OSPEDALE
SAN RAFFAELE

Controversie nella manipolazione invasiva della blastocisti

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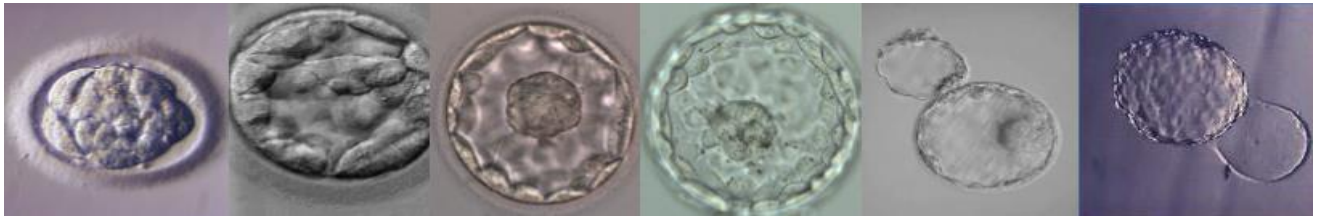
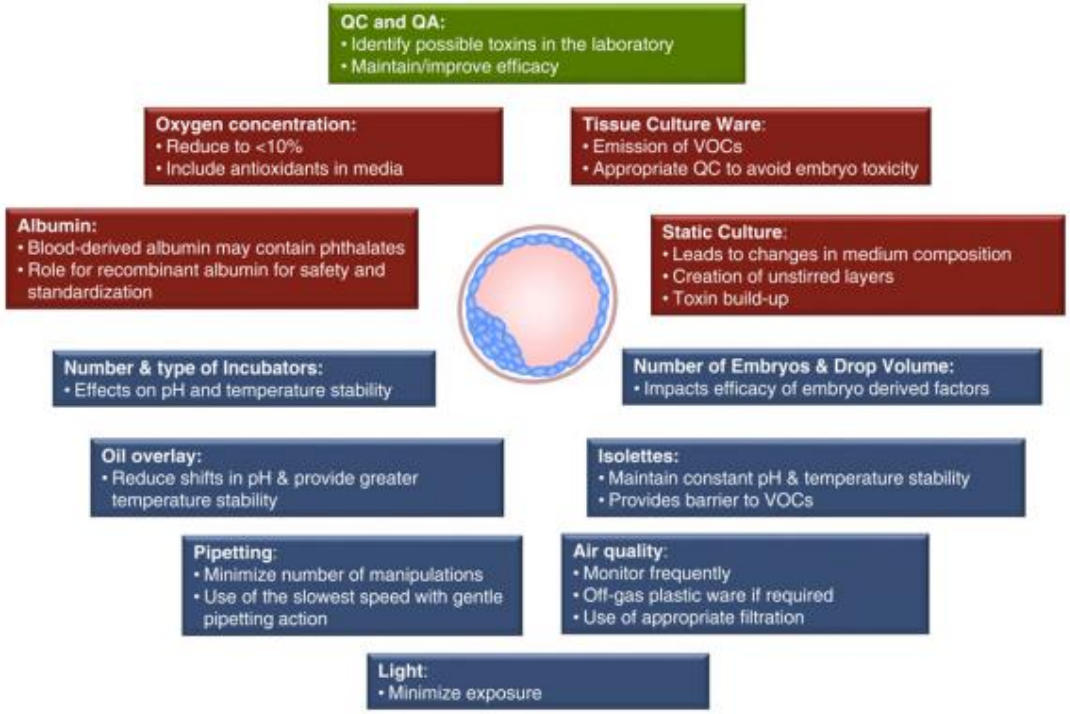
San Raffaele Scientific Institute

Milano

INTRODUZIONE

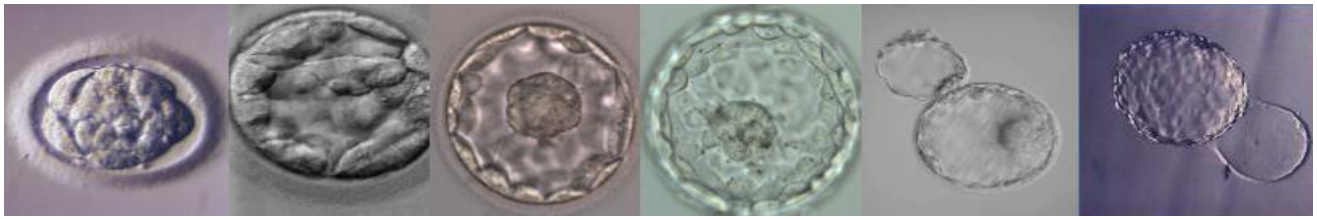


Coltura estesa fino allo stadio di blastocisti = pratica comune



Wale & Gardner, 2016

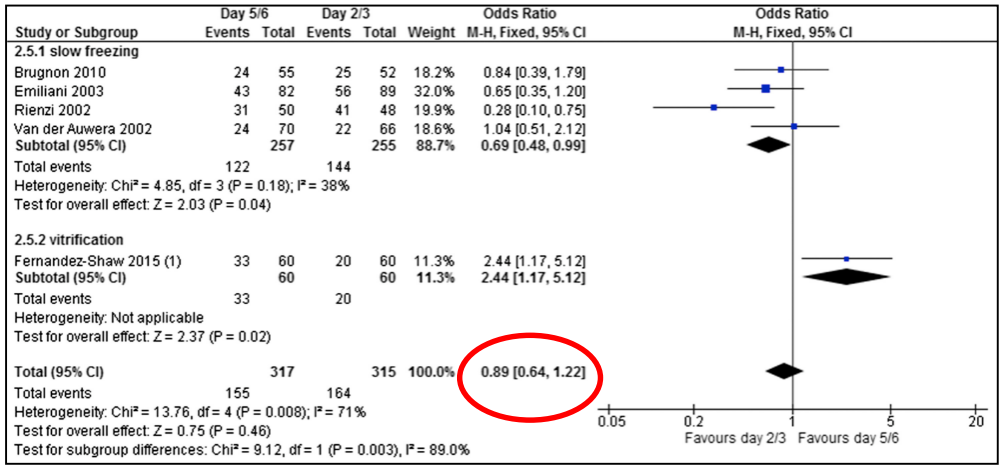
INTRODUZIONE



VANTAGGI DELLA COLTURA A BLASTOCISTI

- ✓ sincronizzazione con l'endometrio
- ✓ migliore selezione dell'embrione vitale
- ✓ minima esposizione dell'embrione all'ambiente uterino iperstimolato (estrogeni e progesterone)
- ✓ incremento nel tasso di impianto
- ✓ permette la PGT

INTRODUZIONE



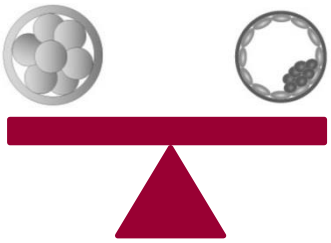
Cochrane Library

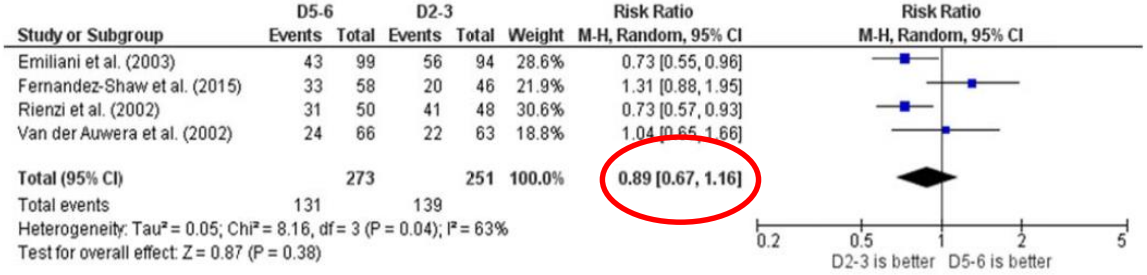
Cochrane Database of Systematic Reviews

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

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

2016





Ultrasound Obstet Gynecol 2017; 49: 583–591

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]

