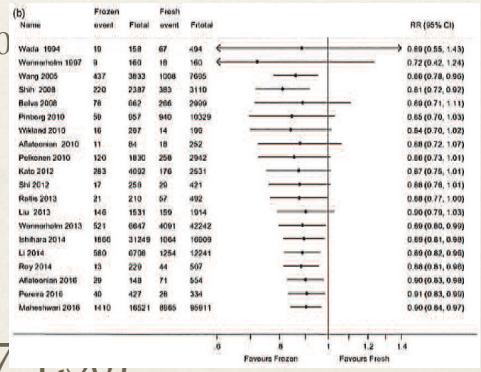


* Include 26 studi: Analizzate le complicanze ostetriche e perinatali nelle gravidanze singole dopo ET di embrioni (freschi e crioconservati)

LA GRANDEZZA DEGLI OUTCOMES INVARIATA NEL TEMPO, AUMENTATA LA PRECISIONE)

* **I NATI SINGOLI da EMBRIONI CRIOCONSERVATI**

Rischio relativo **<** di: (Già NOTO DALLA METANALISI CUMULATIVA DAL 200



parto prematuro (0,90; IC 95% 0,84-0,97)

basso peso alla nascita (0,72; IC 95% 0,67-0,77)

piccolo per età gestazionale (0,61; 95 % CI 0,56-0,67)

Rischio **>** di:

disordini ipertensivi della gravidanza (1,29, IC 95% 1,07

elevato peso per età gestazionale (1,54, IC 95% 1,48- 1,61)

alto peso alla nascita (1,85, IC 95% 1,46-2,33).

No differenza nel rischio di ANOMALIE CONGENITE E MORTALITÀ PERINATALE

15.7 Embryo transfer strategies

Timing of transfer

Royal College of Obstetricians and Gynaecologists
Bringing to life the best in women's health care

Fertility: assessment and treatment for people with fertility problems

February 2013
NICE Clinical Guideline

SET → strategia di I PRIMA per DONNE ≤ 39 ANNI
DET → strategia di I PRIMA per 40 ≤ DONNE ≤ 42 ANNI

* 1 studio (Papanikolaou, 2006): 1 embrione allo stadio di clivaggio con 1 allo stadio di blastocisti.
* 1 studio: la singola blastocisti con un singolo stadio di clivaggio trasferimento di embrioni (Zech et al., 2007).

Table 15.27 GRADE findings for comparison of timing of embryo transfer

CICLI FRESCHI

Number of studies	Number of patients/women: Intervention (Day 2 – 3)	Number of patients/women: Comparator (Day 5 – 6)	Effect: Relative (95% CI)		
Live full-term singleton birth					
Cumulative					
No evidence reported					
Fresh cycle					
DET					
4 (Van der Auwera et al., 2002; Rienzi et al, 2002; Emiliani et al., 2003; Papanikolaou et al., 2005)	121/287 (42.2%)	140/282 (49.6%)	OR 0.74 (0.53 to 1.04)	75 fewer per 1000 (from 153 fewer to 10 more)	Very low
SET					
1 (Papanikolaou et al., 2006)	38/176 (21.6%)	56/175 (32%)	OR 0.59 (0.36 to 0.95)	103 fewer per 1000 (from 11 fewer to 175 fewer)	Moderate

DET

SET

Frozen cycle					
No evidence reported					
Clinical pregnancy					
DET					
7 (Van der Auwera et al., 2002; Rienzi et al., 2002; Emiliani et al., 2003; Papanikolaou et al., 2005; Hreinsson et al., 2004; Bungum et al., 2003; Coskun et al., 2000)	219/525 (41.7%)	232/507 (45.8%)	OR 0.86 (0.67 to 1.1)	37 fewer per 1000 (from 96 fewer to 24 more)	Very low
Clinical pregnancy – SET					
2 (Papanikolaou et al., 2006; Zech et al., 2007)	64/275 (23.3%)	100/303 (33%)	OR 0.62 (0.43 to 0.89)	96 fewer per 1000 (from 29 fewer to 155 fewer)	Moderate

