CULTURA E VITA
Via Buon Pastore 126 - 41100 Modena

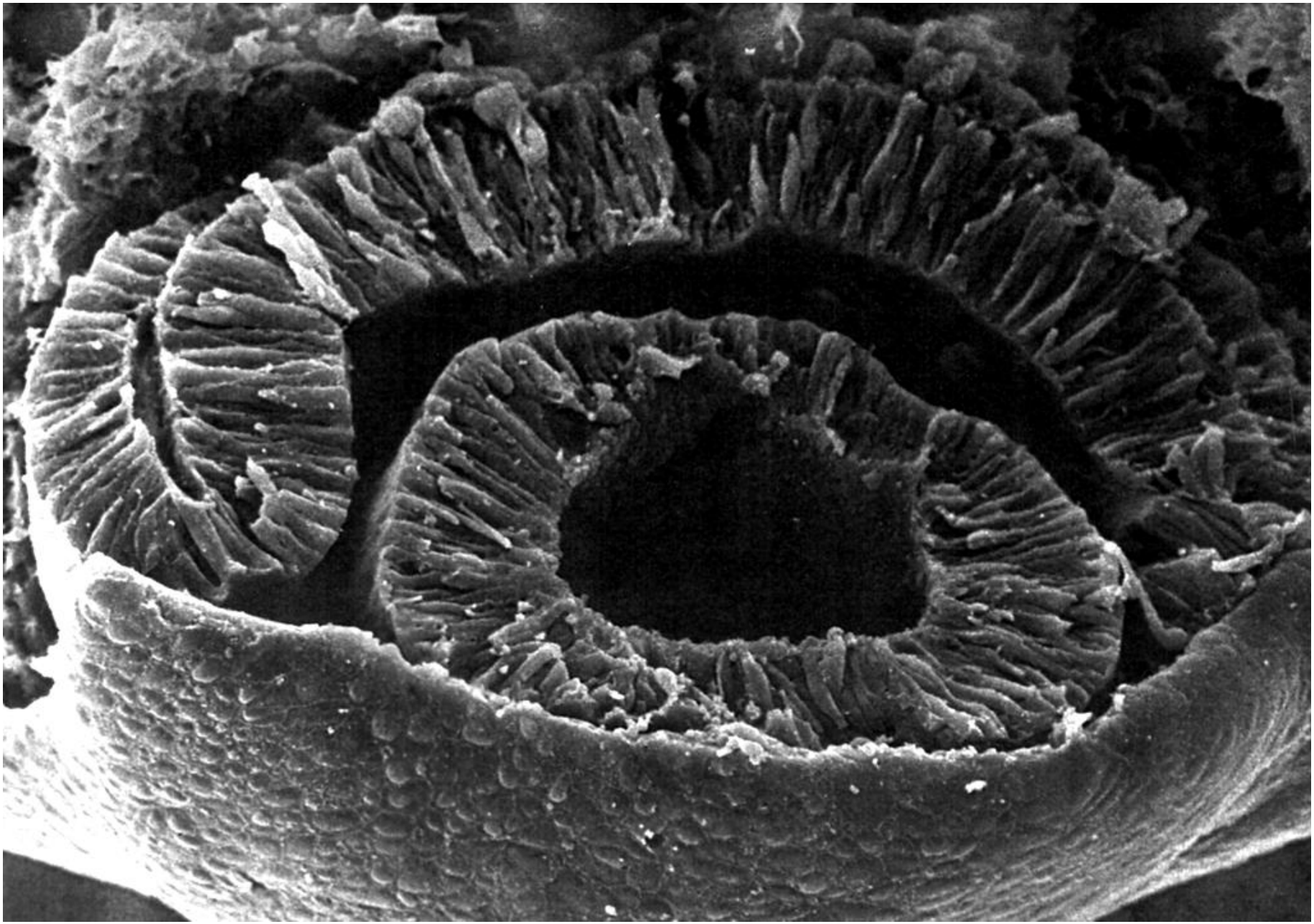
UNIVERSITÀ DEGLI STUDI
DI MODENA E REGGIO EMILIA

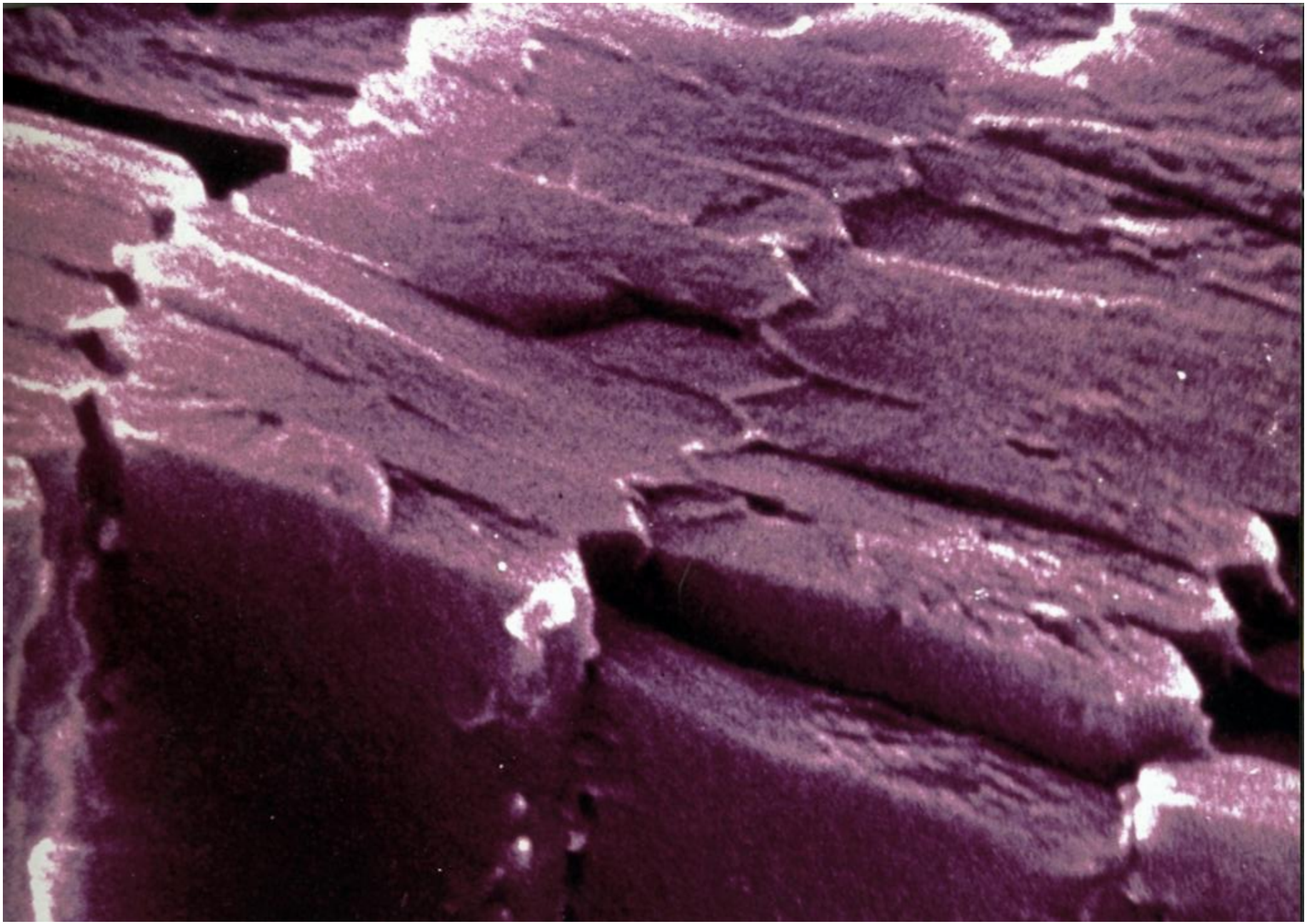
LA VITA NEL GHIACCIO

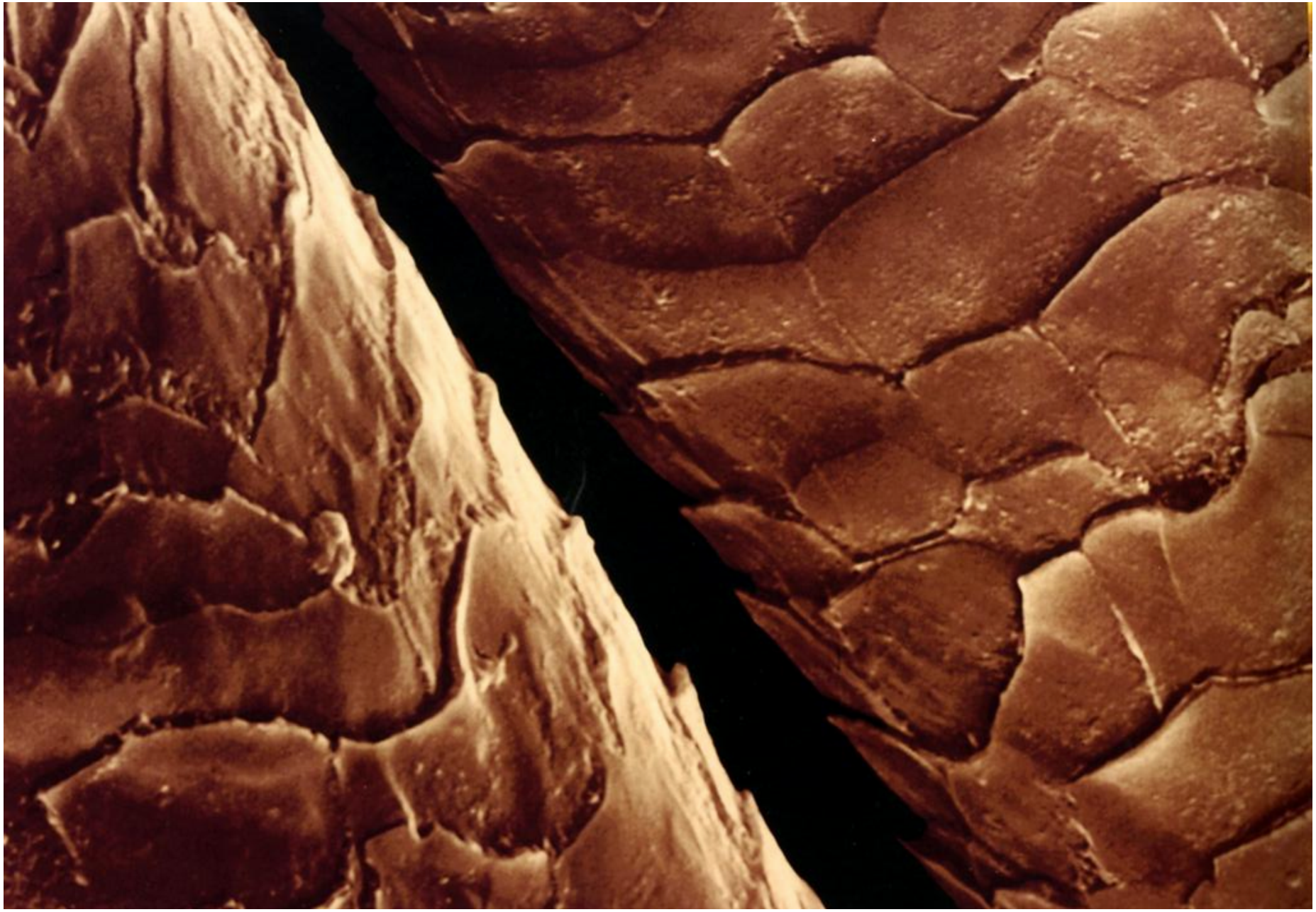
CARLO CIROTTO