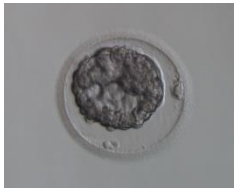
NO differenze nel tasso di gravidanze cliniche cumulative

INTRODUZIONE

La manipolazione della blastocisti allo scopo di ottimizzare i risultati dell'IVF

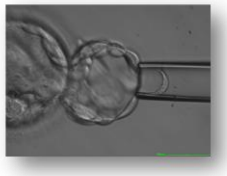
TIPI DI MANIPOLAZIONE DELLA BLASTOCISTI:

- ✓ **Artificial Shrinkage (collapse)**



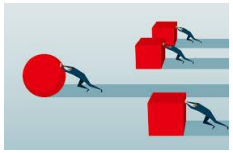
- ✓ **Assisted hatching**

- ✓ **Biopsia del trofectoderma**



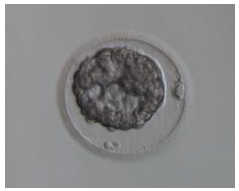
Efficacia?

Efficienza?



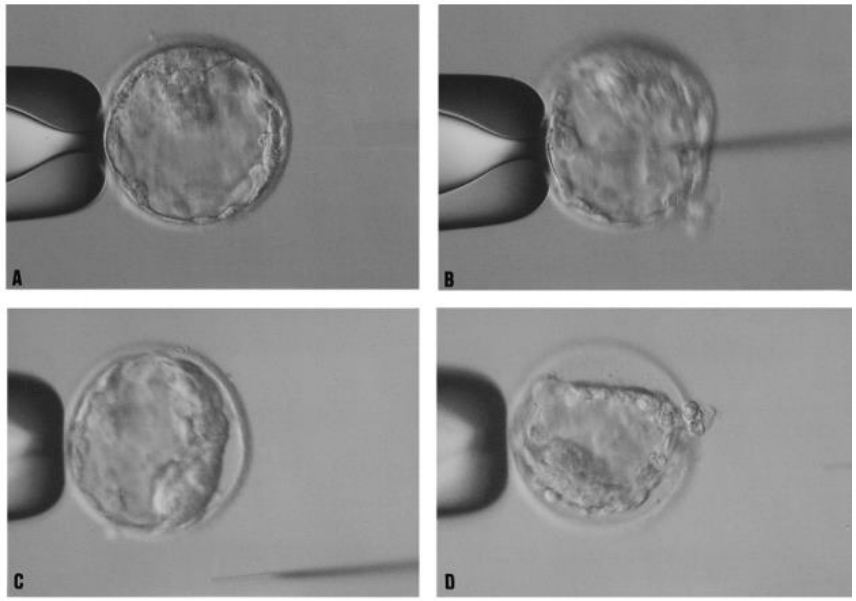
Sicurezza?

ARTIFICIAL SHRINKAGE



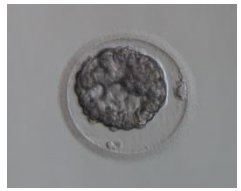
''Artificial shrinkage'' o **''collapse'':**
rimozione parziale del fluido del blastocele prima della vitrificazione della blastocisti

Introdotta da **Vanderzwalmen et al. (2002)**



- ✓ Riduzione del rischio di formazione di cristalli di ghiaccio
- ✓ Incremento del tasso di sopravvivenza

ARTIFICIAL SHRINKAGE



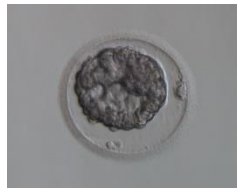
- Molti studi retrospettivi

Mukaida et al. (2006)

✓ Gruppo controllo e gruppo AS in 2 differenti periodi

	control	AS	
N. blastocysts	339	502	
Survival rate	85.0%	97.2%	P<0.05
Clinical PR	34.1%	60.2%	P<0.01

ARTIFICIAL SHRINKAGE



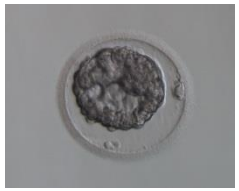
- Studio osservazionale retrospettivo

Cao et al. (2014)

- ✓ AS con 2 metodi diversi: laser vs ago (assente controllo NO AS)
- ✓ 438 blastocisti scongelate
- ✓ NO differenze in tasso di sopravvivenza, IR, CPR
- ✓ Più alto tasso di hatching nel gruppo AS laser
- ✓ Migliori outcome neonatali nel gruppo AS laser

Utilizzo del laser!!!

ARTIFICIAL SHRINKAGE



- Studio retrospettivo con grande numerosità

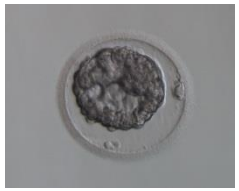
Wang et al. (2017)

- ✓ AS con 2 metodi diversi: laser (LAS) vs ago (MNAS)
- ✓ assente controllo NO AS

	MNAS	LAS	
Warming cycles	743	809	
Survival rate	94%	95.4%	NS
IR	54.4%	60.8%	P=0.01
LBR	45.2%	50.4%	P=0.04
MZT	1.73%	4.07%	P=0.042

Più alto IR e LBR usando il laser ma più alto MZT

ARTIFICIAL SHRINKAGE



➢ Studi retrospettivi

Darwish et al. (2016)

✓ Gruppo di controllo NO AS vs gruppo AS (laser)

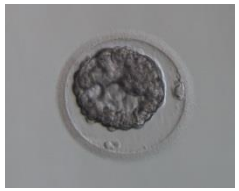
	control	AS	
N° thawed blastocysts	115	309	
Survival rate	79.4%	97.3%	P<0.01
IR	24.5%	41.1%	P<0.01
CPR	39.1%	67.2%	P<0.01

Levi-Setti et al. (2016)

✓ Gruppo di controllo NO AS vs gruppo AS (ago)

	control	AS	
N° thawed blastocysts	625	820	
Survival rate	96.6%	97.8%	NS
IR	23.2%	29.9%	P=0.005
LBR	45.2%	50.4%	P=0.04
Abortion rate	32.0%	23.2%	NS

ARTIFICIAL SHRINKAGE



➢ 1 studio prospettico randomizzato controllato!!!

Human Reproduction, Vol.30, No.11 pp. 2509–2518, 2015

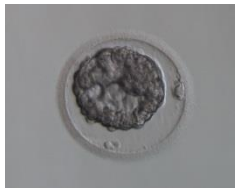
human reproduction

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. Polyzos, N. De Munck, C. Blockeel, H. Van de Velde, and G. Verheyen

ARTIFICIAL SHRINKAGE



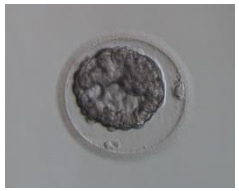
Van Landuyt et al. (2015)

- ✓ Gruppo di controllo NO AS vs gruppo AS (laser)
- ✓ Outcome primario: IR per blasto trasferita nel 1° ciclo di scongelamento

	control	AS		
1° warming cycles	69	69		
Survival rate	91%	100%	NS	
IR	29%	38%	P=0.089	OR: 1.48; 95%CI:0.78-2.83
All warming cycle	115	116		
Survival rate	92%	98%	P=0.007	OR: 4.25; 95%CI:1.19-15.21
High-quality blast	23.5%	36.3%	P=0.016	OR: 1.86; 95%CI:1.12-3.08

Necessità di altri RCT….

ARTIFICIAL SHRINKAGE



E’ una manipolazione sicura per il nascituro???