DET

SET

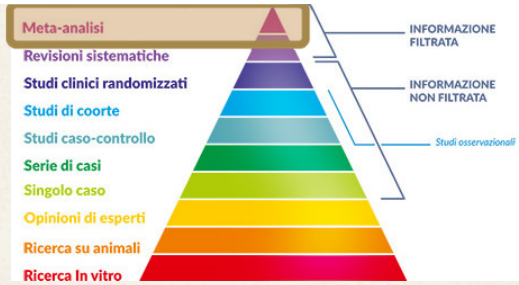
CICLI CRIOCONSERVATI

Number of studies	Number of patients/women: Intervention (Day 2 – 3)	Number of patients/women: Comparator (Day 5 – 6)	Effect: Relative (95% CI)	Effect: Absolute (95% CI)	Quality
Multiple pregnancies (the number of pregnancies with more than one fetus)					
DET					
7 (Kolibianakis et al., 2004; Van der Auwera et al., 2002; Rienzi et al., 2002; Emiliani et al., 2003; Papanikolaou et al., 2005; Hreinsson et al., 2004; Bungum et al., 2003)	72/658 (10.9%)	78/633 (12.3%)	OR 0.9 (0.64 to 1.27)	11 fewer per 1000 (from 41 fewer to 28 more)	Very low
Multiple births (the number of babies born from a multiple pregnancy)					
No evidence reported					
Preterm delivery					
No evidence reported					
Adverse pregnancy outcome (ectopic pregnancy, extrauterine pregnancy, miscarriage)					
DET					
7 (Kolibianakis et al., 2004; Van der Auwera et al., 2002; Rienzi et al., 2002; Emiliani et al., 2003; Papanikolaou et al., 2005; Hreinsson et al., 2004; Bungum et al., 2003)	51/658 (7.8%)	67/633 (10.6%)	OR 0.72 (0.49 to 1.05)	27 fewer per 1000 (from 51 fewer to 5 more)	Very low
SET					
2 (Papanikolaou et al., 2006; Zech et al., 2007)	29/275 (10.5%)	26/303 (8.6%)	OR 1.23 (0.7 to 2.15)	18 more per 1000 (from 24 fewer to 82 more)	Low

CI confidence interval, DET double embryo transfer, OR odds ratio, SET single embryo transfer

Cochrane Library

Cochrane Database of Systematic Reviews



Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection (Review)

Pandian Z, Marjoribanks J, Ozturk O, Serour G, Bhattacharya S

2013

- Inclusi 14 RCT con 2165 donne
- SET RIPETUTO: PARE LA MIGLIOR OPZIONE per la maggior parte delle pazienti, principalmente dimostrato per pazienti giovani a buona prognosi.

SET ripetuto (2 a fresco o 1 fresco + 1 crioconservato: 42%) **vs DET** (1 DET: 31-44%) (3 studi allo stadio di clivaggio):
NO DIFFERENZA in termini di CUMULATIVE PR (LOW QUALITY OF EVIDENCE)
SIGNIFICATIVAMENTE DIFFERENTE LE MULTIPLE PR (0-2% VS 13%) (LOW QUALITY OF EVIDENCE)

SET SINGOLO vs **DET SINGOLO** (2 studi **con ET a BL**):
SIGNIFICATIVAMENTE DIFFERENTE IL LBR (24-33% vs 45%) (HIGH QUALITY OF EVIDENCE)
SIGNIFICATIVAMENTE DIFFERENTE il MULTIPLE PR (1-3% VS 14%) (HIGH QUALITY OF EVIDENCE)

**SET È ASSOCIATO AD UNA RIDUZIONE DEL LBR RISPETTO AL DET.
SE RIPETUTO, NON ASSOCIATO A DIFFERENZE IN TERMINI DI CUM. PR E ASSOCIATO A RIDUZIONE SIGNIFICATIVA DELMULTIPLE PR.**

HAVING HEALTHY BABIES
ONE AT A TIME

How many embryos should I transfer to have one baby?

