MORPHOLOGICAL ASPECTS of HUMAN BLASTOCYSTS BEFORE AND AFTER VITRIFICATION

Marianne Moser

Landes- Frauen- und Kinderklinik Linz, Austria

Blastocyst Scoring

Gardner et al.:

Blastocyst Score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril , Vol 73, No6, 1155-1158, 2000.

- Expansion: Grade I to VI
- Inner Cell Mass: a,b,c
- Trophectoderm: a,b,c

assessment for blastos III-V:

Expansion size of blastocoel, diameter of blastocyst

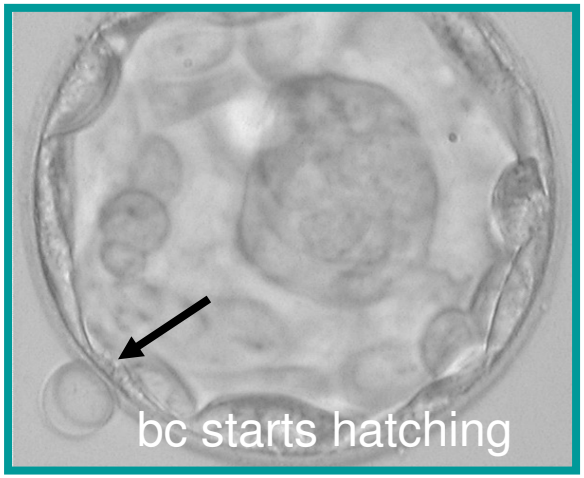
I

II

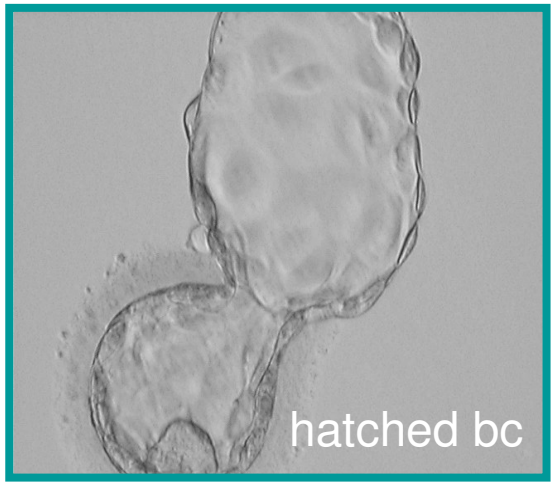
III

IV

V

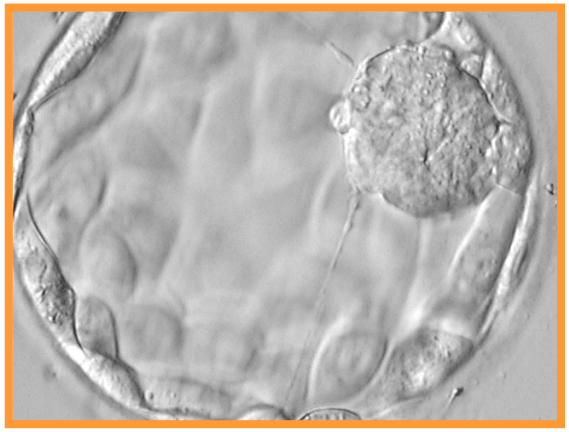


VI



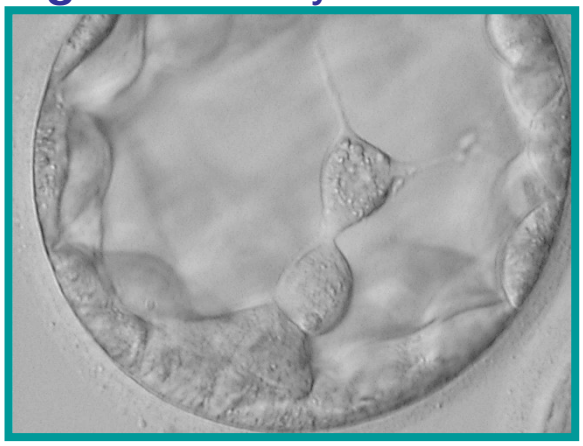
Inner Cell Mass (ICM)