NO studi di follow-up a lungo termine di bambini nati

ASSISTED HATCHING

"Assisted hatching":
rimozione parziale della zona pellucida della blastocisti

Introdotta da **Cohen et al. (1988)**

- ✓ Diverse tipologia di manipolazione
- ✓ Su blastocisti a fresco o crioconservate
- ✓ In differenti gruppi di pz

- ✓ Pratica comune di molti centri di IVF
- ✓ Razionale?
  - Zona ''hardening'' per effetto della crioconservazione
  - ZP spessa
- ✓ No evidenze per AH su blastocisti da studi ben disegnati e metanalisi

ASSISTED HATCHING

FRESH EMBRYO TRANSFER

- Studio retrospettivo

Lu et al. (2015)

- ✓ Thinning al 25%
- ✓ 176 pz nel gruppo controllo e 22 nel gruppo AH
- ✓ NO differenze in CPR, LBR

ASSISTED HATCHING

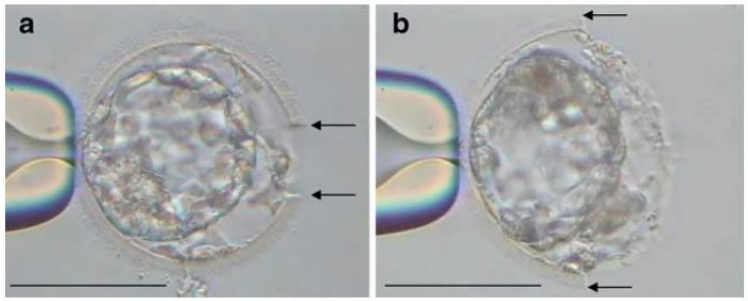
FROZEN EMBRYO TRANSFER

- Studio retrospettivo

Hiraoka et al. (2008)

- ✓ Breaching (40 µm vs 50% ZP)
- ✓ Embrioni scongelati in day 3 ed effettuato AH allo stadio di blastocisti

AH 40 µm AH 50%



	control	AH 40		AH 50	
N° cycles for ET	30	40		31	
CPR	17%	43%	P<0.05	74%	P<0.05
IR	10%	27%	P<0.05	52%	P<0.05
MPR	0%	0%	NS	17%	NS
LBR	13%	38%	P<0.05	65%	P<0.05

ASSISTED HATCHING

FROZEN EMBRYO TRANSFER

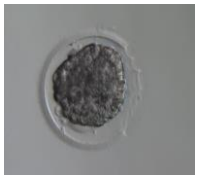
- Studio retrospettivo

Zhang et al. (2009)

- ✓ Thinning (40 µm vs 80 µm)
- ✓ Embrioni scongelati in day 3 ed effettuato AH allo stadio di blastocisti

	control	AH 40		AH 80	
N° cycles for ET	31	34		57	
CPR	16.13%	23.53%	NS	40.35%	P=0.03
IR	7.50%	9.41%	NS	21.54%	P=0.007
MPR	20.0%	0%	NS	21.74%	NS

ASSISTED HATCHING



➢ 2 studi prospettici randomizzati controllati su blastocisti scongelate

Human Reproduction, Vol.26, No.8 pp. 1997–2007, 2011
Advanced Access publication on June 8, 2011 doi:10.1093/humrep/der161

human reproduction ORIGINAL ARTICLE *Embryology*

The effect of modified quarter laser-assisted zona thinning on the implantation rate per embryo in frozen/vitrified-thawed/warmed embryo transfer cycles: a prospective randomized controlled trial†

S. Debrock*, K. Peeraer, C. Spiessens, D. Willemen, P. De Loecker, and T.M. D'Hooghe

Reproductive BioMedicine Online (2014) 28, 582–589

ELSEVIER

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Laser-assisted hatching improves clinical outcomes of vitrified–warmed blastocysts developed from low-grade cleavage-stage embryos: a prospective randomized study

Cai-Yun Wan [1], Cheng Song [1], Liang-Hui Diao, Guan-Gui Li, Zhong-Jian Bao, Xiao-Dong Hu, Hong-Zhan Zhang, Yong Zeng *

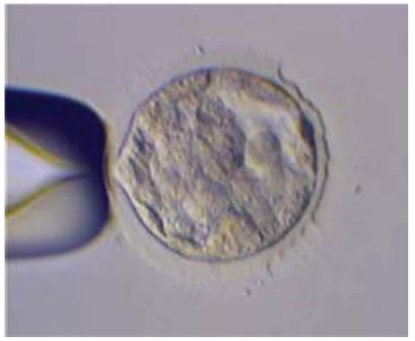
ASSISTED HATCHING

FROZEN EMBRYO TRANSFER

- Studio prospettivo randomizzato

Debrock et al. (2011)

- ✓ Thinning 120°
- ✓ Scongelate blastocisti ed effettuato AH



	A. Total group			B. first cycle group		
	mQLAZT	Control	*P*-value	mQLAZT	Control	*P*-value
Per embryo d5 slow						
Survived (%)[a]	33.3	42.9	>0.99	33.3	42.9	>0.99
No. of FET cycles	1	4		1	4	
No. of transfers (%/FET cycle)	0 (0)	2 (50.0)	>0.99	0 (0)	2 (50.0)	>0.99
No. of pregnancies (%/ET)	0	1 (50.0)	>0.99	0	1 (50.0)	>0.99
No. of impl (IU-EU) (%)[b]	0/0 (0)	1/3 (33.3)	>0.99	0/0 (0)	1/3 (33.3)	>0.99
Per embryo d5 vitr						
Survived (%)[a]	36.1	49.1	0.08	39.6	48.6	0.31
No. of FET cycles	23	35		15	33	
No. of transfers (%/FET cycle)	11(48.8)	20 (57.1)	0.59	7 (46.7)	20 (60.6)	0.53
No. of pregnancies (%/ET)	1 (9.1)	5 (25)	0.38	0 (0)	5 (25.0)	0.28
No. of impl (IU-EU) (%)[b]	0/16 (0)	5/27 (18.5)	0.14	0/10 (0)	5/27 (18.5)	0.14

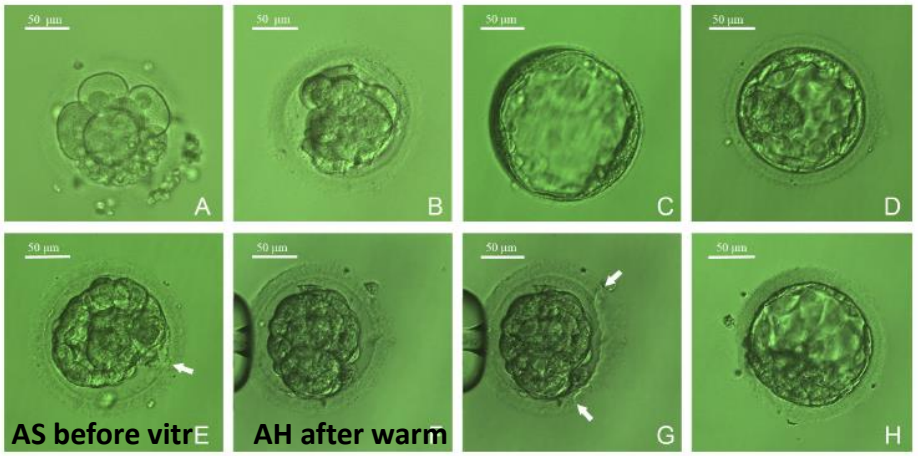
ASSISTED HATCHING

FROZEN EMBRYO TRANSFER

- Studio prospettivo randomizzato

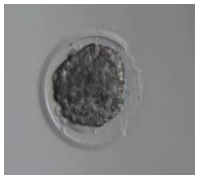
Wan et al. (2014)

- ✓ Breaching 25%
- ✓ AH effettuato solo su blastocisti sviluppate da embrioni in day 3 di pessima qualità



	control	AH	
N° cycles	102	96	
CPR	35.3%	51%	P=0.034
IR	23.6%	34.2%	P=0.021
LBR	28.4%	40.6%	NS

ASSISTED HATCHING



Reproductive BioMedicine Online (2010) 21, 17–25

Zona-free embryo culture: is it a viable option to improve pregnancy rates?