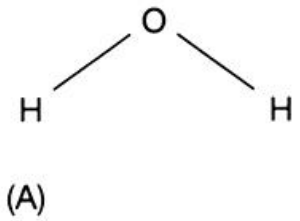
UNIVERSITA' DI PERUGIA

MODENA 11 MAGGIO 2013

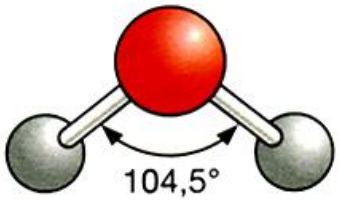




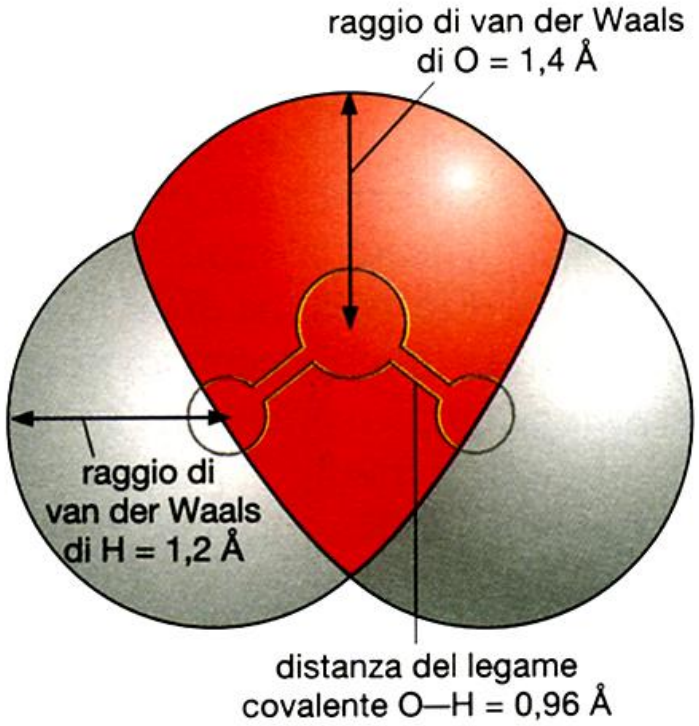


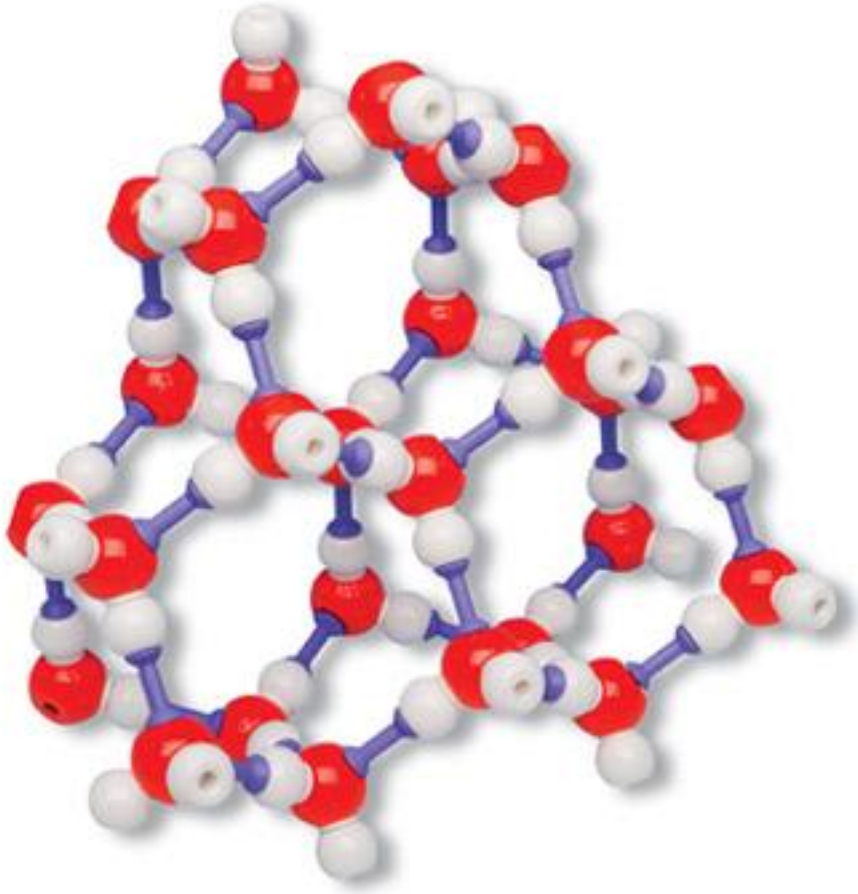

O
H
H

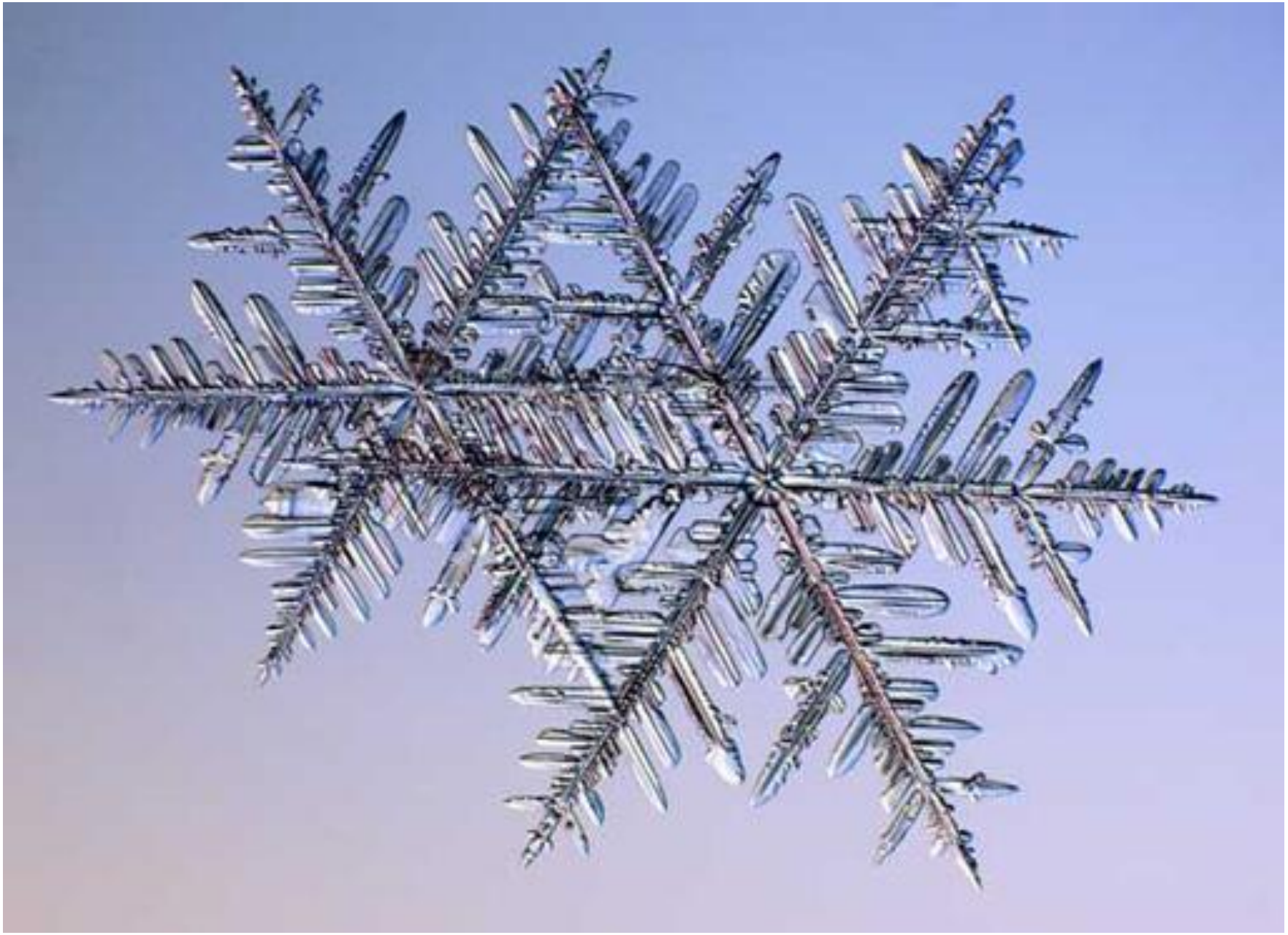
(A)