grade a: tightly packed, many cells



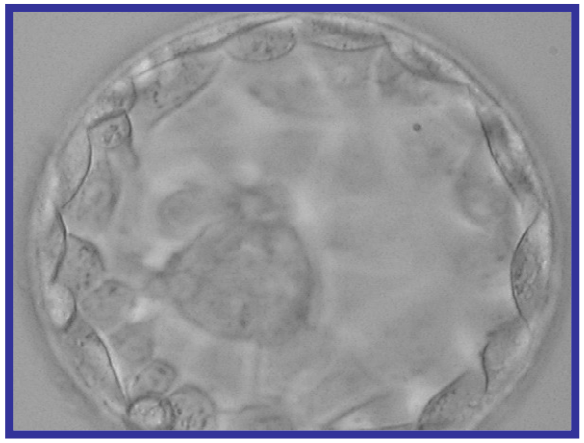
grade b: loosely grouped, several cells

grade c: very few cells

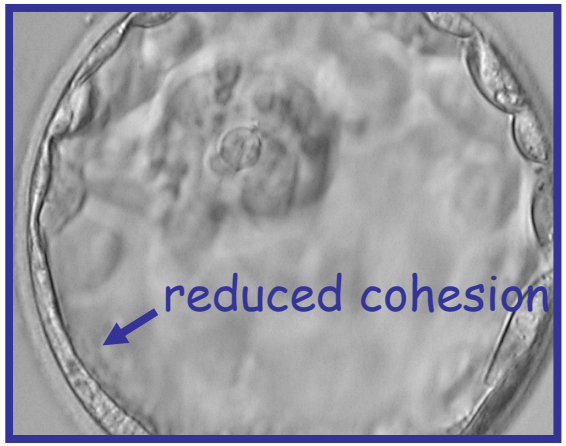


Trophectoderm

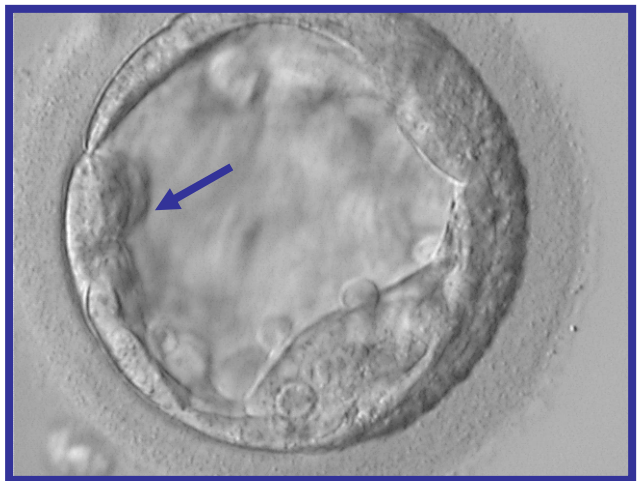
grade a: numerous sickle cell shaped cells forming a cohesive epithelium



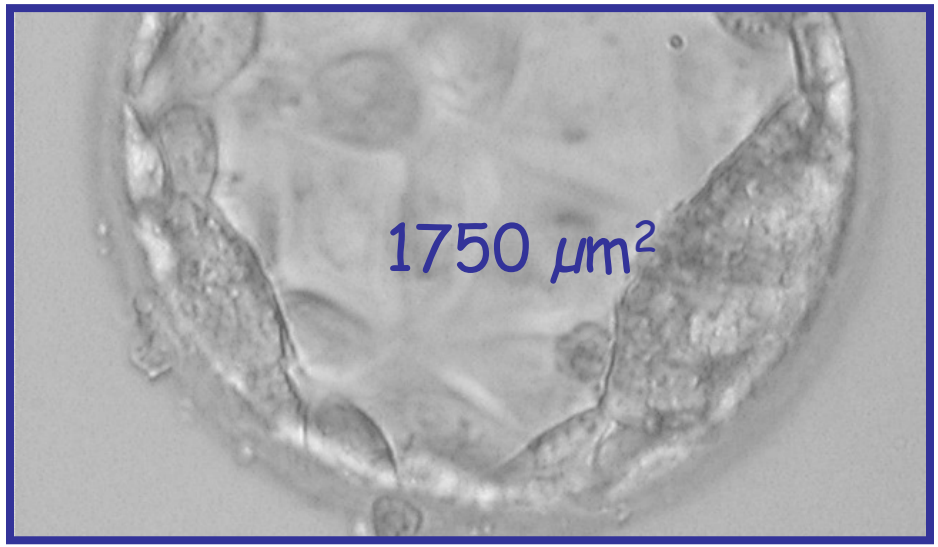
grade b: few cells forming a loose epithelium