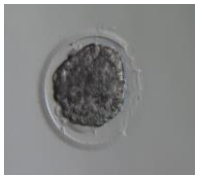
Gábor Vajta [a,*], Laura Rienzi [b], Barry D Bavister [c]

Table 9 Results of ZP total removal using laser assisted hatching compared to zona-intact

	Method	Time of AH	Age (mean ± SD) Zona-free/zona-intact	CPR	IR	AbR	MPR	LBR	P/PR/R study
Fong et al., 1998	pronase	Days 5 blastocysts, few hours before ET	32.6±5.2	n.a.	n.a.	n.a.	n.a.	n.a.	PR
Isik et al., 2000	pronase	Days 5 blastocysts, 30'-60' before ET	30.5±5.2 29.1±3.6	unchanged	unchanged	-	unchanged	unchanged	PR
Urman et al., 2002	pronase	Days 5 blastocysts, 30'-60' before ET	31.8 31.5	unchanged	improved	unchanged	unchanged	-	PR
Kinget et al., 2002	pronase	Day 5 blastocysts, few hours before ET	31.0±3.9 32.0±4.0	improved	improved	-	unchanged	unchanged	PR
Jelinkova et al., 2003	Acidic Tyrode's solution	Day 5 blastocysts, 20' before ET	32.3±4.2 32.1±3.1	improved	improved	-	unchanged	-	PR

Alteri et al., 2019

ASSISTED HATCHING



Fertility and Sterility® Vol. 104, No. 1, July 2015

Risk of major congenital anomalies after assisted hatching: analysis of three-year data from the national assisted reproduction registry in Japan

Junna Jwa, M.D.,[a,b] Seung Chik Jwa, M.D., Ph.D., M.P.H.,[a] Akira Kuwahara, M.D., Ph.D.,[c] Atsumi Yoshida, M.D., Ph.D.,[b] and Hidekazu Saito, M.D., Ph.D.[a]

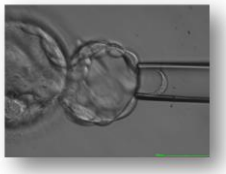
Prevalence and crude and adjusted ORs of assisted hatching (AH) for major congenital anomaly according to multiplicity by organ system.

	All births				Singleton births			
Type of major congenital anomaly	AH (n = 36,033)	Non-AH (n = 37,119)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)	AH (n = 34,949)	Non-AH (n = 36,157)	Crude OR	Adjusted OR[a] (95% CI)
Any major anomalies	490 (1.4)	552 (1.5)	0.91 (0.81–1.03)	0.92 (0.80–1.06)	472 (1.4)	540 (1.5)	0.90 (0.80–1.02)	0.91 (0.79–1.05)
Multiple major anomalies	40 (0.11)	52 (0.14)	0.79 (0.52–1.20)	0.73 (0.47–1.13)	40 (0.11)	50 (0.14)	0.83 (0.55–1.25)	0.76 (0.48–1.19)
Chromosomal abnormalities	130 (0.36)	148 (0.40)	0.90 (0.71–1.15)	0.92 (0.71–1.20)	130 (0.37)	145 (0.40)	0.93 (0.73–1.18)	0.97 (0.73–1.27)
Cardiovascular abnormalities	174 (0.48)	185 (0.50)	0.97 (0.79–1.19)	0.94 (0.75–1.18)	163 (0.47)	182 (0.50)	0.93 (0.75–1.14)	0.91 (0.73–1.15)
Musculoskeletal abnormalities	72 (0.2)	91 (0.25)	0.81 (0.60–1.11)	0.82 (0.58–1.14)	70 (0.2)	89 (0.25)	0.81 (0.59–1.11)	0.82 (0.58–1.16)
Urogenital abnormalities	30 (0.08)	36 (0.10)	0.86 (0.53–1.39)	0.85 (0.50–1.43)	29 (0.08)	36 (0.10)	0.83 (0.51–1.36)	0.80 (0.47–1.36)
Gastrointestinal abnormalities	38 (0.11)	33 (0.09)	1.19 (0.74–1.89)	1.19 (0.71–2.00)	36 (0.10)	29 (0.08)	1.28 (0.79–2.10)	1.29 (0.75–2.21)
Central nervous system abnormalities	36 (0.10)	48 (0.13)	0.77 (0.50–1.19)	0.79 (0.48–1.27)	34 (0.10)	47 (0.13)	0.75 (0.48–1.16)	0.76 (0.47–1.25)
Respiratory abnormalities	3 (0.01)	7 (0.02)	0.44 (0.11–1.71)	0.33 (0.085–1.27)	3 (0.01)	7 (0.02)	0.44 (0.11–1.71)	0.33 (0.081–1.33)
Orofacial abnormalities	46 (0.13)	42 (0.12)	1.13 (0.74–1.71)	1.13 (0.73–1.75)	46 (0.13)	42 (0.12)	1.13 (0.75–1.72)	1.13 (0.71–1.79)
Eye, ear, neck abnormalities	13 (0.04)	18 (0.05)	0.74 (0.36–1.52)	0.96 (0.50–1.86)	13 (0.04)	18 (0.05)	0.75 (0.37–1.52)	0.97 (0.43–2.16)
Others	1 (0.00)	5 (0.01)	0.21 (0.02–1.76)	0.16 (0.02–1.23)	1 (0.00)	5 (0.01)	0.21 (0.02–1.77)	0.16 (0.02–1.41)

Note: CI = confidence interval; OR = odds ratio.
[a] Adjusted for maternal age, calendar year, fetal sex, embryo stage at transfer, and status of cryopreservation.

Jwa. Assisted hatching and major birth defects. Fertil Steril 2015.

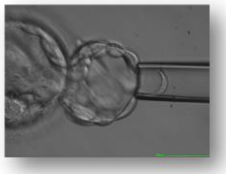
BIOPSIA DEL TROFECTODERMA



- ✓ Solo per test genetici (**PGT-M, PGT-SR, PGT-A**)

- ✓ Dal 2010: passaggio dalla biopsia in day 3 alla biopsia allo stadio di blastocisti
  - Meno invasiva (meno impatto sulla totalità dell'embrione)
  - Più cellule disponibili per la valutazione genetica (4-10 cells)
  - Più basso tasso di mosaicismo

BIOPSIA DEL TROFECTODERMA



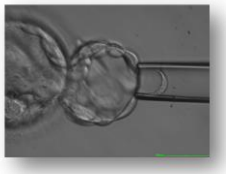
- ✓ Molti studi riportano risultati positivi dopo transfer di blastocisti sottoposte a biopsia del TE per PGT

- ✓ L'impatto della manipolazione della blastocisti effettuando una **biopsia di 4-10 cellule** del TE sullo sviluppo embrionale: pochi studi

    - Variabili inter-paziente
    - Variabili intra-paziente (cicli)

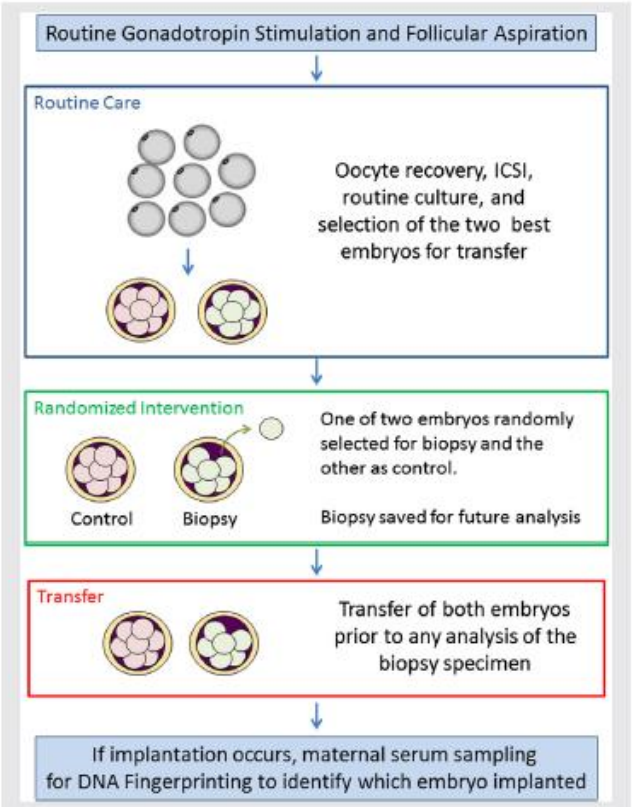
        - Risposta alla stimolazione ormonale
        - Recettività endometriale
        - Laboratorio
        - Terreni di coltura