104,5°

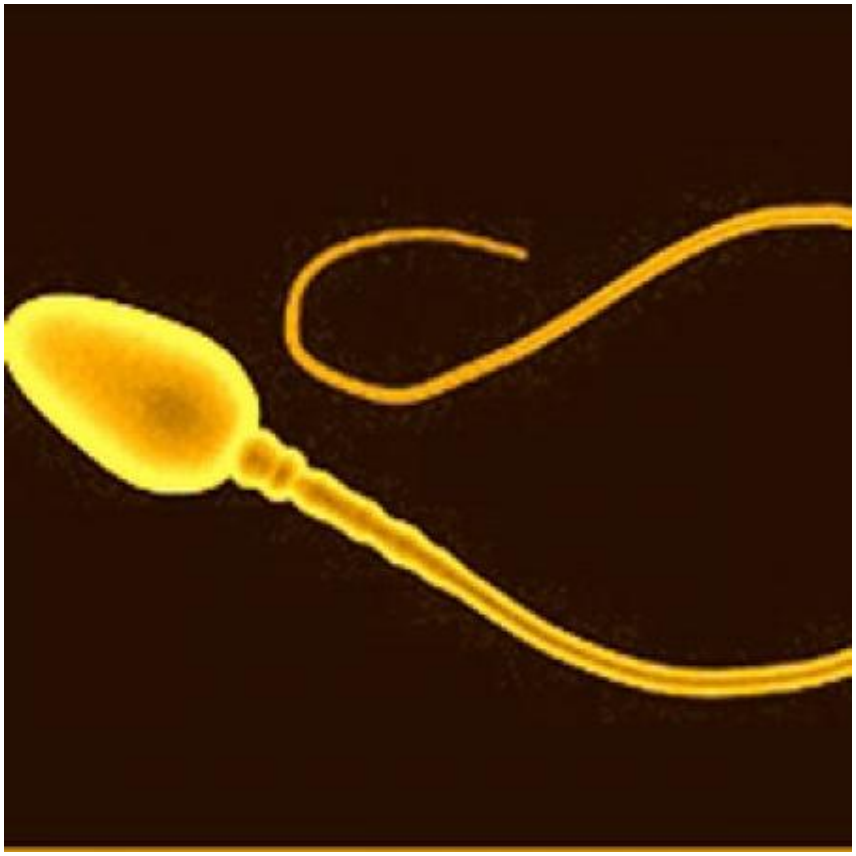
(B)


raggio di van der Waals
di O = 1,4 Å
raggio di
van der Waals
di H = 1,2 Å
distanza del legame
covalente O—H = 0,96 Å