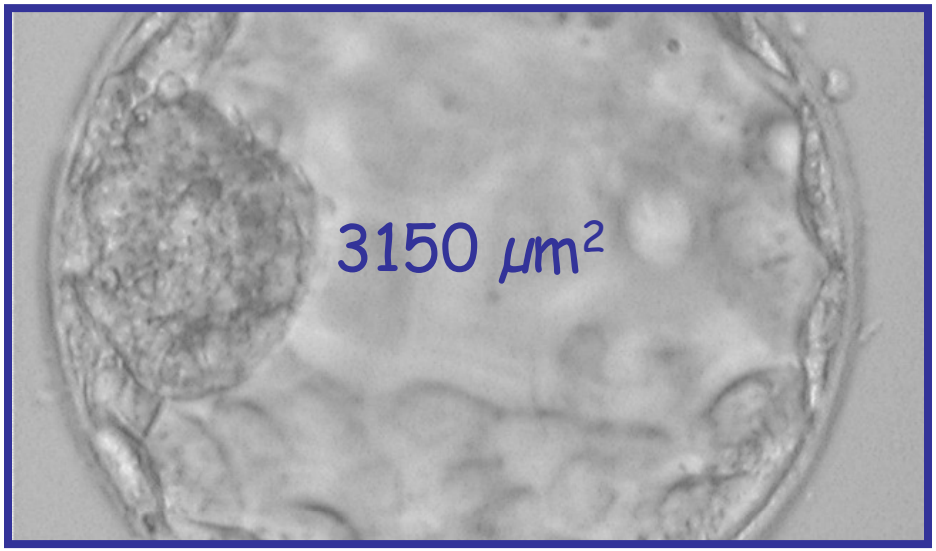


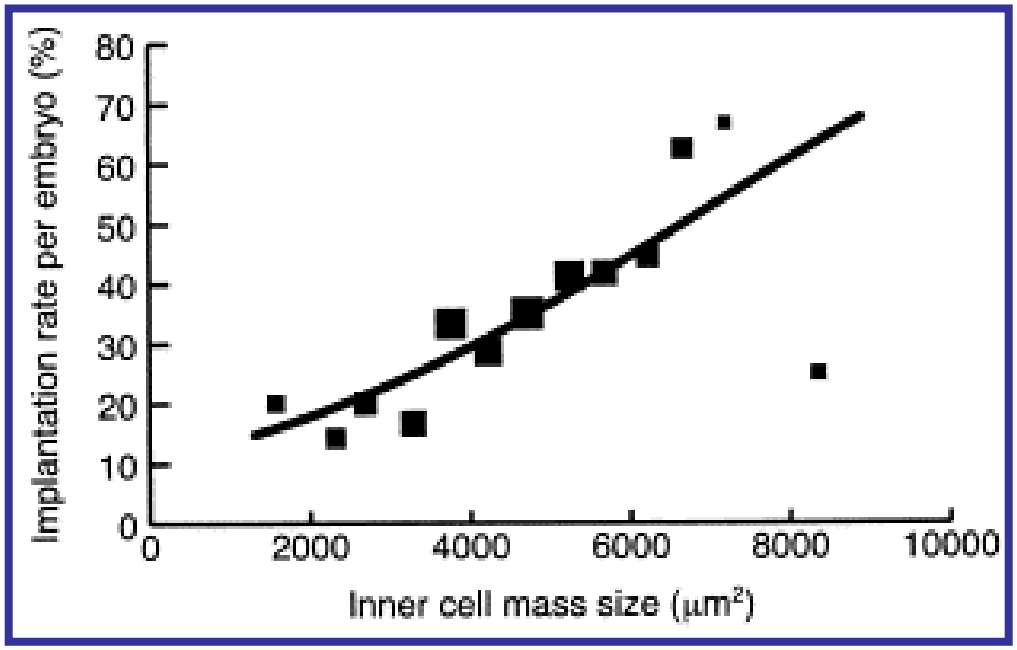
grade c: very few large cells



Size of inner cell mass



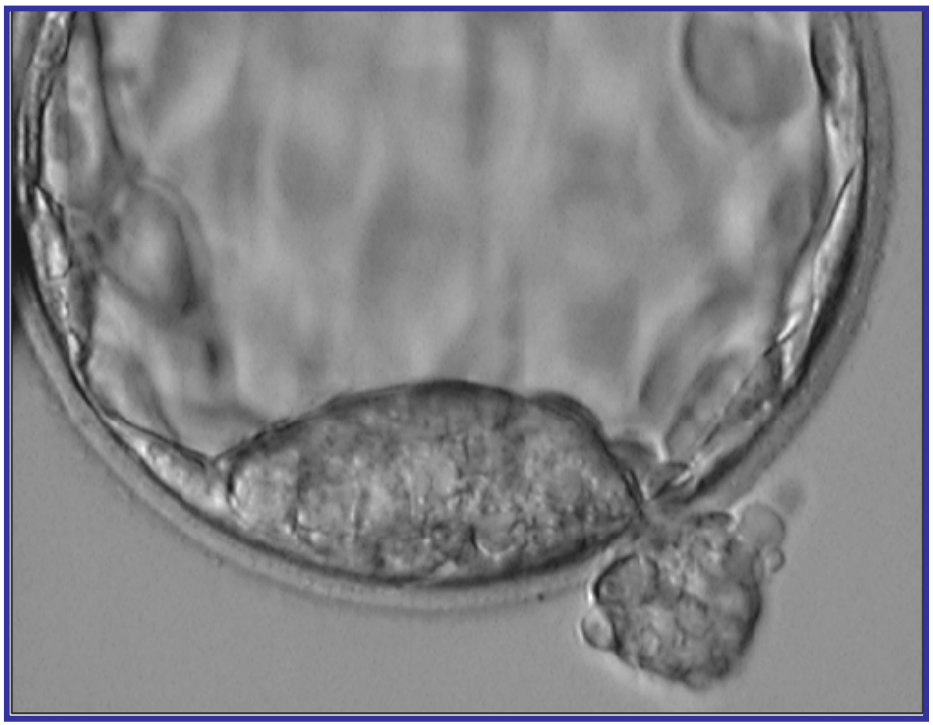


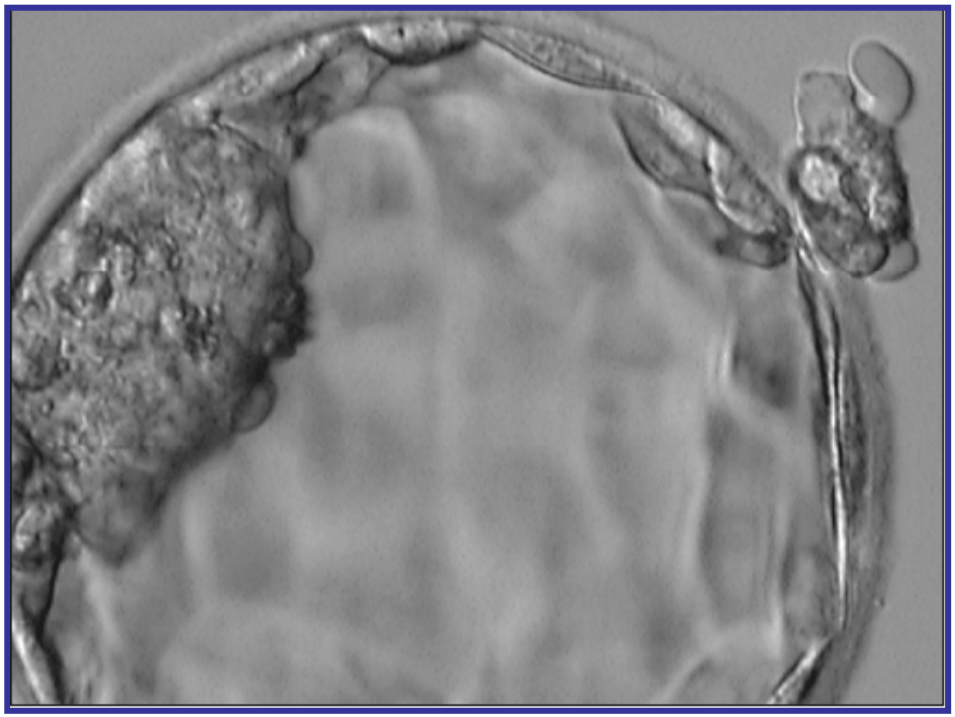


Richter et al., F&St, 2001

Location of herniation

Vaa





Ebner et al., 2007; JTGGA

Table 1. Implantation behaviour of blastocysts hatching at different spots around the zona pellucida