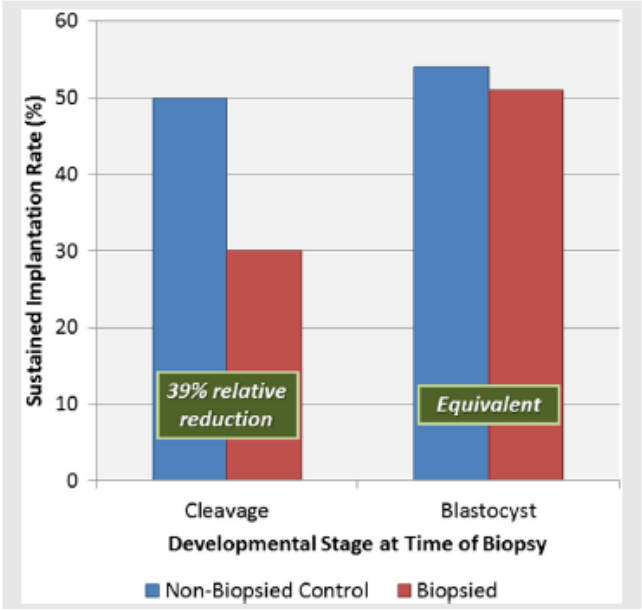
BIOPSIA DEL TROFECTODERMA



- Studio prospettivo randomizzato accoppiato

Scott et al. (2013)

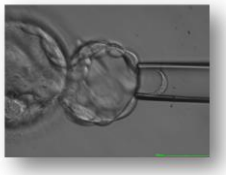




- ✓ Approccio che esclude la variabilità del paziente e del ciclo
- ✓ Valutazione solamente dell'effetto della biopsia

No impatto della biopsia del TE sullo sviluppo e sull'impianto

BIOPSIA DEL TROFECTODERMA

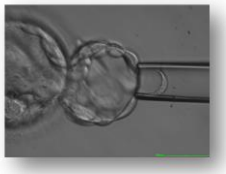


- Importanza della tecnica di biopsia

Swain et al. (2016)

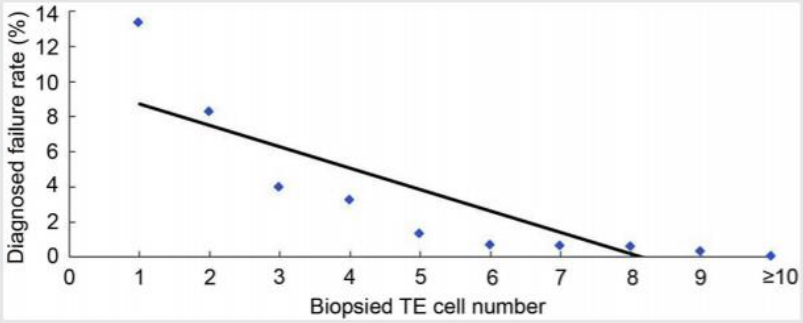
- ✓ No utilizzo di terreni Ca/Mg-free per la biopsia del TE: meno stress
- ✓ ZP breaching in day 3 o 5 per facilitare l'hatching
- ✓ No un eccessivo prelievo di cellule
- ✓ Biopsia di blastocisti espansa
- ✓ Ottima competenza dell'operatore

BIOPSIA DEL TROFECTODERMA



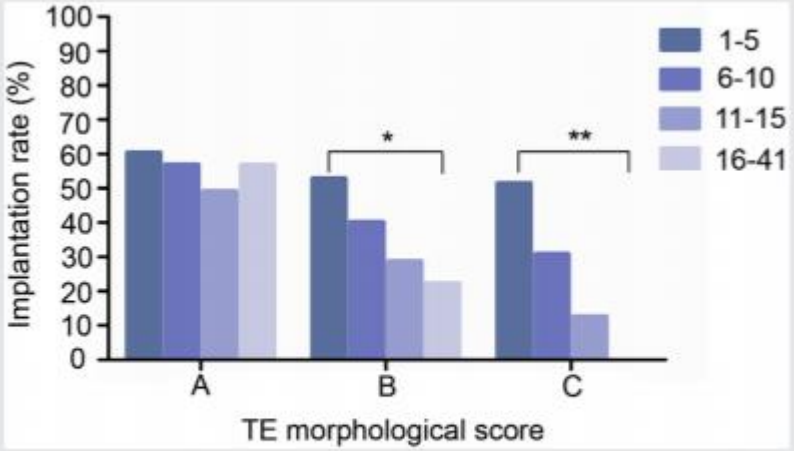
- Impatta il numero di cellule di TE sul potenziale di sviluppo della blastocisti?

Zhang et al. (2016)



Diagnosed failure rates decline along with the increasing biopsied TE cell mumber. Continuous line shows trend line. *P* trend < .001.

Zhang. Blastocyst biopsy and implantation. Fertil Steril 2016.

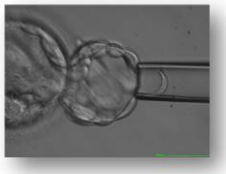


Implantation rate according to the different biopsied TE cell number stratified by TE morphological score. **P* trend < .05; ***P* trend < .01.

Zhang. Blastocyst biopsy and implantation. Fertil Steril 2016.

Il potenziale di impianto è negativamente influenzato dal numero di cellule del TE bioptizzate in blastocisti con un TE di scarsa qualità

BIOPSIA DEL TROFECTODERMA



- Impatta il numero di cellule di TE sul potenziale di sviluppo della blastocisti?

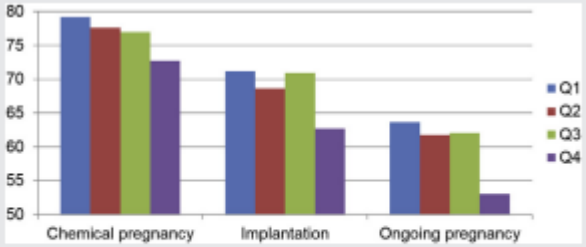
Neal et al., (2017)

Demographic information, cycle characteristics, pregnancy outcomes, and neonatal outcomes by quartile (Q).

	DNA content			
Variable	**Q1 (n = 264)**	**Q2 (n = 290)**	**Q3 (n = 282)**	**Q4 (n = 311)**
Oocyte age (y), mean (range)	35.9 (23.2–44.2)	35.7 (25.6–45.8)	36.2 (22.6–44.9)	36.4 (22.0–44.5)
Body mass index (kg/m^2), mean (range)	25.5 (17.5–46.2)	24.5 (16.7–46.5)	24.9 (16.8–50.4)	24.7 (16.1–61.4)
Endometrial thickness (mm), mean (range)	9.5 (6–16)	9.7 (6–17)	9.8 (6–18)	9.6 (6–18)
Frozen embryo transfers, n (%)	173 (65.5)	192 (66.2)	180 (63.8)	232 (74.6)
Embryo quality, n (%)				
Good	49 (18.6)	61 (21.0)	85 (30.1)	125 (40.2)
Fair	195 (73.9)	217 (74.8)	189 (67.0)	171 (55.0)
Poor	20 (7.6)	12 (4.1)	7 (2.5)	13 (4.2)
Mean initial hCG (mIU/mL)	158.3	160.0	153.9	158.3
Relative rise hCG	2.7	2.6	2.6	2.9
Chemical pregnancy, n (%)	209 (79.2)[a]	225 (77.6)[b]	217 (77.0)[b]	226 (72.7)[c]
Implantation, n (%)	188 (71.2)[d]	199 (68.6)[e]	201 (70.9)[d]	195 (62.7)[f]
Ongoing pregnancy, n (%)	168 (63.6)[g]	179 (61.7)[g]	175 (62.1)[g]	165 (53.1)[h]
Live birth, n (%)	163 (61.7)[i]	171 (59.0)[j]	172 (61.0)[i]	159 (51.1)[k]
Mean gestation age at delivery (wk)	37.7	38.0	38.0	38.1
Mean birth weight (g)	3,335.6	3,341.7	3,397.7	3,437.5
Fresh transfer (n = 226)	3,295.6	3,244.1	3,361.2	3,294.5
Frozen transfer (n = 433)	3,356.6	3,402.2	3,421.8	3,483.2
Female (n = 310)	3,348.5	3,294.0	3,170.8	3,325.3
Male (n = 349)	3,326.2	3,387.3	3,601.7	3,548.4