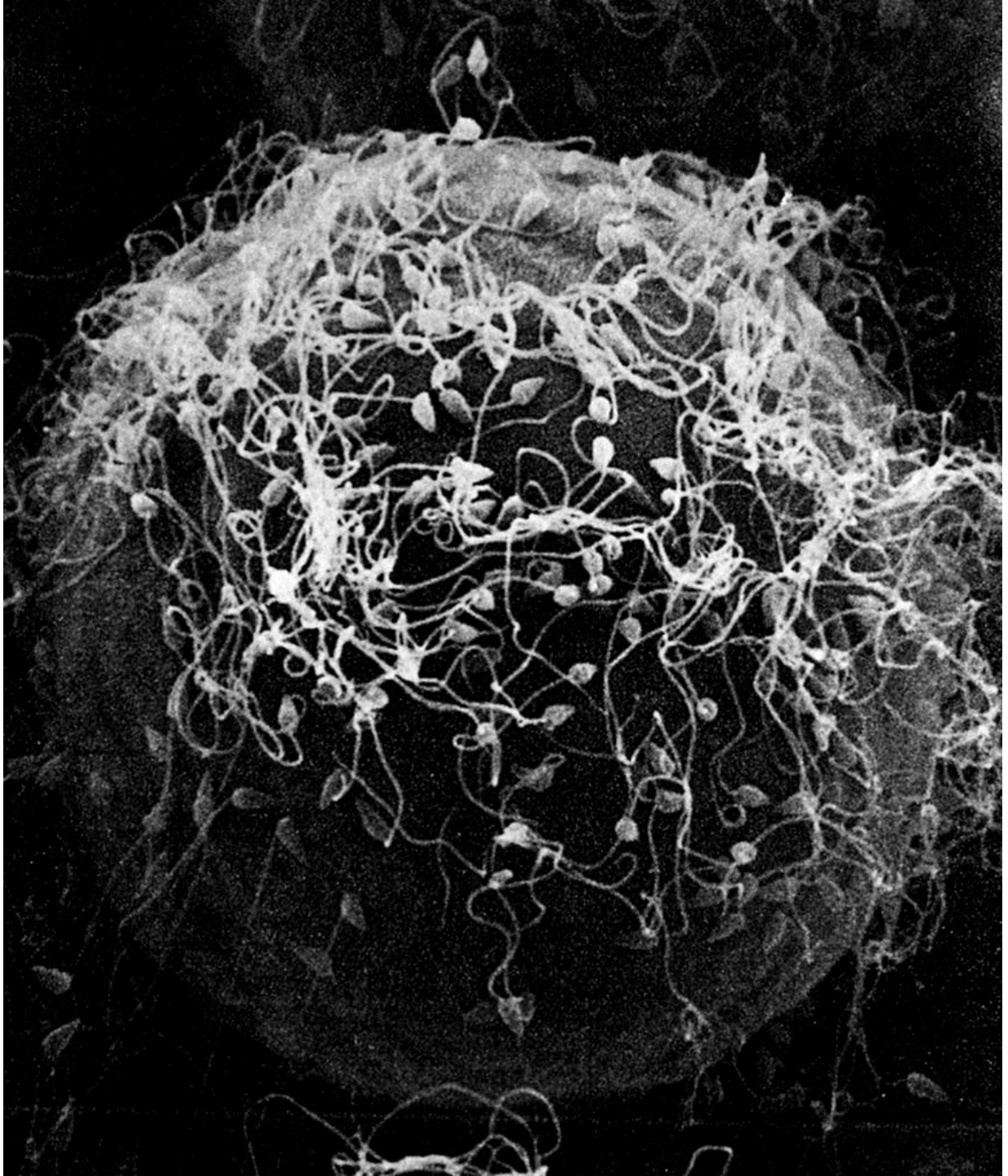


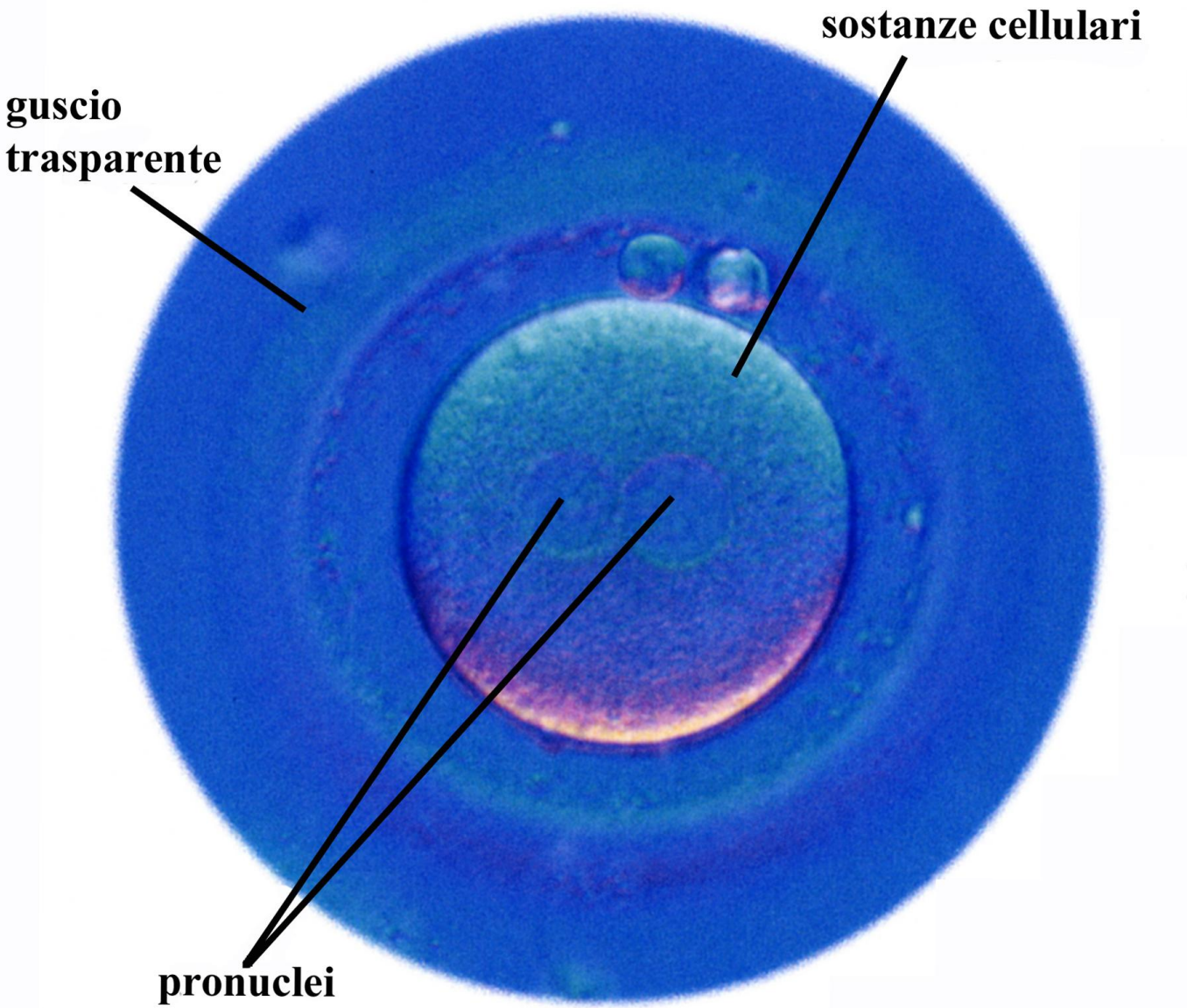


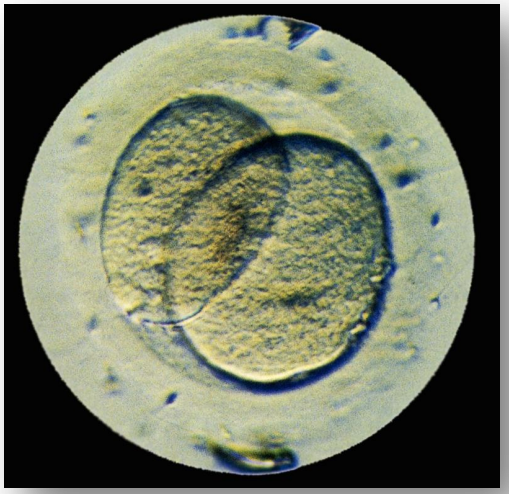
CELLULE GERMINALI



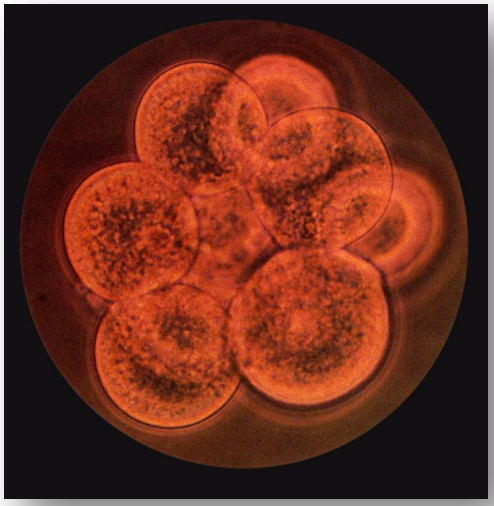
SPERMATOZOI

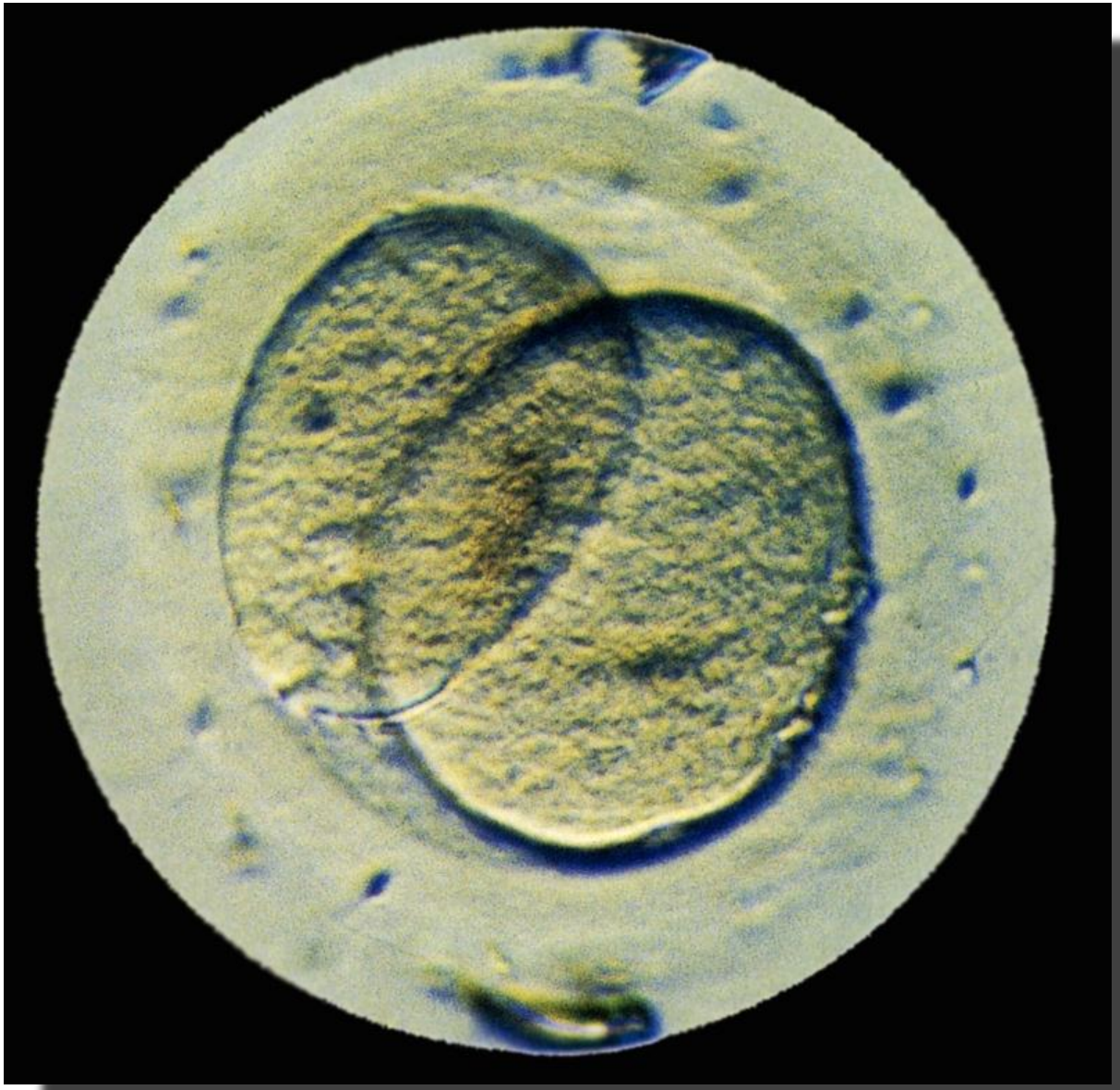


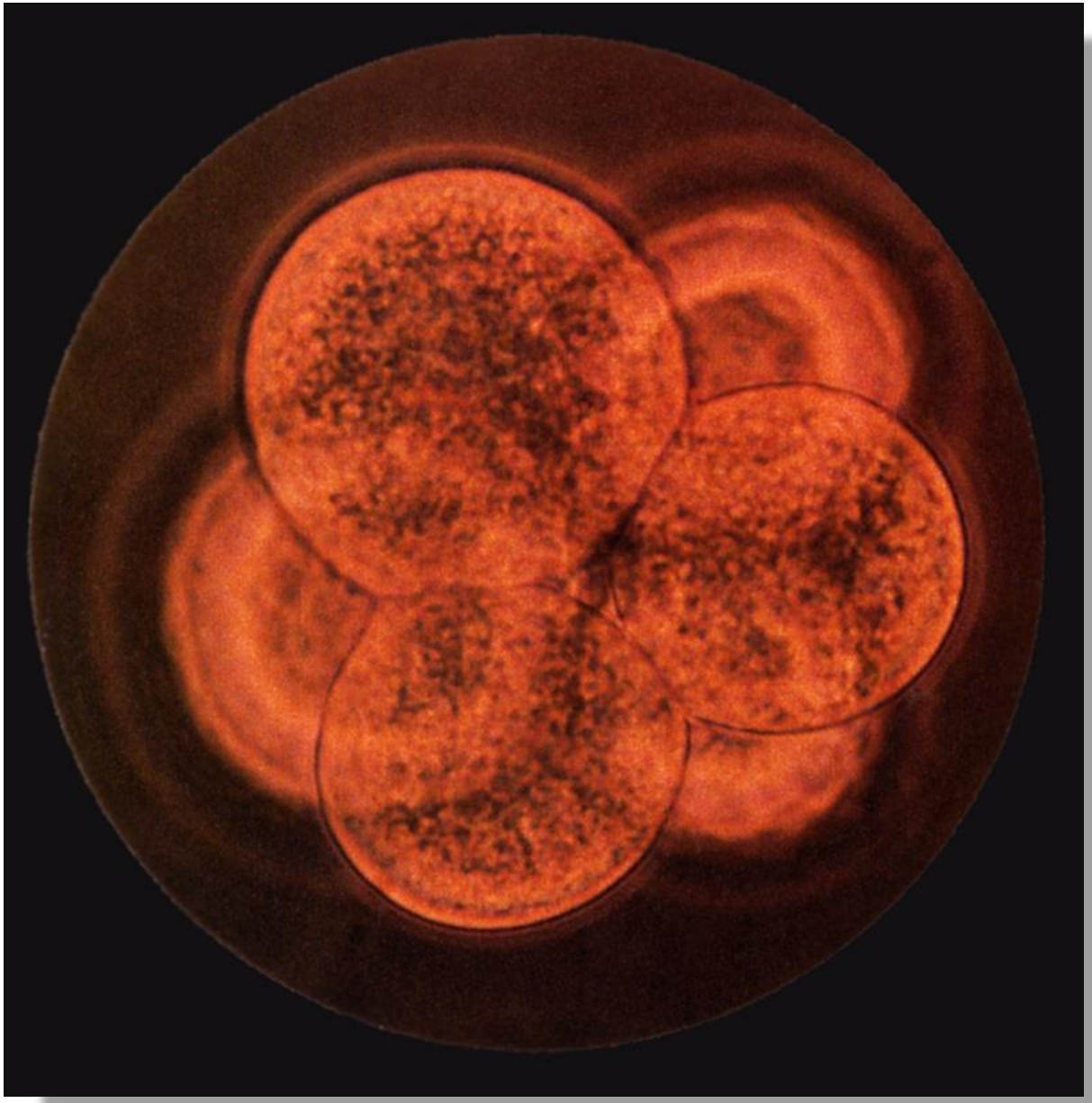

sostanze cellulari
guscio
trasparente
pronuclei



EMBRIONI DA 2 A 8 CELLULE







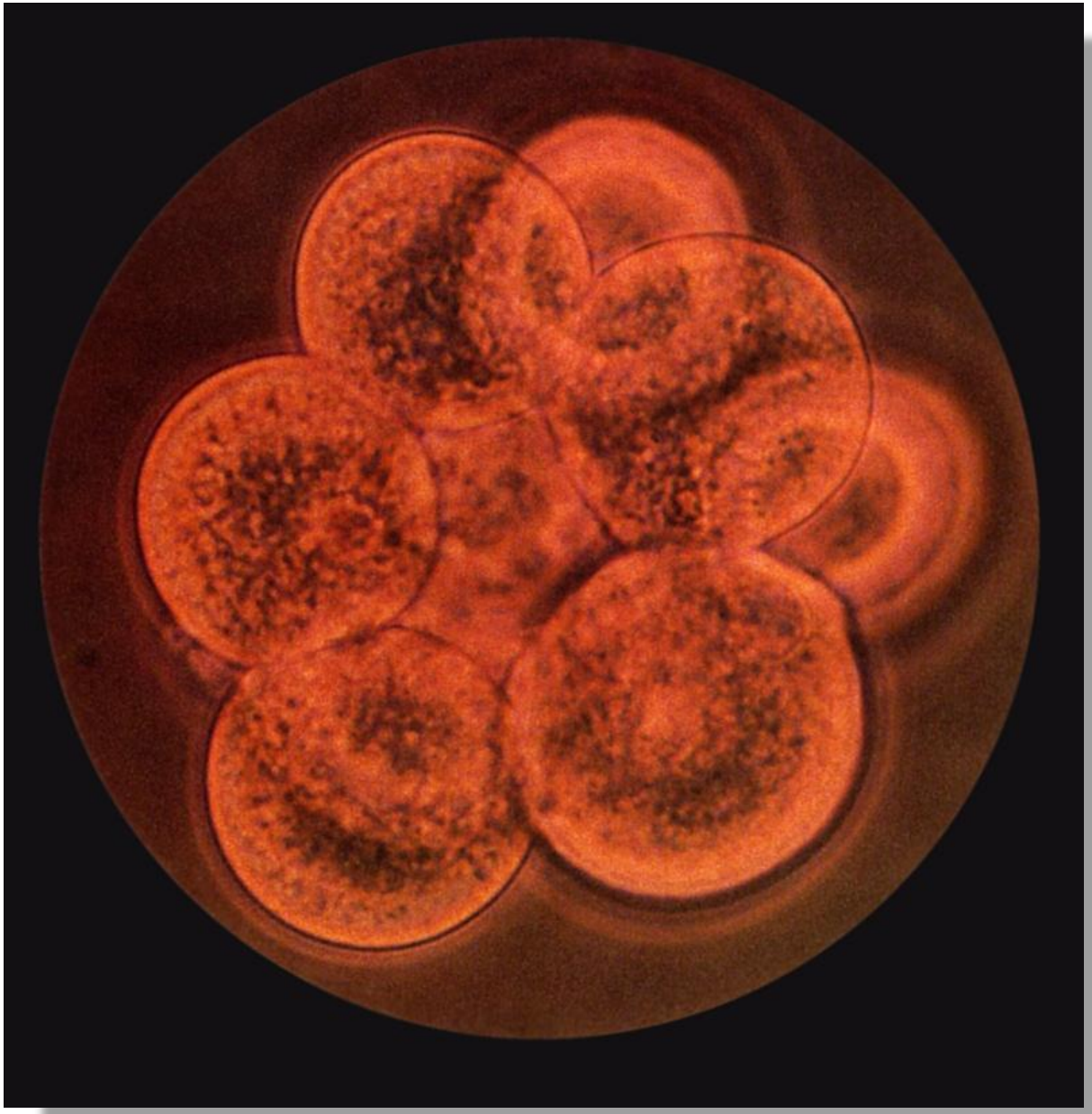


Table 1
Protocols: concentration, time and properties of the vitrification solution for day 2-3 human embryo cryopreservation.

Author and reference	Mukaida et al.[10]	Desai et al.[19]	Rama Raju et al.[20]	Kuwayama et al.[21]
Type of container	Cryostraw	Cryoloop[a]	Cryoloop[b]	Cryotop
Temperature	Room (25–27 °C)	Warm stage (37 °C)	Warm stage (37 °C)	Room (25–27 °C)
Equilibration step	Ethylene glycol, Ficoll, sucrose 20 (2 min): 20% EG	7.5% ethylene glycol + 7.5% DMSO (2 min)	10%EG (5 min)	7.5% ethylene glycol + 7.5% DMSO (5-10 min[c])
Vitrification step	Ethylene glycol, Ficoll, sucrose 40 (1 min): 40% EG	15% EG + 15% DMSO + F.+S. (35 s)	40%EG + S. (30 sec)	15% ethylene glycol + 15% DMSO + sucrose (1 min)
Cooling system	Vapor phase LN_2 (3 min), then plunged into LN_2	Plunged into LN_2 directly (ultra-rapid cooling)	Plunged into LN_2 directly (Ultra-rapid cooling)	Plunged into LN_2 directly (ultra-rapid cooling)
Warming step	One step 0.5 M sucrose (5 min)	Two steps 0.25 M S. (2 min) 0.125 M S. (3 min)	Four Steps 1 M S. (2.5 min) 0.5 M S. (2.5 min) 0.25 M S. (2.5 min) 0.125 M S. (2.5 min)	Two Steps 1 M sucrose (1 min) 0.5 M sucrose (3 min)

DMSO, dimethylsulphoxide; LN_2, nitrogen oxide.

[a] Reported by Desai et al., in 2007.

[b] Reported by Raju et al, in 2005.

[c] The duration of equilibrium is adjusted according to the time needed for re-expansion of the vitrified embryos.