	Study group haching from ICM	Mixed group	Control group hatching from TE
n	29	26	53
Clinical PR	21 (72.4)	16 (61.5)	27 (50.9)
MPR	5 (23.8)	5 (19.2)	4 (14.8)
IR	26/39 (66.7)[a,b]	21/52 (40.4)[b]	31/76 (40.8)[a]

[a] p=0.009; [b] p=0.01

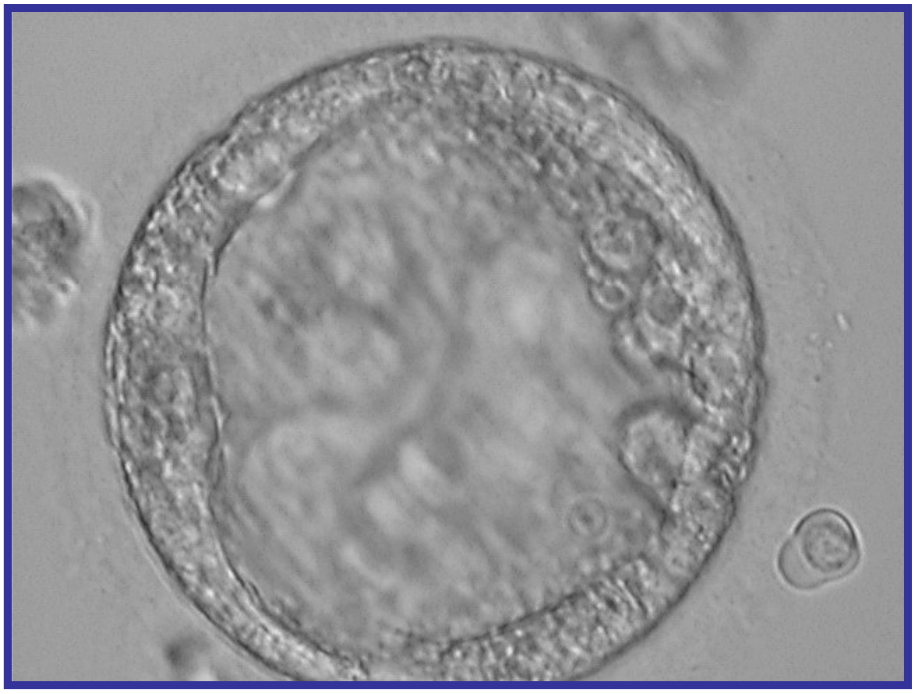
Values in parentheses are percentages. Mixed group had two blastocyst with different hatching spots transferred.

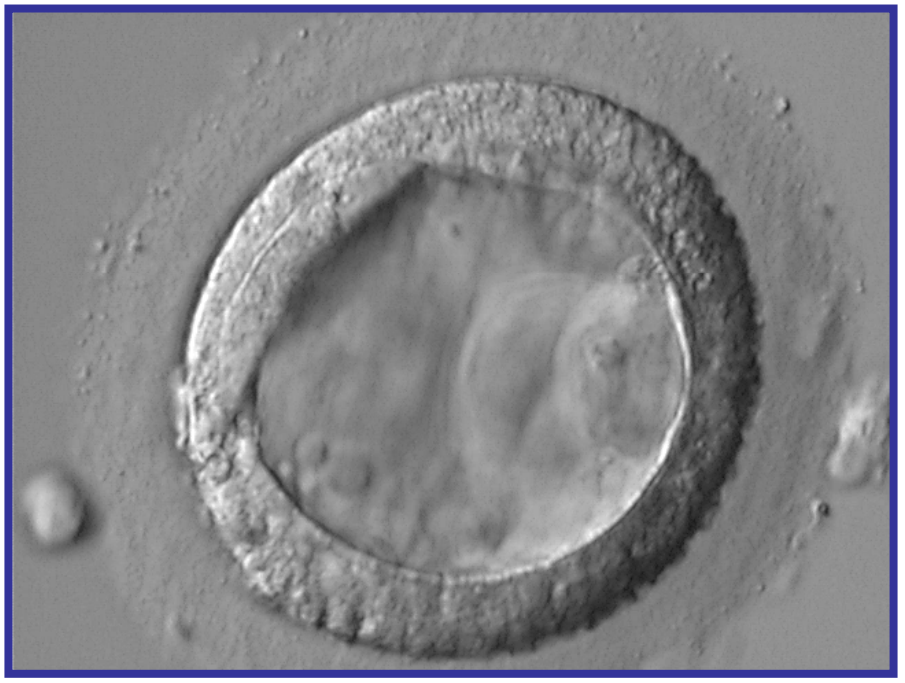
ICM: inner cell mass; IR: implantation rate; MPR: multiple pregnancy rate; n: number of patients; PR: pregnancy rate; TE: trophectoderm