Note: Population numbers (n) for hCG parameters are lower owing to exclusion of subjects who did not have hCG drawn at the specified time points (Q1, n = 142; Q2, n = 143; Q3, n = 153; Q4, n = 134). Population numbers (n) for neonatal outcomes are lower owing to exclusion of subjects who did not have a singleton live birth (Q1, n = 163; Q2, n = 170; Q3, n = 169; Q4, n = 157). There were no differences in age, body mass index, or endometrial thickness across quartiles. Frozen embryo transfer rates were higher in the quartile of highest DNA content (Q4) ($P < .01$). This quartile also had a higher proportion of good-quality embryos. There was no difference in pregnancy outcomes among quartiles 1–3. Pregnancy rates were lower for the biopsy group with highest DNA content (Q4). There were no differences seen in initial hCG, rise of hCG, or neonatal outcomes across quartiles.
Superscript letters a vs. c, d vs. f, g vs. h, i vs. k: $P < .05$.

Neal. Embryo biopsy size and pregnancy outcome. Fertil Steril 2016.

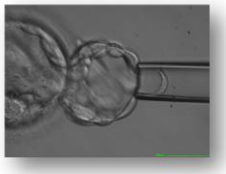


Pregnancy outcomes across quartiles of trophectoderm biopsy DNA content. The biopsy quartile with highest DNA content (Q4) demonstrated poorer outcomes across all measured outcomes.

Neal. Embryo biopsy size and pregnancy outcome. Fertil Steril 2016.

Le biopsie del TE con alta quantità di DNA sono associate con una riduzione del tasso di nati vivi

BIOPSIA DEL TROFECTODERMA



- Impatta il numero di cellule di TE sul potenziale di sviluppo della blastocisti?

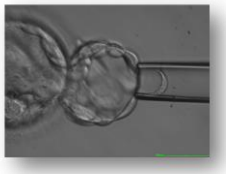
Guzman et al. (2019)

	Overall		*p* value
	Group 1	Group 2	
A			
Biochemical pregnancy rate	68% (182/267)	56% (121/215)	0.005
Good-quality embryo	79% (85/108)	64% (63/99)	0.01
Fair-quality embryo	61% (97/158)	50% (58/116)	0.04
Clinical pregnancy rate	66%(175/267)	53% (115/215)	0.005
Good-quality embryo	75% (81/108)	61% (60/99)	0.02
Fair-quality embryo	59% (94/159)	47% (55/116)	0.04
B			
Biochemical pregnancy rate	68% (182/267)	56% (121/215)	0.005
≤35 years	70% (53/76)	54% (49/91)	0.02
>35 years	68% (129/191)	58% (72/124)	0.02
Clinical pregnancy rate	66%(175/267)	53% (115/215)	0.005
≤35 years	67% (51/76)	54% (49/91)	0.01
>35 years	65% (124/191)	53% (66/124)	0.02

NO differenze nel tasso di aborto

Il tasso di impianto è negativamente influenzato dal numero di cellule del TE bioptizzate

BIOPSIA DEL TROFECTODERMA



- Riproducibilità della biopsia

Human Reproduction, Vol.31, No.1 pp. 199–208, 2015
Advanced Access publication on December 4, 2015 doi:10.1093/humrep/dev294

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

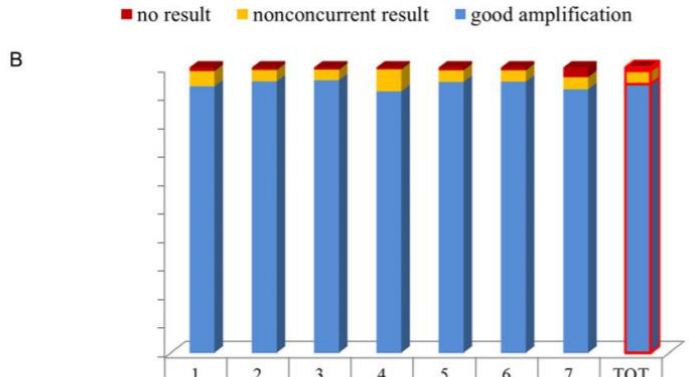
Consistent and reproducible outcomes of blastocyst biopsy and aneuploidy screening across different biopsy practitioners: a multicentre study involving 2586 embryo biopsies

Antonio Capalbo[1,2,*], Filippo Maria Ubaldi[1,3,4], Danilo Cimadomo[1,2], Roberta Maggiulli[1], Cristina Patassini[2], Ludovica Dusi[3], Federica Sanges[4], Laura Buffo[3], Roberta Venturella[4], and Laura Rienzi[1,3,4]

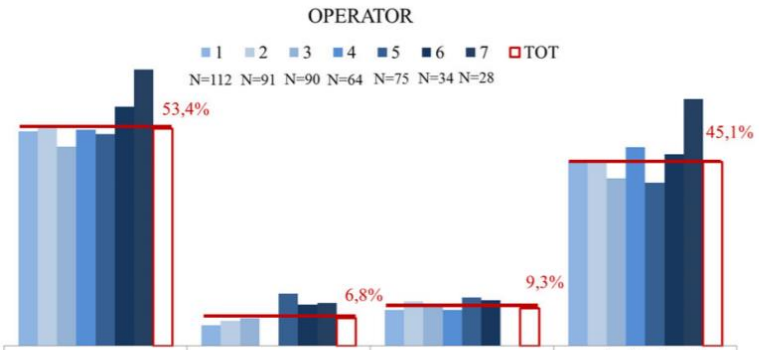
A

Operator	1	2	3	4	5	6	7	TOT
Number of biopsied blastocysts	474	426	591	271	417	200	207	2586
%	(18,3)	(16,4)	(22,8)	(10,5)	(16,1)	(7,7)	(8,0)	
95% CI	432-518	386-468	544-640	239-305	377-459	173-229	179-237	

B

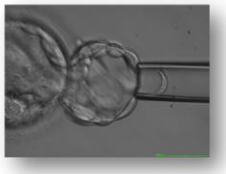


	1	2	3	4	5	6	7	TOT
no result	1,3%	0,9%	0,8%	0,7%	1,0%	1,0%	3,4%	1,2%
nonconcurrent result	5,3%	4,0%	3,7%	7,7%	4,0%	4,0%	4,3%	4,6%
good amplification	93,4%	95,1%	95,5%	91,6%	95,0%	95,0%	92,3%	94,2%



	positive pregnancy test	biochemical pregnancy	miscarriage rate	ongoing implantation rate (>20 weeks)
1	52,7%	5,1%	8,9%	45,5%
2	53,8%	6,1%	10,9%	45,1%
3	48,9%	6,8%	9,8%	41,1%
4	53,1%	0,0%	8,8%	48,8%
5	52,0%	12,8%	11,8%	40,0%
6	58,8%	10,0%	11,1%	47,1%
7	67,9%	10,5%	0,0%	60,7%
TOT	53,4%	6,8%	9,3%	45,1%