Embryo Stage (Day of Transfer)	EMBRYO TRANSFER OPTION		
	1 SET (fresh)	1 SET (fresh) + 1 SET (frozen)	1 DET (fresh)
Cleavage (2-3 days)	At least one baby: 38% Twins: Less than 1%	At least one baby: 55% Twins: Less than 1%	At least one baby: 49% Twins: 16%
Blastocyst (5-6 days)	At least one baby: 51% Twins: Less than 1%	At least one baby: 66% Twins: 1%	At least one baby: 60% Twins: 27%

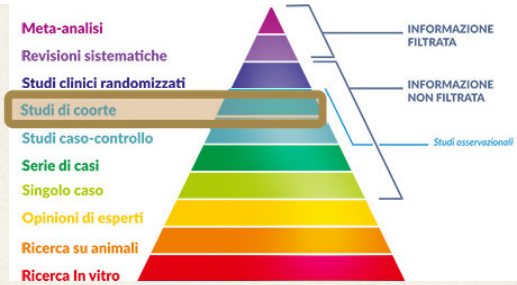
Source: Centers for Disease Control and Prevention, National Assisted Reproductive Technology Surveillance System (NASS), 2010-2012.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

SOCIETY FOR ASSISTED REPRODUCTIVE TECHNOLOGY
sart
FOUNDED 1985

RBM ONLINE

ELSEVIER



Article

Artificial cryopreserved embryo transfer cycle success depends on blastocyst developmental rate and progesterone timing

Kemal Ozgur [a], *Hasan Bulut* [a], *Murat Berkkanoglu* [a], *Peter Humaidan* [b], *Kevin Coetzee* [a,*]

[a] *Antalya IVF, Antalya 07080, Turkey*
[b] *The Fertility Clinic, Skive Regional Hospital and Faculty of Health, Aarhus University, Aarhus, Denmark*

KEY MESSAGE

The timing of blastocyst transfer in frozen embryo transfers must take into consideration the developmental rate of the blastocyst being transferred.

Table 3 – Comparison of reproductive outcomes.[a]

Parameter	Day-5 group (standard) Day-6 progesterone	Day-5 group (short) Day-5 progesterone	Day-4 group (short) Day-5 progesterone	Day-5 group; standard versus short		Day-5 standard versus day-4 short	
Number of single blastocyst transfers	139	104	152	RR (95% CI)	*P*	RR (95% CI)	*P*
Blastocyst quality							
Grade 2–3[b]	39.6 (55)	31.7 (33)	65.8 (100)		NS		0.017
Grade 4–5[b]	60.4 (84)	68.3 (71)	34.2 (52)		NS		0.009
Pregnancy[c]	69.1 (96)	51.0 (53)	71.1 (108)	1.5 (1.147 to 2.028)	0.004	0.955 (0.748 to 1.220)	NS
Clinical pregnancy[d]	60.4 (84)	43.3 (45)	65.8 (100)	1.5 (1.105 to 1.993)	0.008	0.9 (0.707 to 1.132)	NS
Live birth[e]	52.5 (73)	37.5 (39)	60.5 (92)	1.4 (1.049 to 1.936)	0.020	0.9 (0.680 to 1.073)	NS
Total pregnancy loss	24.0 (23)	26.4 (14)	14.8 (16)	0.9 (0.567 to 1.494)	NS	1.4 (0.911 to 2.028)	NS

[a] Data presented as number or percentage (number) with statistical comparison carried out with the chi-squared test, with the standard-protocol day-5 sub-group used as a reference group in comparisons RR (95% CI).
[b] Blastocyst expansion grades 2–5 (early to hatching), with inner cell mass and trophectoderm scores of AA, AB, BA, and BB

- Conferma dell'Importanza della Somministrazione del Progesterone:
  RISPETTARE IL TIMING DELL'EMBRIONE
- Considera l'ET a BL PRECOCE
- LA BL PRECOCE potrebbe presentare un minor tasso di aneuploidie (minor abortività)

  - Retrospettivo / non privo di fattori confondenti / Timing della BL è centro dipendente

A quando l' ET..per chi? Per cosa? ..
Qual è l'END-POINT?

