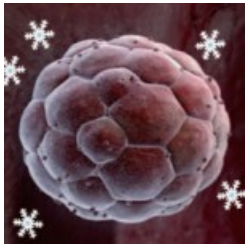
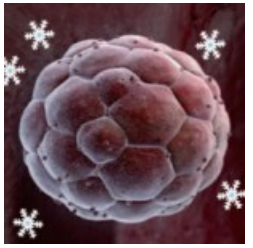
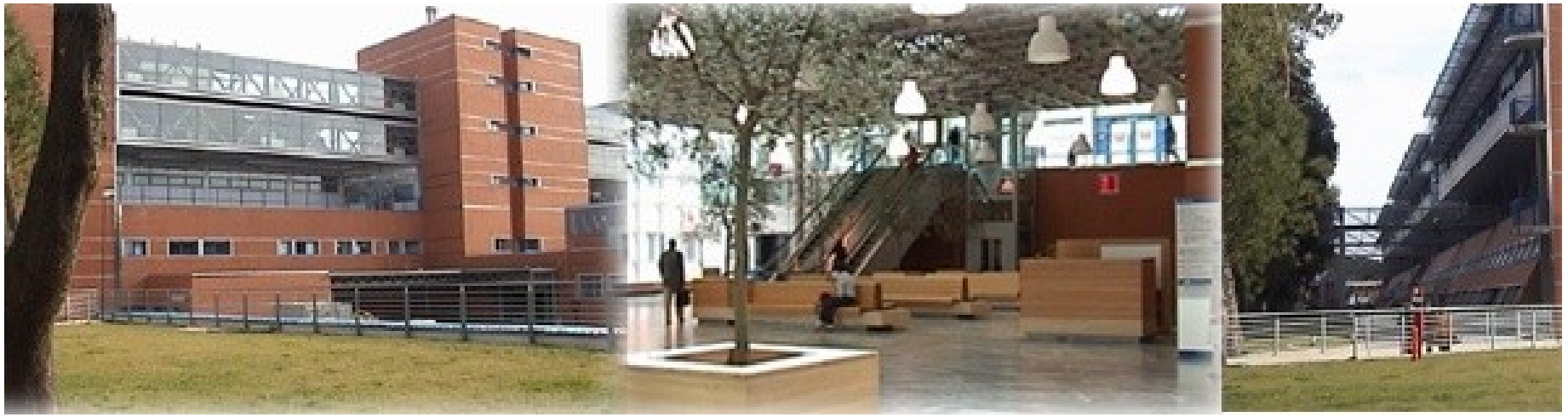