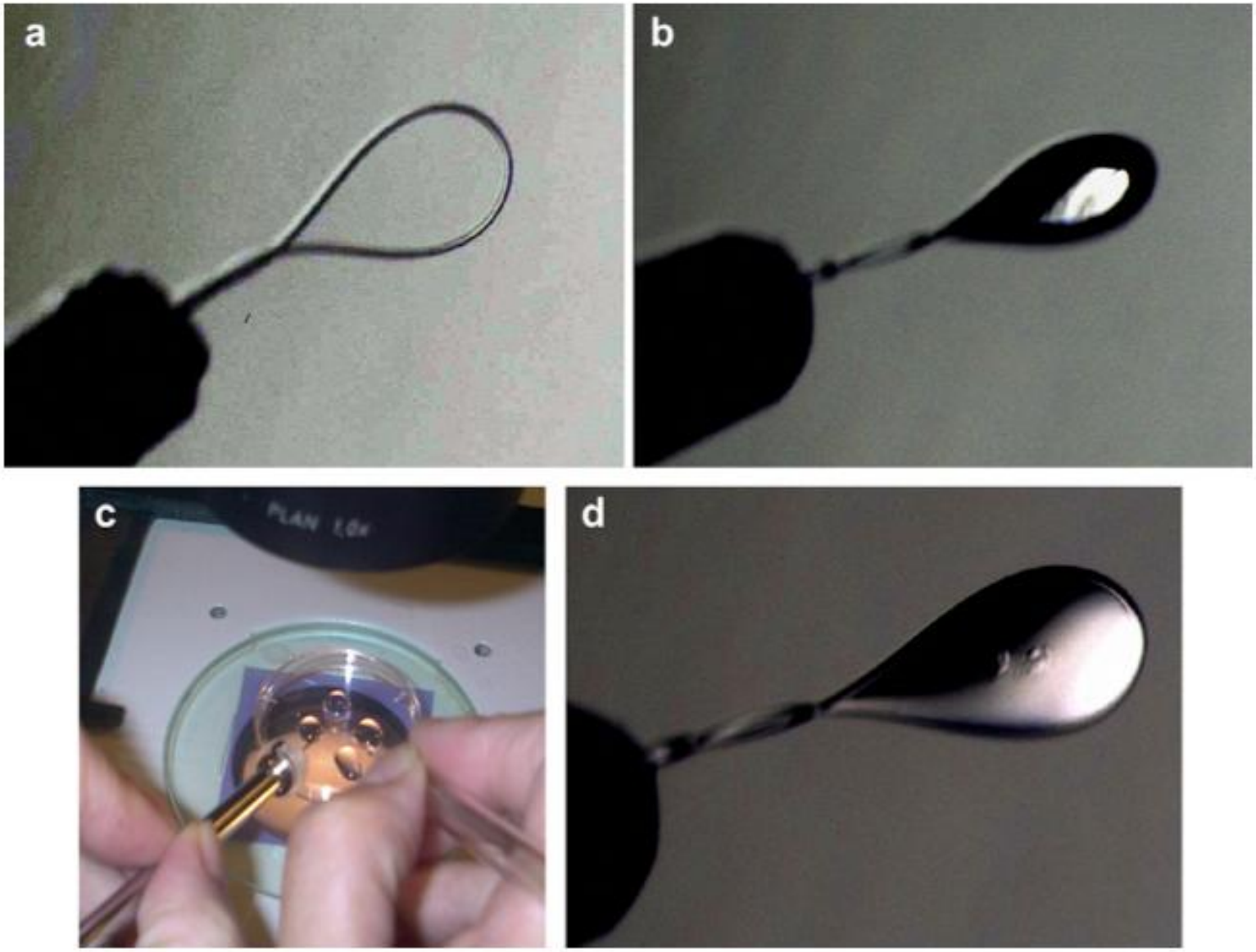


Fig. 1. (a) A minute nylon loop (20 μm wide, 0.5–0.7 mm in diameter) mounted on a stainless steel pipe under ×100 magnification; (b) a thin layer of the vitrification solution on the nylon loop after dipping the loop into the solution; (c) under a dissecting microscope, the capping portion of cryovial with the cryoloop attached with a stainless steel handling rod for manipulation is held in the left hand, and a pulled Pasteur pipette with blastocysts is held in the right hand. Before loading, blastocysts are rinsed several times in small drops of final vitrification solution (solution II) on the lid of a culture dish; (d) blastocysts on the loop with thin layer of vitrification solution.

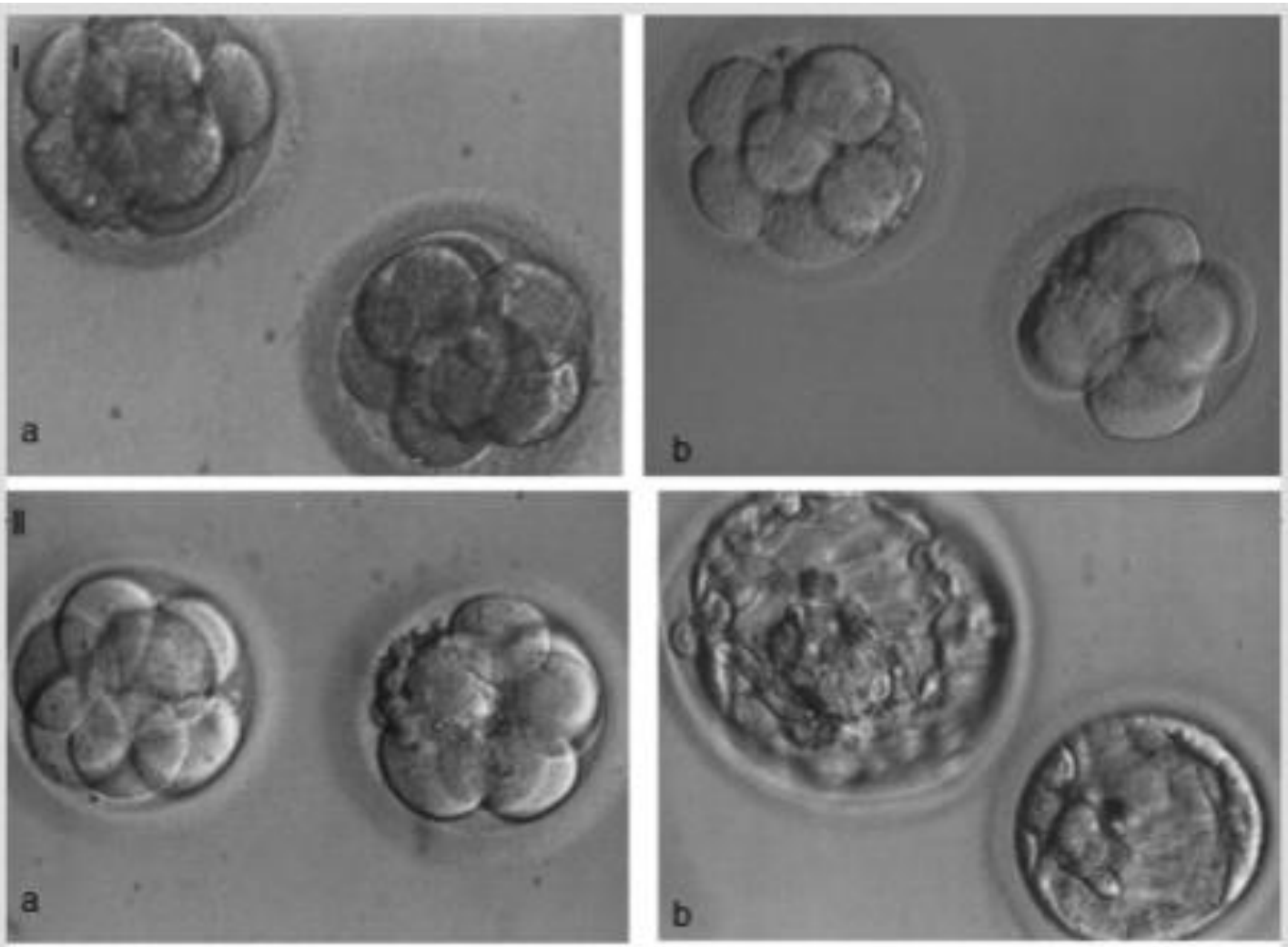
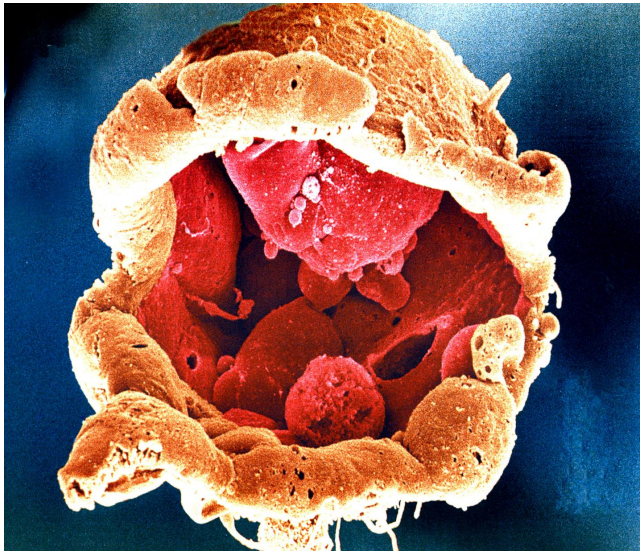

I
a
b
II
a
b

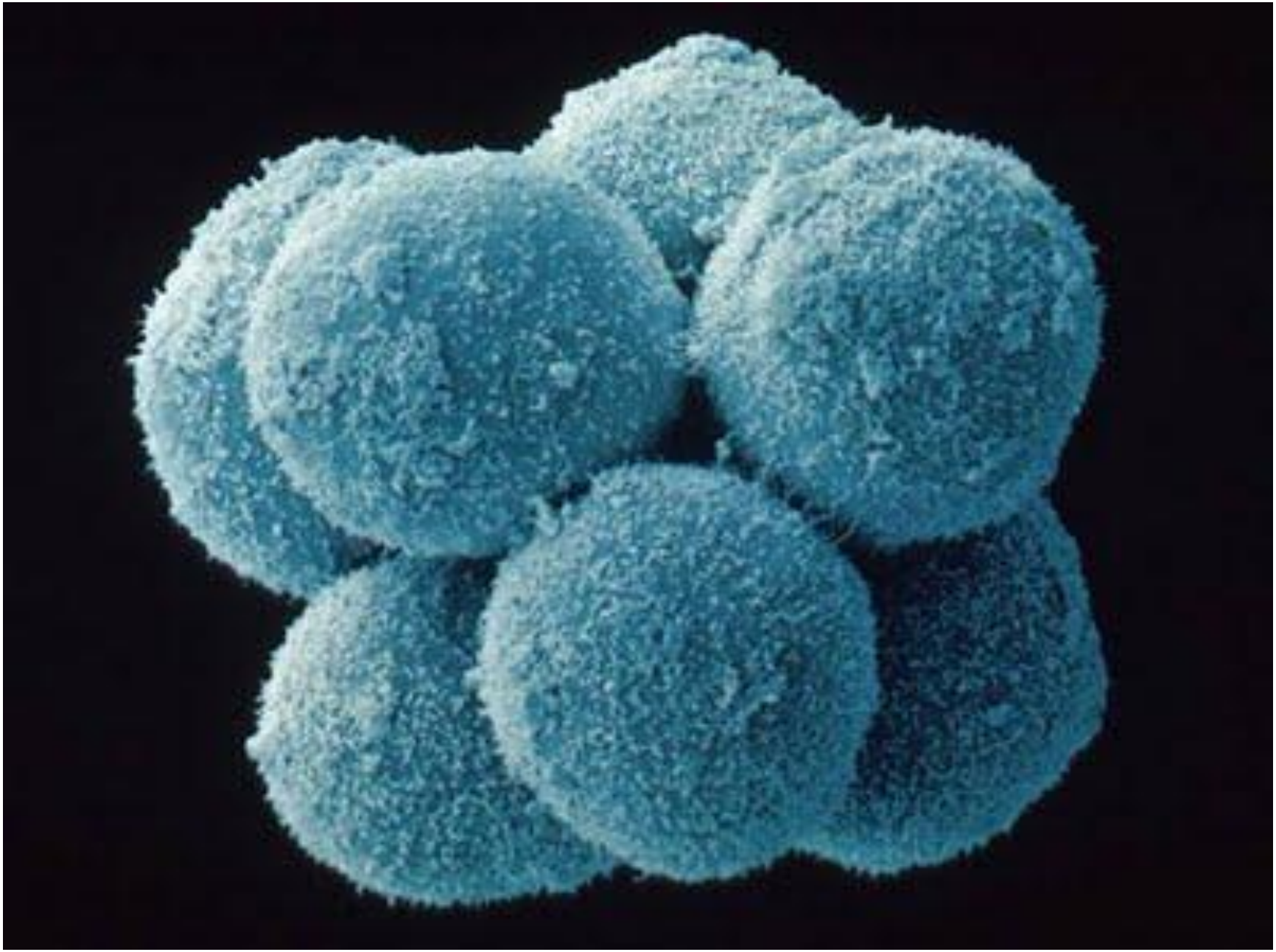
Table 2
Clinical results in each vitrification approach for Day 2–3 embryos.