* Clinical Pregnancy Rate?
* Live birth rate?
* Cumulative Pregnancy Rate?
* Healthy Child?
* Helthy Mother?

UNIVERSITY of York
Centre for Reviews and Dissemination

NHS
National Institute for Health Research

E-FREEZE: a randomised controlled trial evaluating the clinical and cost-effectiveness of a policy of freezing all embryos followed by thawed frozen embryo transfer, compared with a policy of fresh embryo transfer in women undergoing in-vitro fertilization

Date abstract record published
24/08/2015

Proposta Studio Multicentrico (10 centri IVF in UK - 1086 donne – 543 per braccio – durata 4 anni). L'Outcome primario è stato definito: **UN NATO SINGOLO A TERMINE E NORMOPESO**

CONCLUSIONI....

* Se l'end-point è la gravidanza cumulativa → INTUITIVAMENTE ET in fase di CLIVAGGIO (Metanalisi con bassa qualità di evidenzia ma non lo conferma)

* Se l'end-point è il peso del bambino alla nascita → ET da Embrioni crioconservati

* Se l'end-point è la riduzione di complicanze della paziente (Riduz. Preclapsie, Riduz. Aborti, Riduz. Rischio gemellarità monozigote) → CLEAVAGE da fresco

* Se l'end-point è evitare i nati prematuri e ridurre l'abortività → evitare l'ET a BL

COMPATIBILMENTE COI LIMITI DI QUESTI STUDI E L'ETEROGENEITÀ DEGLI OUTCOME CLINICI TENUTI IN ESAME COME END POINT:

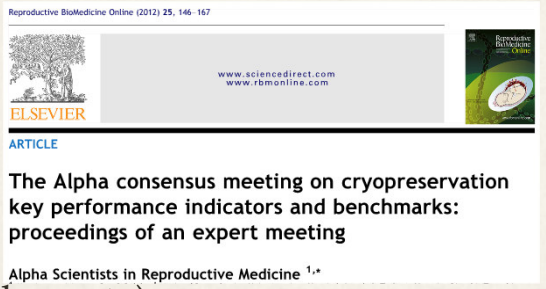
- SE PROBLEMATICHE LEGATE ALLA COLTURA IN VITRO → IMPLEMENTAZIONE DEGLI STUDI CHE NE STANNO VALUTANDO LA CORRELAZIONE.
- SE PROBLEMATICHE LEGATE ALL'INFERTILITA' (ED ALL'ETÀ) → IMPLEMENTARE MAGGIORMENTE ALTRE STRATEGIE

STANDARDIZZAZIONE del LAB di Embriologia Clinica

★ Ottimali condizioni di Coltura (uomo e animale) influenzano

- ★ MORFOLOGIA embrionaria
- ★ La % di Blastulazione
- ★ Il Success Rate
- ★ SOPRAVVIVENZA EMBRIONARIA:

(IL PRINCIPALE PARAMETRO per misurare l'efficienza dello scongelamento)


Reproductive BioMedicine Online (2012) 25, 146–167

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www.sciencedirect.com
www.rbmonline.com

ARTICLE

The Alpha consensus meeting on cryopreservation key performance indicators and benchmarks: proceedings of an expert meeting

Alpha Scientists in Reproductive Medicine [1,*]

→ Scelta della BASSA TENSIONE di ossigeno (

→ Scelta di incubatori TLI, Conventional or Benchtop *(Sciorio R, JARG 2017)*

→ Scelta terreni di coltura

(Rinnovare o meno il terreno al D3) e (Sequenziali vs Singoli)

(Sfontouris et al, J Assist Reprod Genet 2016, A systematic review and meta-analysis of randomized controlled trials)

→ Utilizzo di Ridotti Volumi (per minimizzare diluizione di fattori benefici)

→ Implementazione di controlli adeguati: EMOGAS (pH)

SONDE AD IMMERSIONE (T) e GasAnalyzer (CO2)

→ Ottimali Training e Re-training con Monitoraggio delle Competenze

(Operatore clinico e di Laboratorio)

Harper J et al, HR 2017.
Adjuncts in the IVF laboratory: where is the evidence for 'add-on' interventions?