Ospedale Evangelico Internazionale - Genova

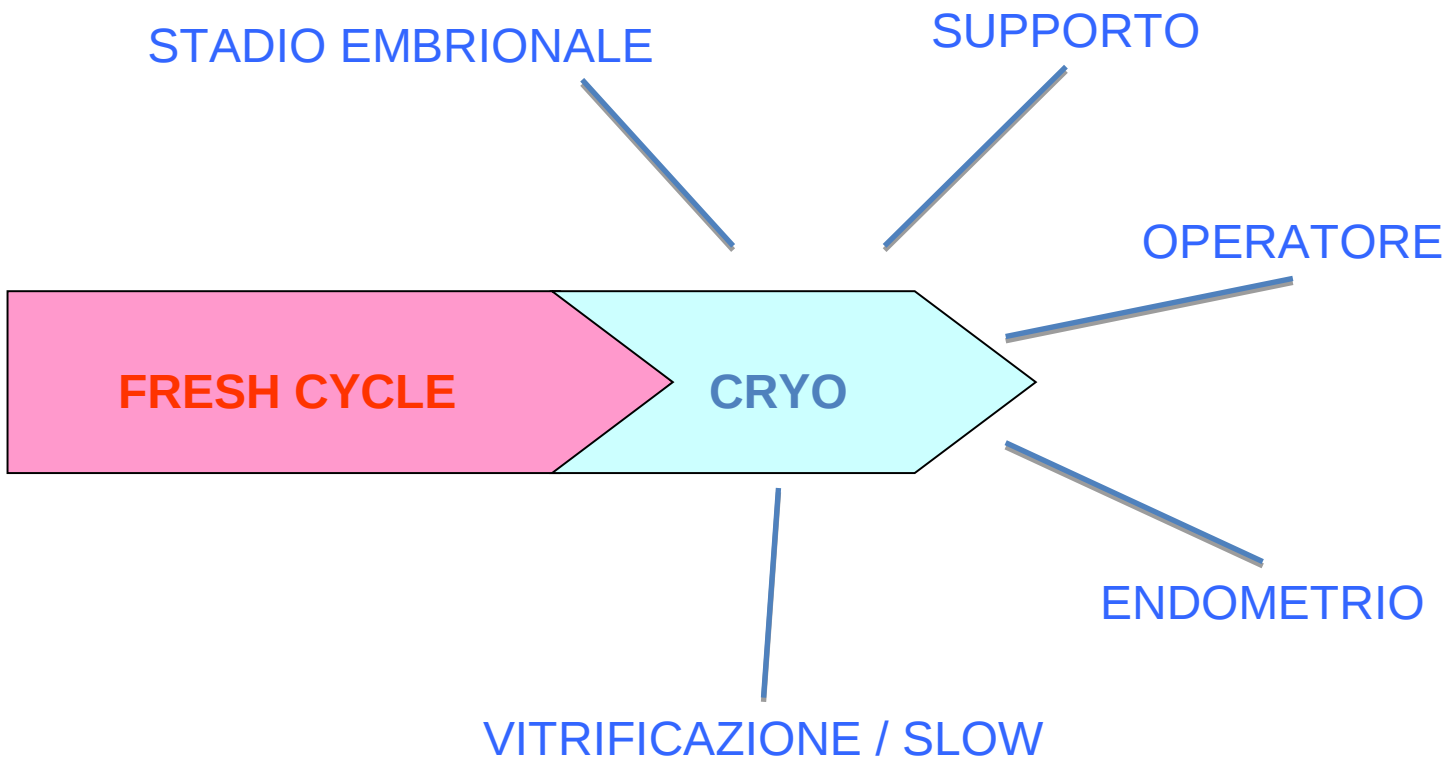


Crioconservazione Embrionaria

D1 – D5



Crioconservazione



STADIO EMBRIONALE

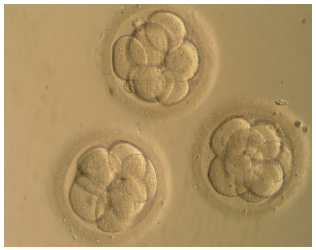
Ai diversi stadi embrionali corrispondono diverse percentuali di successo della tecnica….

… relazione inversamente proporzionale tra volume cellulare e sopravvivenza

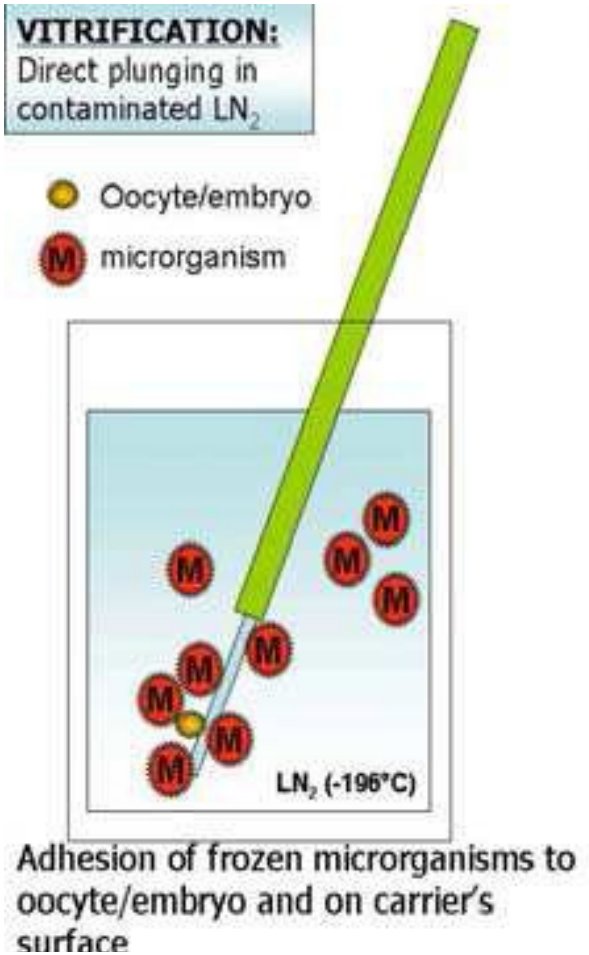
- Maggiore indiziata di essere il miglior stadio per crioconservare
- Per politica del laboratorio il 90 % degli embrioni crioconservati sono blastocisti
- Se sopravvissuta al thawing maggiore tasso di impianto

SUPPORTO

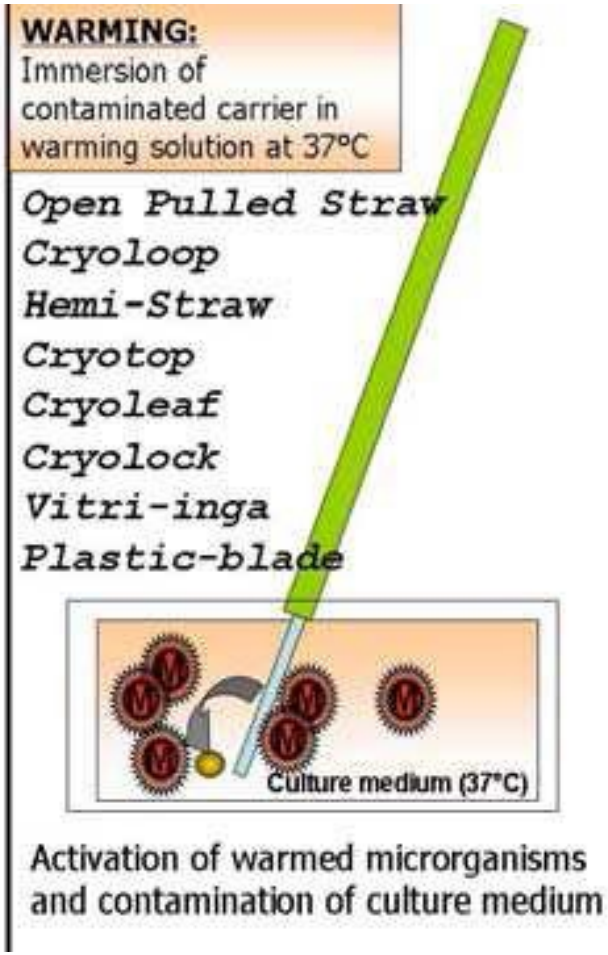
Scelta fondamentale, ogni laboratorio deve trovare il protocollo ed il supporto che meglio si adatta alla propria organizzazione e capacità, provvedendo altresì alla sicurezza dei campioni crioconservati.