Author and reference	Mukaida et al.[10]	Desai et al.[19]	Ramu Raju et al.[20]	Results of Nagata Clinic[52]
Type of container	Cryostraw	Cryoloop[a]	Cryoloop[b]	Cryotop
Age (years)	Not available	34.1 ± 4.5	31.3 ± 4.5	35.0 ± 4.5
Number of cycles	127	77	40	604; 346 women
Survival rate	Not available	201/236	121/127	1701 out of 1774
		85%	95%	95.9%
Cleavage rate[c]	486 out of 661	184/236	Not available	1289 out of 1774
	76%	78%		72.7%
Pregnancy rate	34 out of 127	34/77	14/40	164 out of 604
	26.8%	44.2%	35.0%	27.2%
Implantation rate	Not available	40/201	18/121	192 out of 1442
		19.9%	14.9%	13.3%
Delivery rate[d]	22 out of 127	Not available	13/40	118 out of 604
	17%		32.5%	19.5%

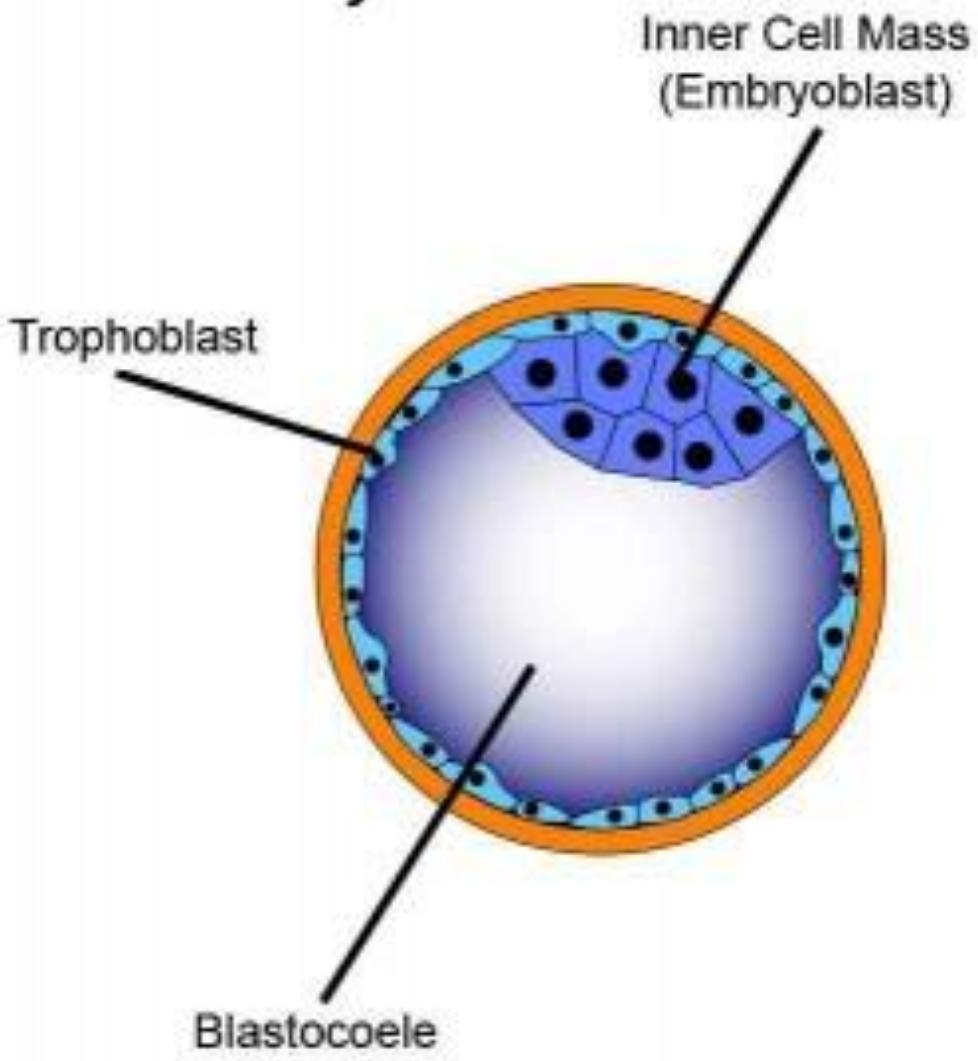
[a] Reported by Desai et al., 2007.
[b] Reported by Raju et al., 2005.
[c] Including survival and further cleavage rate.
[d] Luding on-going pregnancy.