BIOPSIA DEL TROFECTODERMA



- Si possono bioptizzare blastocisti già bioptizzate?
- Qual è l'impatto di biopsie multiple sulla gravidanza?
- Indicazione ri-biopsia: risultato di PGT non conclusivo

Fertility and Sterility® Vol. 102, No. 6, December 2014

Blastocysts can be rebiopsied for preimplantation genetic diagnosis and screening

Shuoping Zhang, M.Sc.,[a,b,c] Ke Tan, M.Sc.,[a,d] Fei Gong, M.D., Ph.D.,[a,b,c] Yifan Gu, Ph.D.,[a,b,c] Yueqiu Tan, Ph.D.,[a,b,c] Changfu Lu, Ph.D.,[a,b,c] Keli Luo, M.D., Ph.D.,[a,b,c] Guangxiu Lu, M.D.,[a,b,c,d] and Ge Lin, M.D., Ph.D.[a,b,c,d]

Fertility and Sterility® Vol. 108, No. 6, December 2017

Impact of multiple blastocyst biopsy and vitrification-warming procedures on pregnancy outcomes

Cara K. Bradley, Ph.D., Mark Livingstone, C.R.E.I., Maria V. Traversa, M.Sc.Med., and Steven J. McArthur, B.Sc.

The effect of repeated biopsy on pre-implantation genetic testing for monogenic diseases (PGT-M) treatment outcome

Shira Priner[1,2] · Gheona Altarescu[2,3] · Oshrat Schonberger[1] · Hananel Holzer[1] · Esther Rubinstein[1] · Nava Dekel[1] · Aharon Peretz[1] · Talia Eldar-Geva[1,2]

Inconclusive results in preimplantation genetic testing: go for a second biopsy?

Monica Parriego, Lluc Coll, Francesca Vidal, Montserrat Boada, Marta Devesa, Buenaventura Coroleu & Anna Veiga

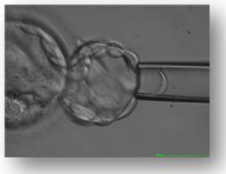
Human Reproduction, Vol.33, No.10 pp. 1839–1846, 2018
Advanced Access publication on September 18, 2018 doi:10.1093/humrep/dey282

human reproduction ORIGINAL ARTICLE *Embryology*

Inconclusive chromosomal assessment after blastocyst biopsy: prevalence, causative factors and outcomes after re-biopsy and re-vitrification. A multicenter experience

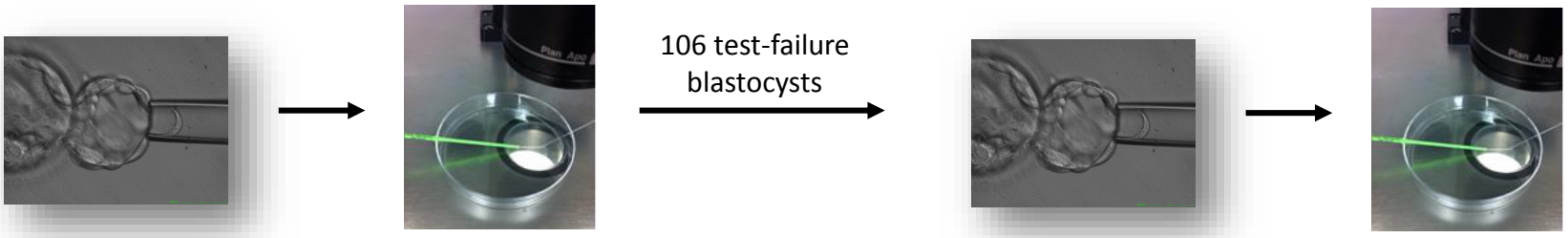
Danilo Cimadomo[1,2,3,4], Laura Rienzi[1,2,3,4], Valeria Romanelli[5], Erminia Alviggi[2], Paolo Emanuele Levi-Setti[6], Elena Albani[6], Ludovica Dusi[3], Letizia Papini[4], Claudia Livi[7], Francesca Benini[7], Antonella Smeraldi[6], Cristina Patassini[5], Filippo Maria Ubaldi[1,2,3,4], and Antonio Capalbo[5,8,*]

BIOPSIA DEL TROFECTODERMA



- Studio retrospettivo

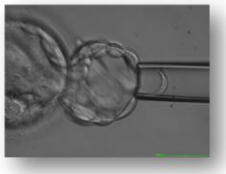
Zhang et al. (2014)



- ✓ 70/106 blastocisti pronte per la seconda biopsia
- ✓ 44.3% blastocisti euploidi
- ✓ 19 blastocisti scongelate, 18 sopravvissute/ET, 9 impiantate (50%)

Per le blastocisti con risultati non conclusivi, vale la pena effettuare un'ulteriore biopsia

BIOPSIA DEL TROFECTODERMA

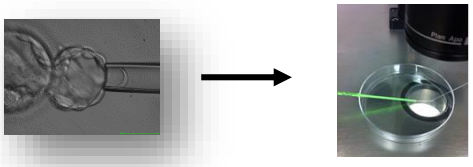


- Studio retrospettivo

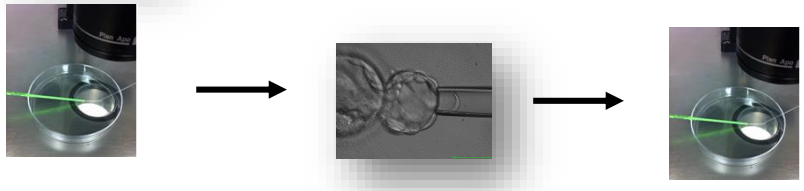
Bradley et al. (2017)

✓ 3 condizioni:

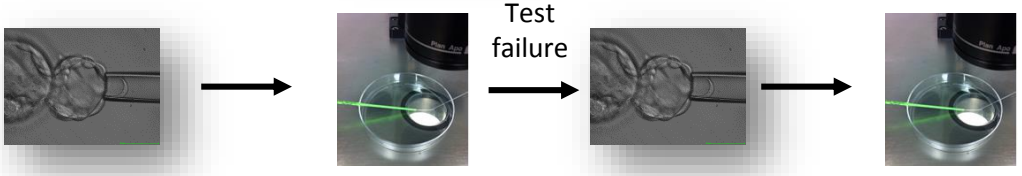
Controllo (n=2130)



Gruppo 2 (n=34)



Gruppo 3 (n=29)

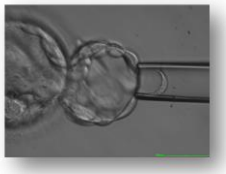


✓ Tasso di gravidanza dopo ET di blastocisti euploide

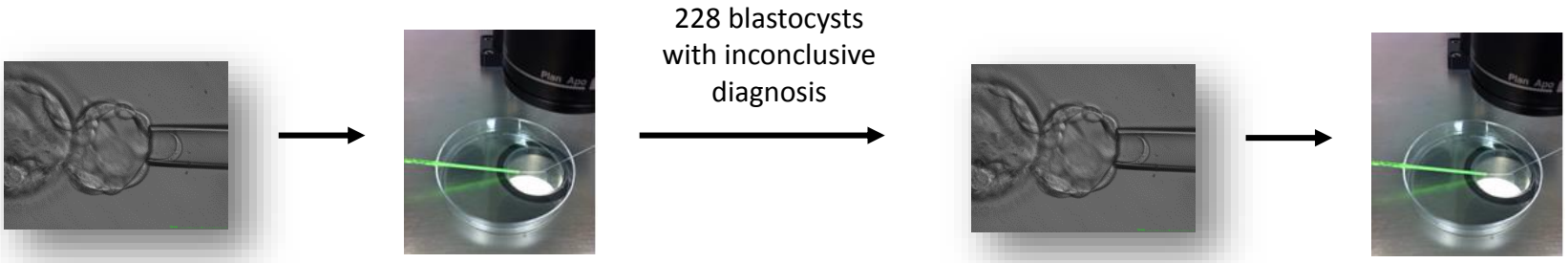
Controllo	54.3%	
Gruppo 2	47.1%	P=0.013
Gruppo 3	31.0%	