Supporto



Adhesion of frozen microrganisms to oocyte/embryo and on carrier's surface

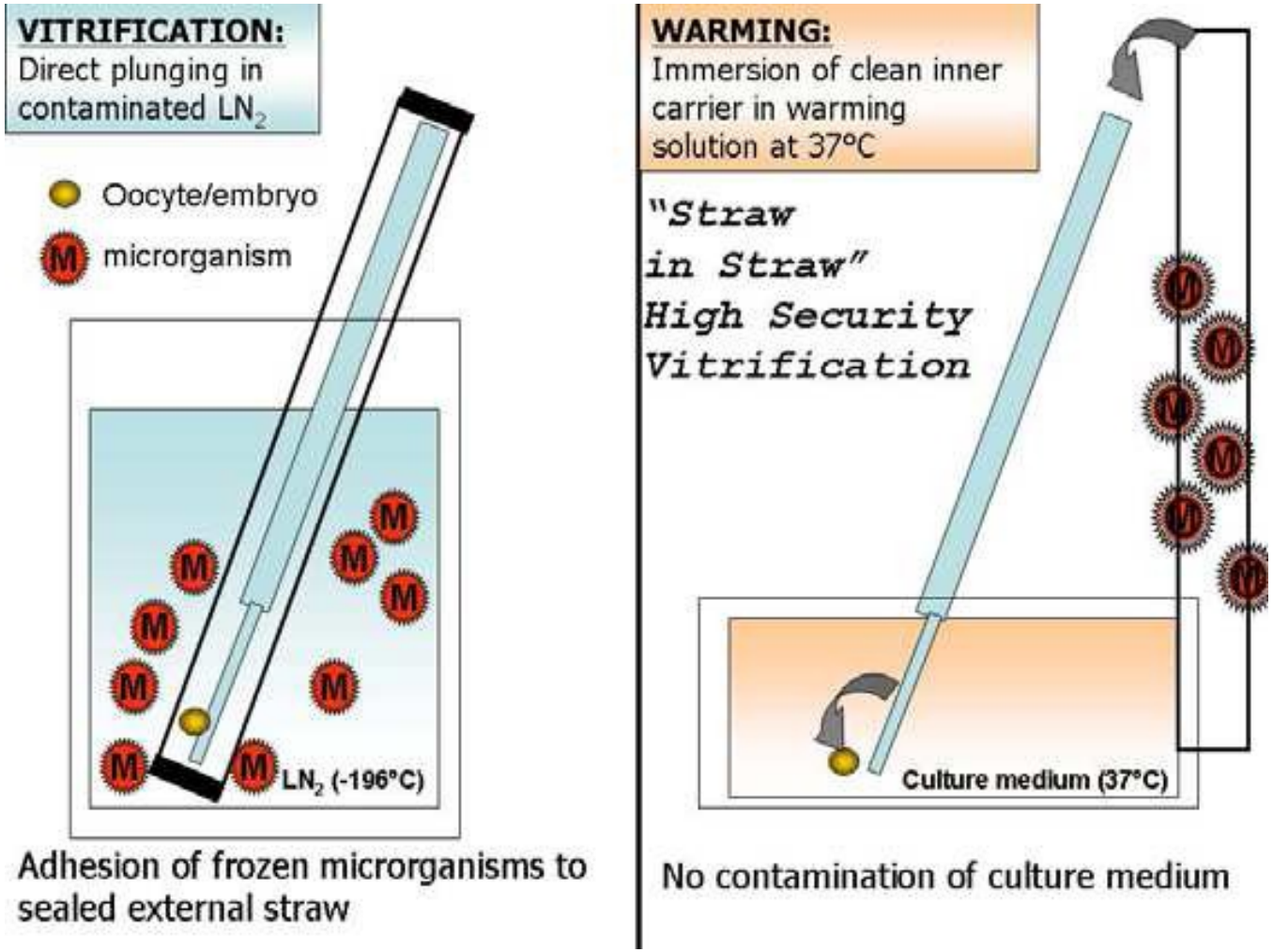


Activation of warmed microrganisms and contamination of culture medium

Open Systems

Parmegiani RBM Online 2011

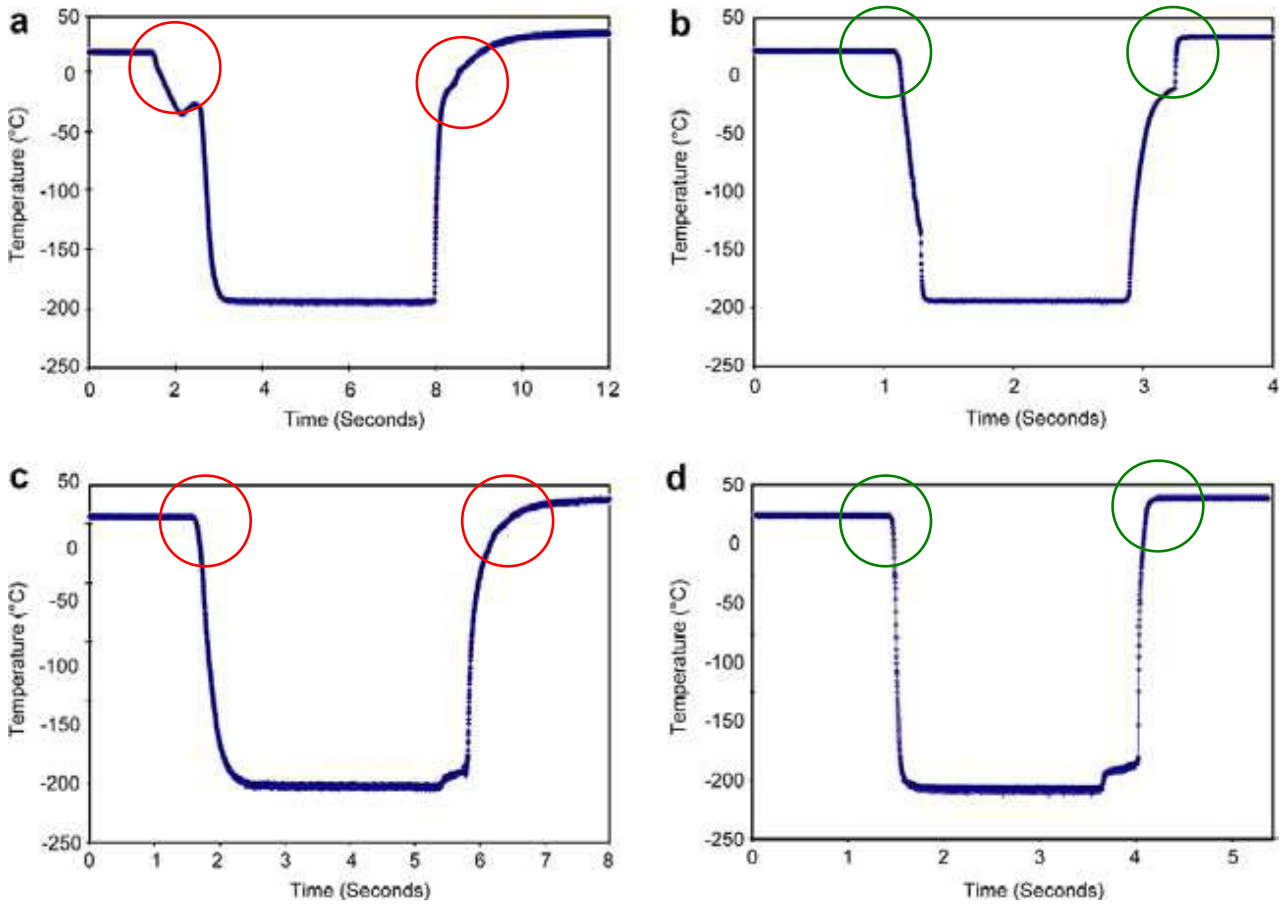
Supporto



Closed Systems

Supporto

DEVICE	VOLUME	COOLING RATE
CRYOLOOP	>1 µl	20.000 ºC/min
HEMI-STRAW	>1 µl	>20.000 ºC/min
CRYOLEAF	>1 µl	23.000 ºC/min
VITRI-INGA	1 µl	20.000 ºC/min
CVM-RING	>1 µl	10.000 ºC/min
VITRISAFE	>1 µl	1.300 ºC/min
HSS	0.5 µl	2.000 ºC/min
0.25 ML STRAW	25 µl	2.500 ºC/min
OPS	1 µl	16.700 ºC/min
CRYOTOP	0.1 µl	23.000 ºC/min
CRYOTIP	1 µl	12.000 ºC/min
RAPID-I	0.5 µl	1.200 ºC/min
CRYOPETTE	1.2µl	23.700 ºC/min
ULTRAVIT	0.2 µl	250.000 ºC/min



PVC Vs QUARZO

Equazione di Yavin & Arav

$$\text{Probabilità di Vitrificazione} = \frac{\text{Cooling and warming rates} \times \text{Viscosity (CPA concentration)}}{\text{Volume}}$$