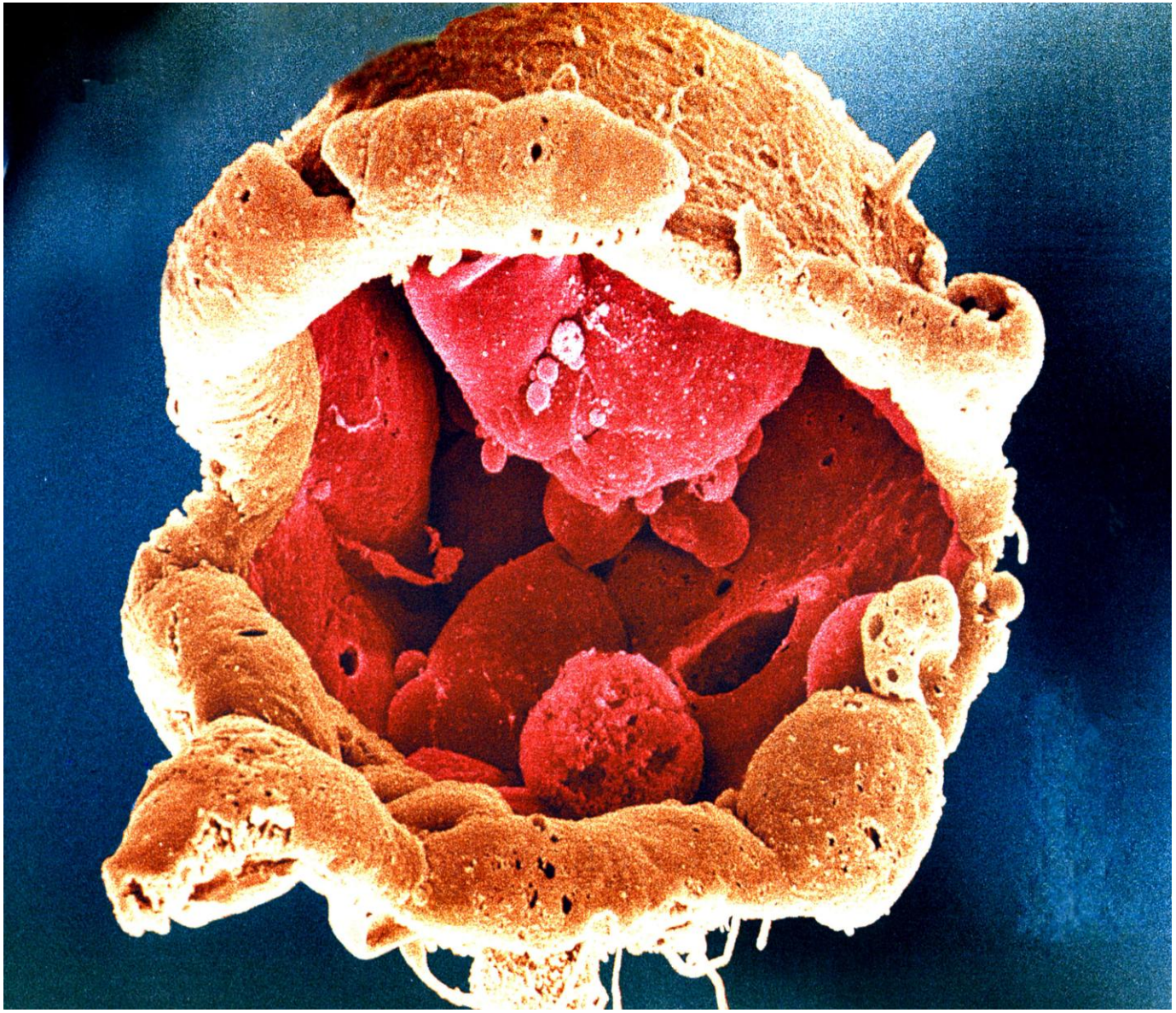


BLASTOCISTI



The Blastocyst





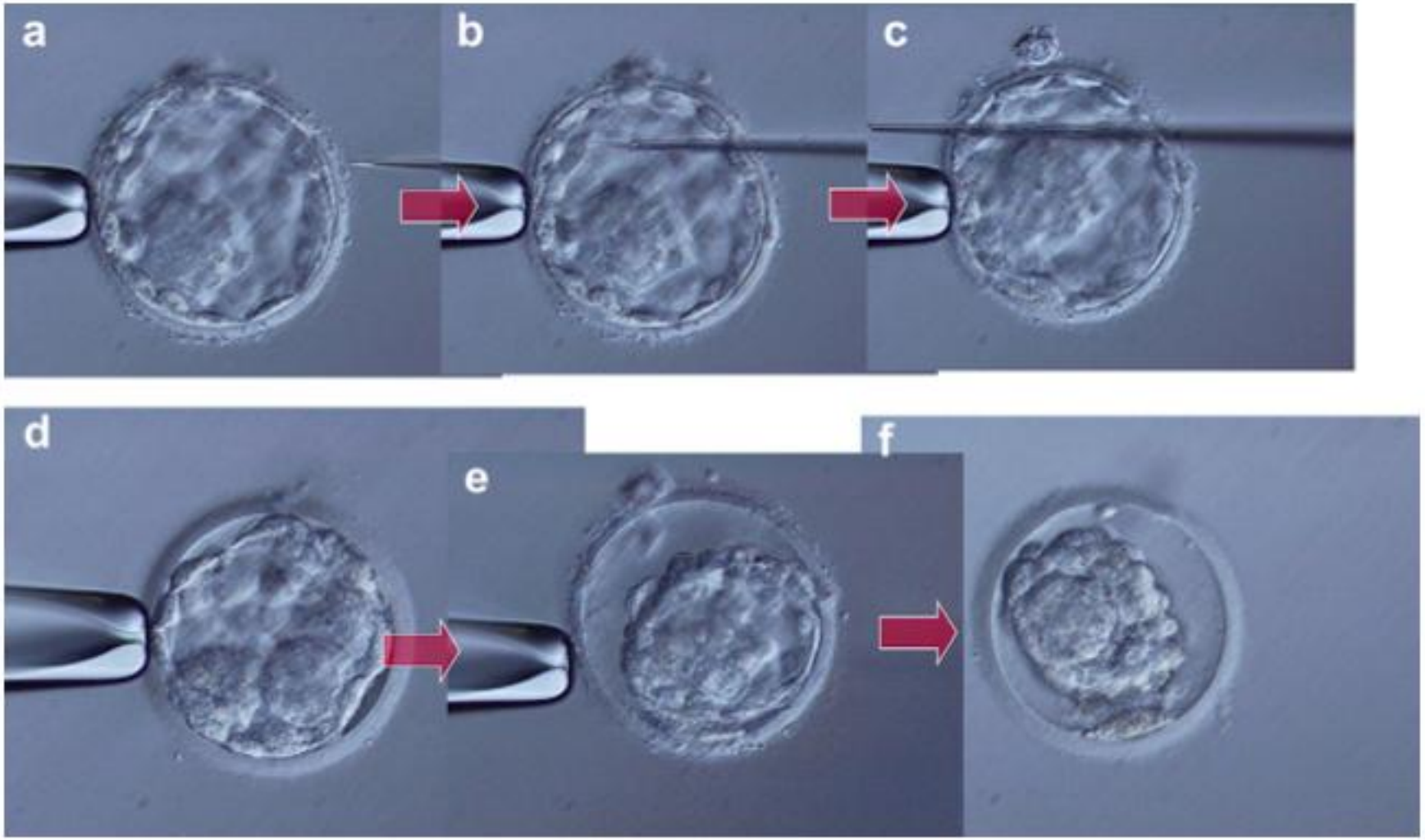
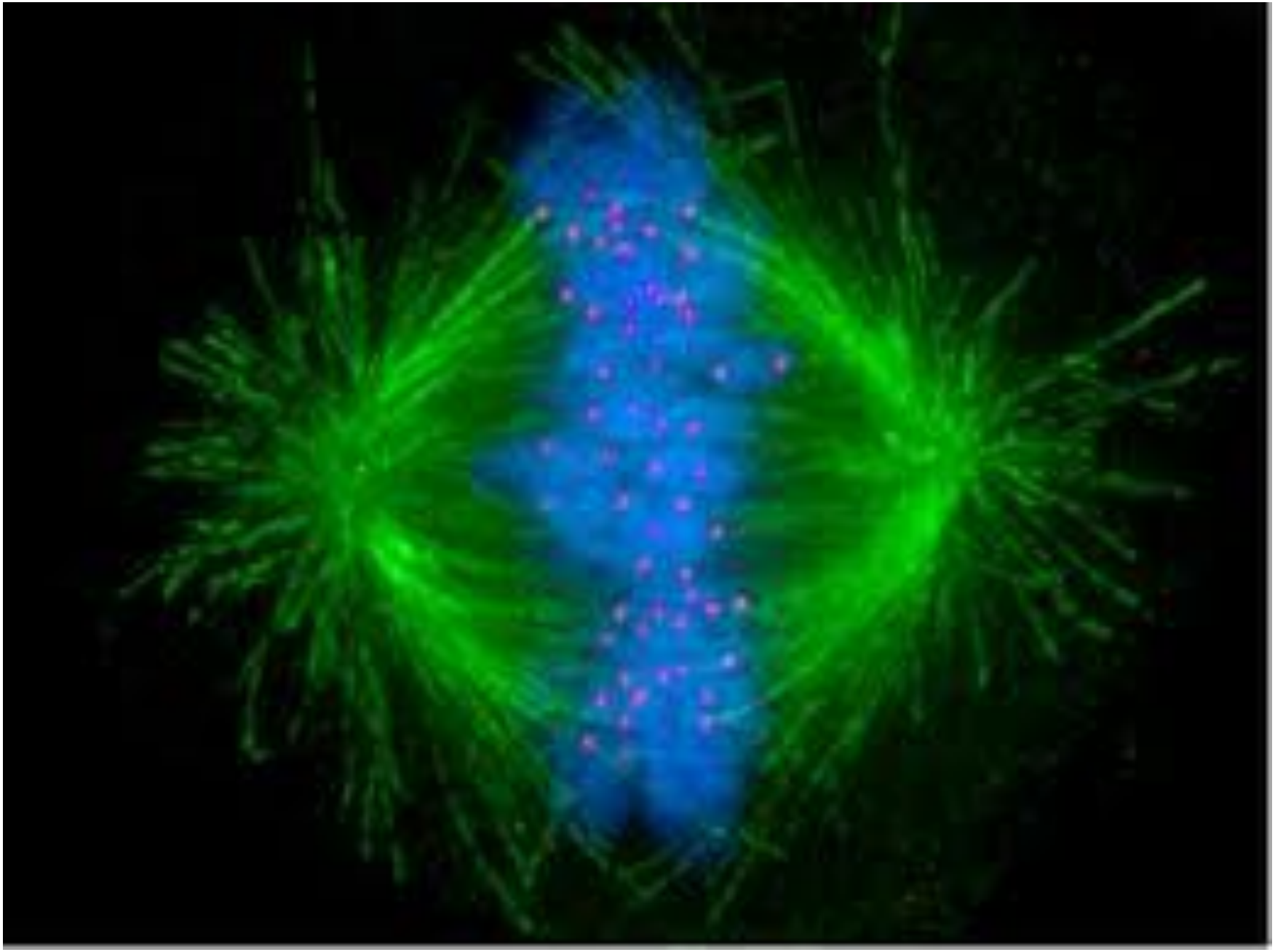


Fig. 2. Artificial shrinkage of expanded blastocyst with the micro-needle; (a) holding the expanded blastocyst with holding micro-pipette connected to micromanipulation; (b) insertion of the micro-needle inside the blastocoele at the point of away from the inner cell mass; (c) puncture through the blastocoele and removing the micro-needle gradually; (d) beginning of shrinkage 10 s after puncture; (e) partial shrinkage 30 s after puncture; and (f) complete shrinkage 1 min after puncture.

Table 3 Assisted reproduction outcomes of fresh cycles, slow freezing and vitrification

Parameters	Fresh cycle	Slow freezing	Vitrification	P-value (Fresh vs. Slow)	P-value (Fresh vs. Vitrification)	P-value (Slow vs. Vitrification)
General clinical data						
Embryos Transferred	2.2±0.9	2.9±0.90	2.6±0.7	0.07	0.23	0.45
Implantation rate (%)	298/1448(20.6)	48/334(14.4)	142/719(19.8)	0.010	0.651	0.035
Clinical pregnancy rate (%)	239/657(36.4)	39/151(25.8)	109/300(36.3)	0.014	0.989	0.025
Multiple pregnancy rate(%)	58/657(8.8)	9/151(6.0	29/300(9.7)	0.249	0.675	0.181
Live birth (LB) rate (%)	225/657(34.3)	35/151(23.2)	106/300(35.3)	0.009	0.743	0.009
Loss of pregnancy						
Early abortion rate(%)	23/239(9.6)	3/39(7.7)	18/109(16.5)	0.701	0.064	0.175
Late abortion rate(%)	15/239(6.3)	3/39(7.7)	2/109(1.8)	0.739	0.075	0.082
Induced abortion rate(%)	2/239(0.8)	1/39(2.6)	0	0.895	1.000	0.264
Ectopic pregnancy rate(%)	9/239(3.8)	0	4/109(3.7)	0.619	1.000	0.573
Mode of delivery						
Spontaneous vaginal delivery rate(%)	34/190(17.9)	5/32(15.6)	6/85(7.1)	0.755	0.019	0.157

OOCITI



SPERO DI NON AVERVI ANNOIATO

E VI RINGRAZIO PER L'ATTENZIONE

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