Ri-biopsia può essere considerata per la PGT-M, ma dovrebbe essere considerata con cautela per la PGT-A

BIOPSIA DEL TROFECTODERMA



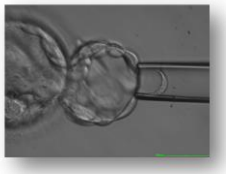
Cimadomo et al. (2018)



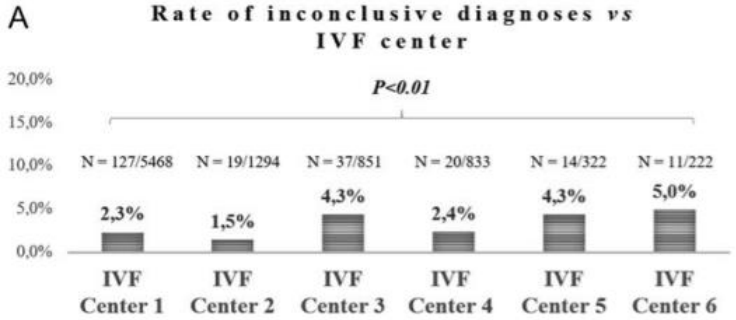
- ✓ 213 blastocisti scongelate e 206 sottoposte a seconda biopsia (96.7% survival rate)
- ✓ 51.9% blastocisti euploidi
- ✓ 49 blastocisti scongelate, 49 sopravvissute/ET, 19 nati (38.8%)

Per le blastocisti con risultati non conclusivi, vale la pena effettuare un'ulteriore biopsia

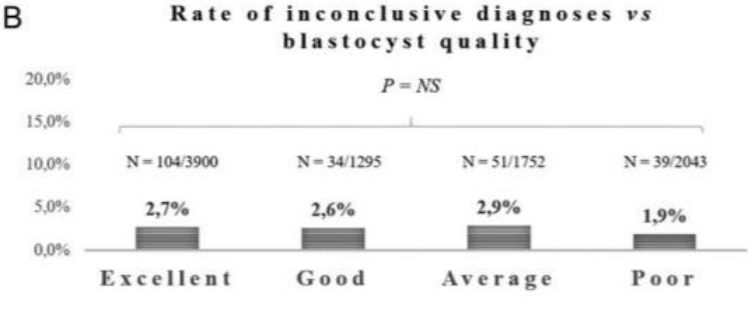
BIOPSIA DEL TROFECTODERMA



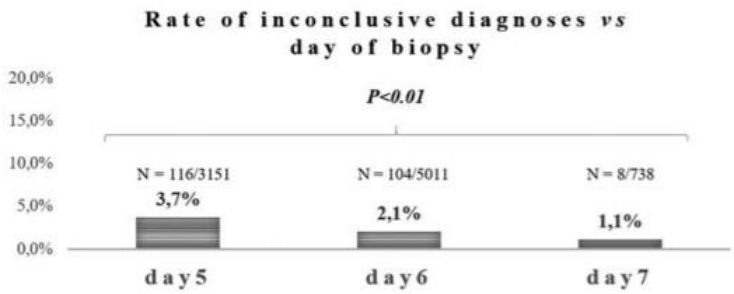
A



B



C

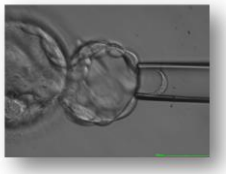


D

Outcome: inconclusive diagnosis	Multivariate OR, 95%CI	Adjusted p-value
IVF center: 1		<0.01
2	0.58, 0.36–0.94	
3	1.76, 1.2–2.6	
4	1.25, 0.77–2.0	
5	2.0, 1.15–3.6	
6	2.5, 1.3–4.8	
Day of biopsy: day5		<0.01
day6	0.52, 0.39–0.68	
day7	0.29, 0.14–0.60	

- ✓ 8 è l'ideale numero di cellule per limitare il rischio di ottenere diagnosi non conclusive
- ✓ Il timing ideale per la biopsia del TE è la sesta giornata
- ✓ TE di scarsa qualità può essere caratterizzato da DNA genomico di buona qualità

BIOPSIA DEL TROFECTODERMA



E' una manipolazione sicura per il nascituro???

Fertility and Sterility® Vol. 106, No. 1, July 2016

Obstetric and neonatal outcomes in blastocyst-stage biopsy with frozen embryo transfer and cleavage-stage biopsy with fresh embryo transfer after preimplantation genetic diagnosis/screening

Shuang Jing, M.Sc.,[a,b,c] Keli Luo, M.D., Ph.D.,[a,b,c] Hui He, M.Sc.,[a,b,c] Changfu Lu, Ph.D.,[a,b,c] Shuoping Zhang, M.D.,[a,b,c] Yueqiu Tan, Ph.D.,[a,b,c] Fei Gong, M.D., Ph.D.,[a,b,c] Guangxiu Lu, M.D.,[a,b,c] and Ge Lin, M.D., Ph.D.[a,b,c]

Più alta incidenza di ipertensione gestazionale nelle gravidanze in seguito a biopsia da blastocisti

controllo??

Am J Obstet Gynecol 2014;210:157.e1-6.

RESEARCH www.AJOG.org

REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY

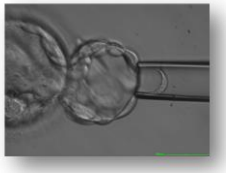
Obstetrical and neonatal outcomes from the BEST Trial: single embryo transfer with aneuploidy screening improves outcomes after in vitro fertilization without compromising delivery rates

Eric J. Forman, MD; Kathleen H. Hong, MD; Jason M. Franasiak, MD; Richard T. Scott Jr, MD

SET blastocisti euploide:
migliori outcome ostetrici rispetto al DET

controllo??

BIOPSIA DEL TROFECTODERMA



E’ una manipolazione sicura per il nascituro???

NO studi di follow-up a lungo termine di bambini nati dopo biopsie allo stadio di blastocisti

CONCLUSIONI

La manipolazione delle blastocisti migliora i risultati dell' ART

- ✓ **Artificial shrinkage**
  - Solo 1 RCT
  - Benefici in termini di tassi di sopravvivenza
  - No dati di follow-up

- ✓ **Assisted hatching**
  - Basso livello di evidenza
  - No popolazione di pazienti definita
  - Non dannosa per la blastocisti ne' per il bambino

- ✓ **Biopsia del trofectoderma**
  - ''self evident' per PGT-M e PGT-SR
  - Basso livello di evidenze per PGT-A
  - Tecnica robusta che non influisce sui risultati
  - No dati di follow-up

WORK IN PROGRESS…..

…a multicentric RCT!!!

pArtiaL zonA pelluciDa removal by assisteD hatchINg of blastocysts the ALADDIN study

ClinicalTrials.gov Identifier: NCT03623659

Study Design

Study Type :	Interventional (Clinical Trial)
Estimated Enrollment :	700 participants
Allocation:	Randomized
Intervention Model:	Parallel Assignment
Masking:	Triple (Participant, Care Provider, Outcomes Assessor)
Primary Purpose:	Treatment
Official Title:	Does Partial Zona Pellucida Removal From Vitrified-warmed Human Blastocysts Improve Delivery Rate in IVF? A Multicentric RCT on Laser **Assisted Hatching**
Actual Study Start Date :	September 5, 2018
Estimated Primary Completion Date :	April 1, 2020
Estimated Study Completion Date :	April 1, 2020

Outcome Measures

Primary Outcome Measures :

1. Delivery rate [Time Frame: 38 weeks after embryo transfer]

   Number of deliveries, that result in a live birth, per transferred blastocyst

Locations

Italy

IRCCS San Raffaele	**Recruiting**
Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico	**Recruiting**

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