Ultravitrificazione

Slush Nitrogen - 210 °C

Semi Closed Systems

Lee H, Elmoazzen H, Wright D, Biggers J, Rueda BR, Heo YS, et al. Ultra-rapid vitrification of mouse oocytes in low cryoprotectant concentrations. Reprod Biomed Online 2010;20:201–8.

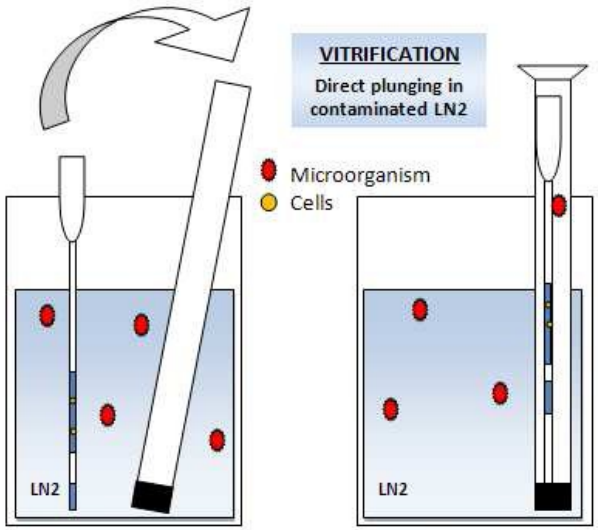
Concentrazione dei crioprotettori equiparabile a quella dello slow freezing

SLUSH NITROGEN

Model	Survival slush (%)	Survival LN (%)	Sig.	Publication
Bovine MII	48	28	$P<0.05$	Arav & Zeron (1997)
Ovine GV	25	5	$P<0.05$	Isachenko *et al.* (2001)
Porcine blastocysts	83	62	$P<0.05$	Beebe *et al.* (2005)
Bovine MII	48	39	$P<0.05$	Santos *et al.* (2006)
Mouse four-cell embryos with biopsied blastomere	87	50	$P<0.05$	Lee *et al.* (2007)
Rabbit embryos	92	83	NS	Papis *et al.* (2009)
Porcine blastocysts	89	93	NS	Cuello *et al.* (2004)
Mouse MII	>80	>80	NS	Seki & Mazur (2009)
Rabbit oocytes	82	83	NS	Cai *et al.* (2005)

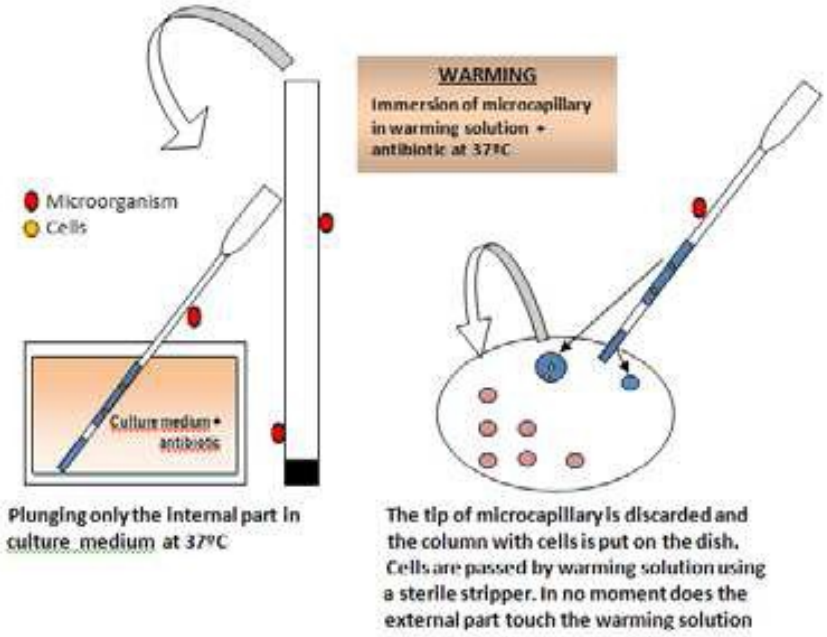
LN, liquid nitrogen; GV, germinal vesicle; Sig., statistical significance; NS, not significant.

ULTRAVIT



Adhesion of frozen microorganims to microcapillary surface

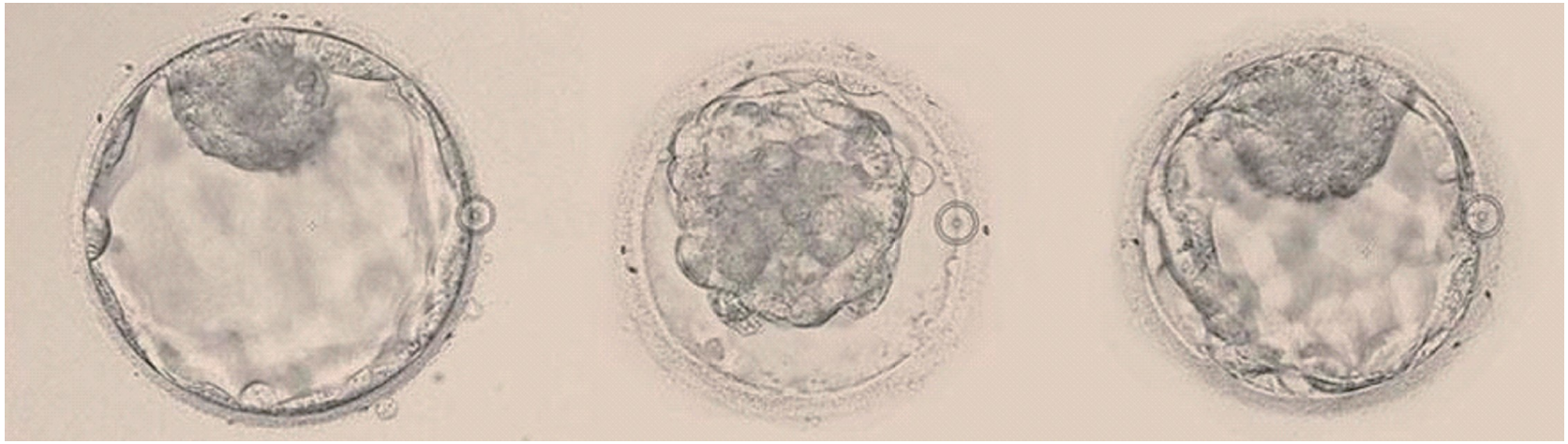
Microcapillary into the seal protective sheet avoiding cross-contamination



Plunging only the internal part in culture medium at 37ºC

The tip of microcapillary is discarded and the column with cells is put on the dish. Cells are passed by warming solution using a sterile stripper. In no moment does the external part touch the warming solution

BLASTO Shrinkage



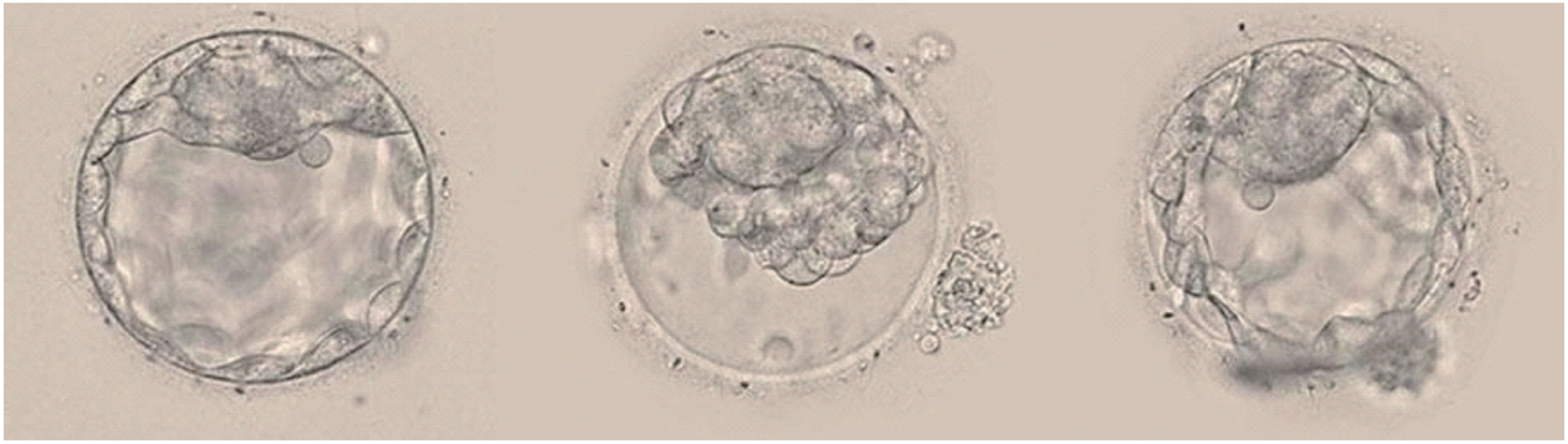
- Micro Needle
- 29 gauge needles – (insulina)
- Micropipetting (175 µm)
- Saccarosio (0.125 – 0.25 M)
- Laser Pulse

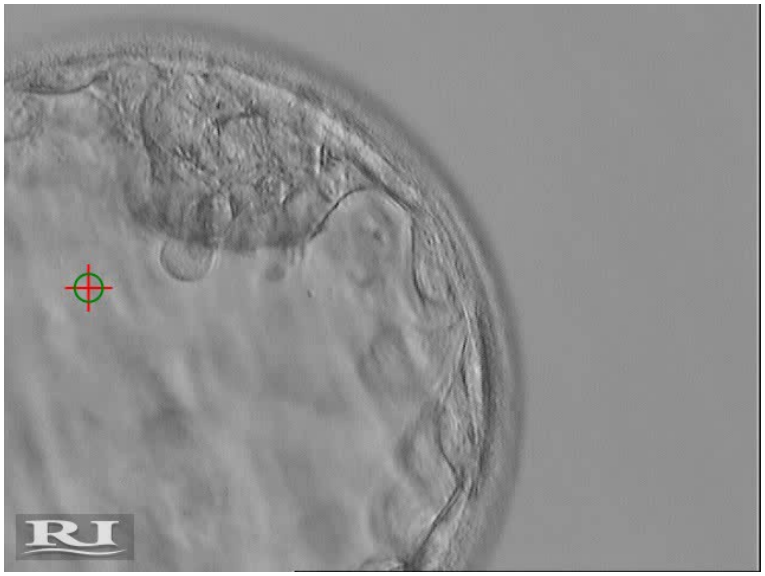
BLASTO Shrinkage

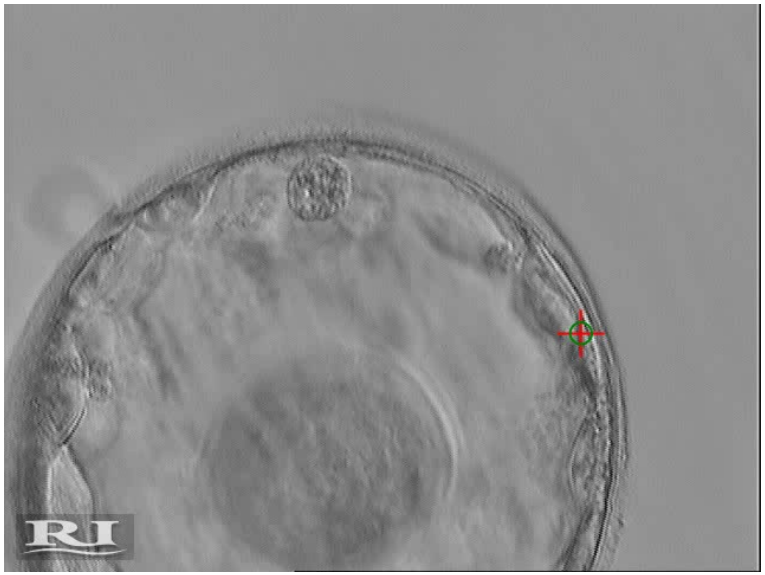
J Assist Reprod Genet. 2014 May;31(5):577-81.

Retrospective clinical analysis of two artificial shrinkage methods applied prior to blastocyst vitrification on the outcome of frozen embryo transfer.

Cao S, Zhao C, Zhang J, Wu X, Guo X, Ling X







BLASTO Shrinkage

Laser Pulse 200 mS

Giunzione tra due cellule del trofoectoderma

Zona pellucida

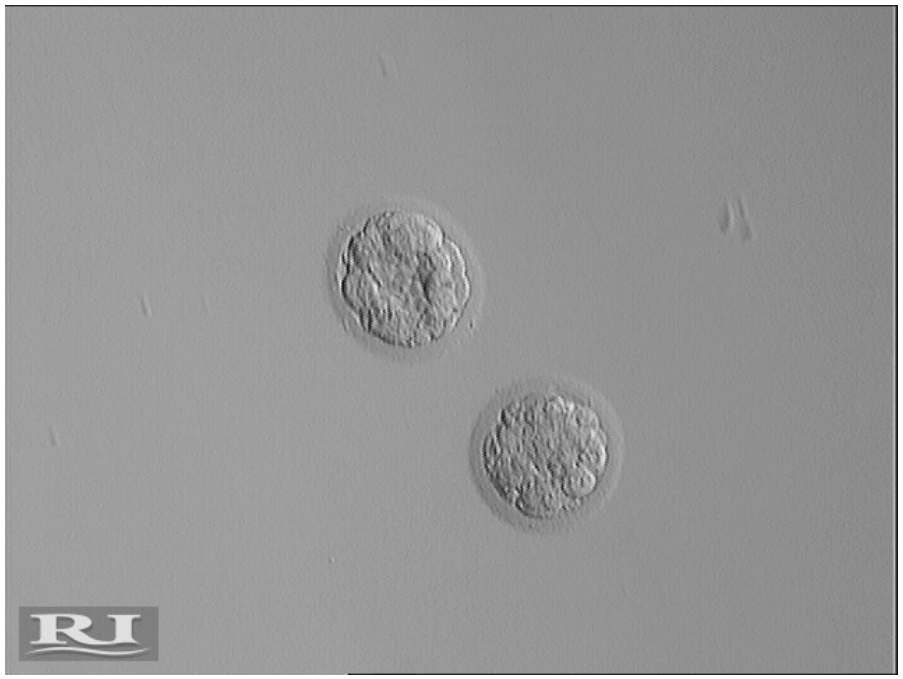
BLASTO

Journal of Assisted Reproduction and Genetics May 2014

Possible selection of viable human blastocysts after vitrification by monitoring morphological changes

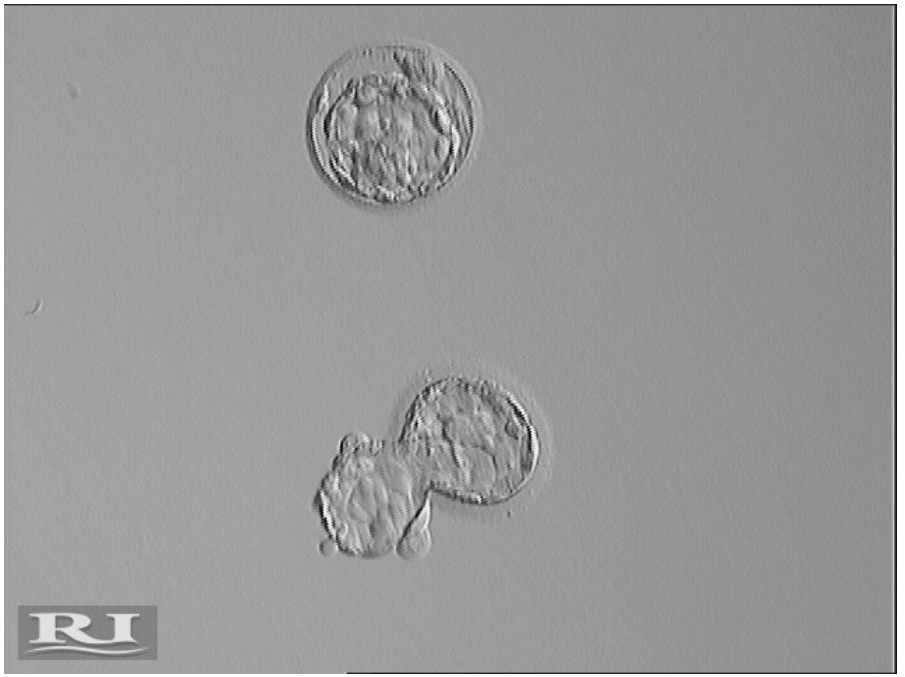
T. Maezawa, M. Yamanaka, S. Hashimoto, A. Amo, A. Ohgaki,
Y. Nakaoka, A. Fukuda, T. Ikeda, M. Inoue, Y. Morimoto

Quando Devitrificare?



16 ORE POST THAWING

2 ORE POST THAWING



Fertil Steril. 2014 May;101(5):1294-1301.e2.

Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers.

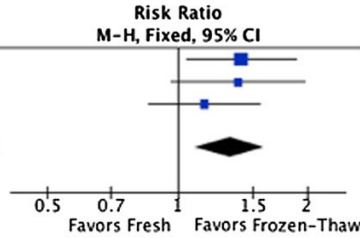
Roy TK, Bradley CK, Bowman MC, McArthur SJ.

Endometrio

A

Study or Subgroup	Frozen-Thawed Events	Total	Fresh Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Aflatoonian 2010	73	187	52	187	46.0%	1.40 [1.05, 1.88]
Shapiro 2011 - Normal	39	70	27	67	24.4%	1.38 [0.97, 1.98]
Shapiro 2011 -High	38	60	34	62	29.6%	1.15 [0.86, 1.55]
Total (95% CI)		**317**		**316**	**100.0%**	**1.32 [1.10, 1.59]**
Total events	150		113			

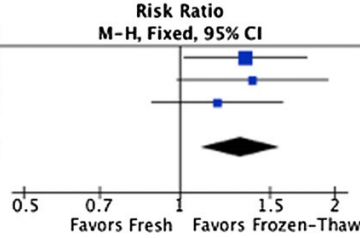
Heterogeneity: $Chi^2 = 1.03$, $df = 2$ ($P = 0.60$); $I^2 = 0\%$
Test for overall effect: $Z = 3.00$ ($P = 0.003$)



B

Study or Subgroup	Frozen-Thawed Events	Total	Fresh Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Aflatoonian 2010	78	187	58	187	47.9%	1.34 [1.02, 1.77]
Shapiro 2011 - Normal	42	70	29	67	24.5%	1.39 [0.99, 1.94]
Shapiro 2011 -High	39	60	34	62	27.6%	1.19 [0.88, 1.59]
Total (95% CI)		**317**		**316**	**100.0%**	**1.31 [1.10, 1.56]**
Total events	159		121			

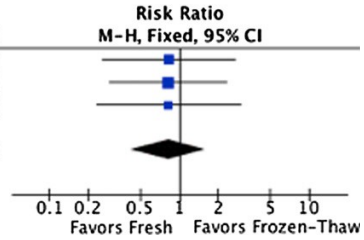
Heterogeneity: $Chi^2 = 0.60$, $df = 2$ ($P = 0.74$); $I^2 = 0\%$
Test for overall effect: $Z = 3.04$ ($P = 0.002$)



C

Study or Subgroup	Frozen-Thawed Events	Total	Fresh Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Aflatoonian 2010	5	187	6	187	33.2%	0.83 [0.26, 2.68]
Shapiro 2011 - Normal	6	70	7	67	39.6%	0.82 [0.29, 2.32]
Shapiro 2011 -High	4	60	5	62	27.2%	0.83 [0.23, 2.93]
Total (95% CI)		**317**		**316**	**100.0%**	**0.83 [0.43, 1.60]**
Total events	15		18			

Heterogeneity: $Chi^2 = 0.00$, $df = 2$ ($P = 1.00$); $I^2 = 0\%$
Test for overall effect: $Z = 0.56$ ($P = 0.57$)



Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis

Matheus Roque, M.D.,Et. Al – Fert & Steril 99, 1 - 2013

CONCLUSIONI

- Ottenere: elevate cooling/warming rates – elevate survival rates – sicurezza per il paziente
- Non accontentarsi di una “sopravvivenza” morfologica.
- Ridurre il volume del blastocele migliora la sopravvivenza alla tecnica

Grazie per l’attenzione

FREEZE ALL

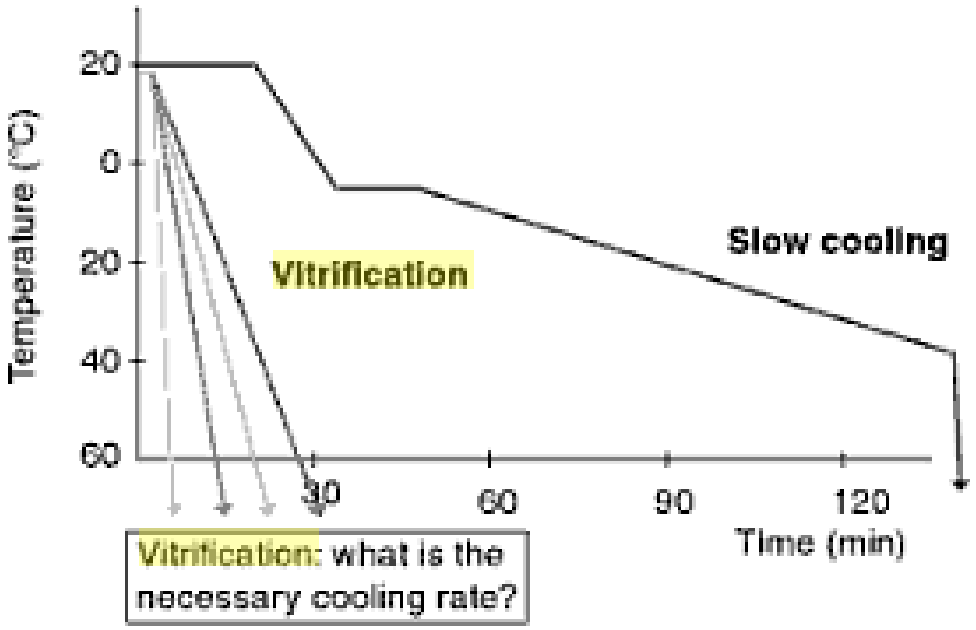
Again, “good” blastocysts show better cryosurvival and subsequent developmental competence.

•Always Day 5 blastocysts? — or are Day 6 blastocysts OK to freeze?

•What are the optimum criteria for selecting blastocysts for freezing?
•Early cavitating stage?
•Expanding blastocysts?
•Expanded blastocysts?

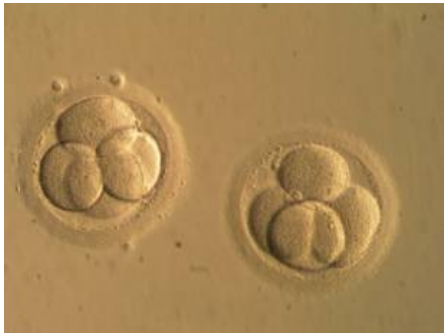
•Many labs freeze “less than ideal” blastocysts, often because that’s all we / the patient have

Sistema


Temperature (°C)
20
0
20
40
60
Vitrification
Slow cooling
30
60
90
120
Time (min)
Vitrification: what is the necessary cooling rate?

ZIGOTE

La crioconservazione degli zigoti rappresenta una alternativa in quelle nazioni dove crioconservare embrioni ad uno stadio più avanzato è proibito dalla legge o da motivi religiosi.



EMBRIONI D2-D3

In alcune nazioni la crioconsevazione al G+3 sta avendo un ritorno di fiamma, con lo slow-freezing