Ebner et al., 2007; JTGGA

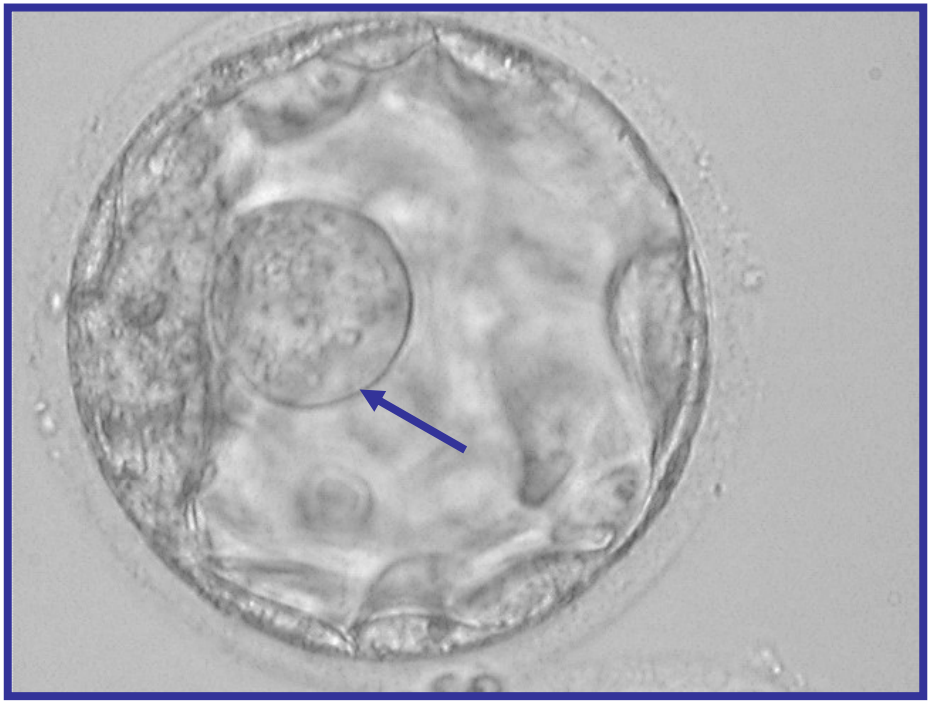
Abnormalities

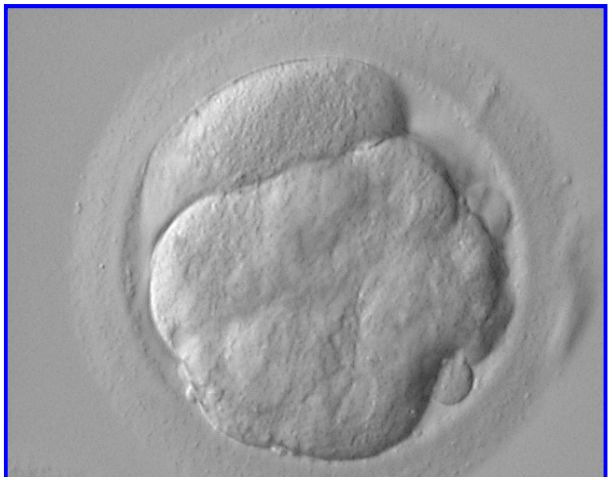
Pseudoblastocysts





Exclusion of blastomeres,

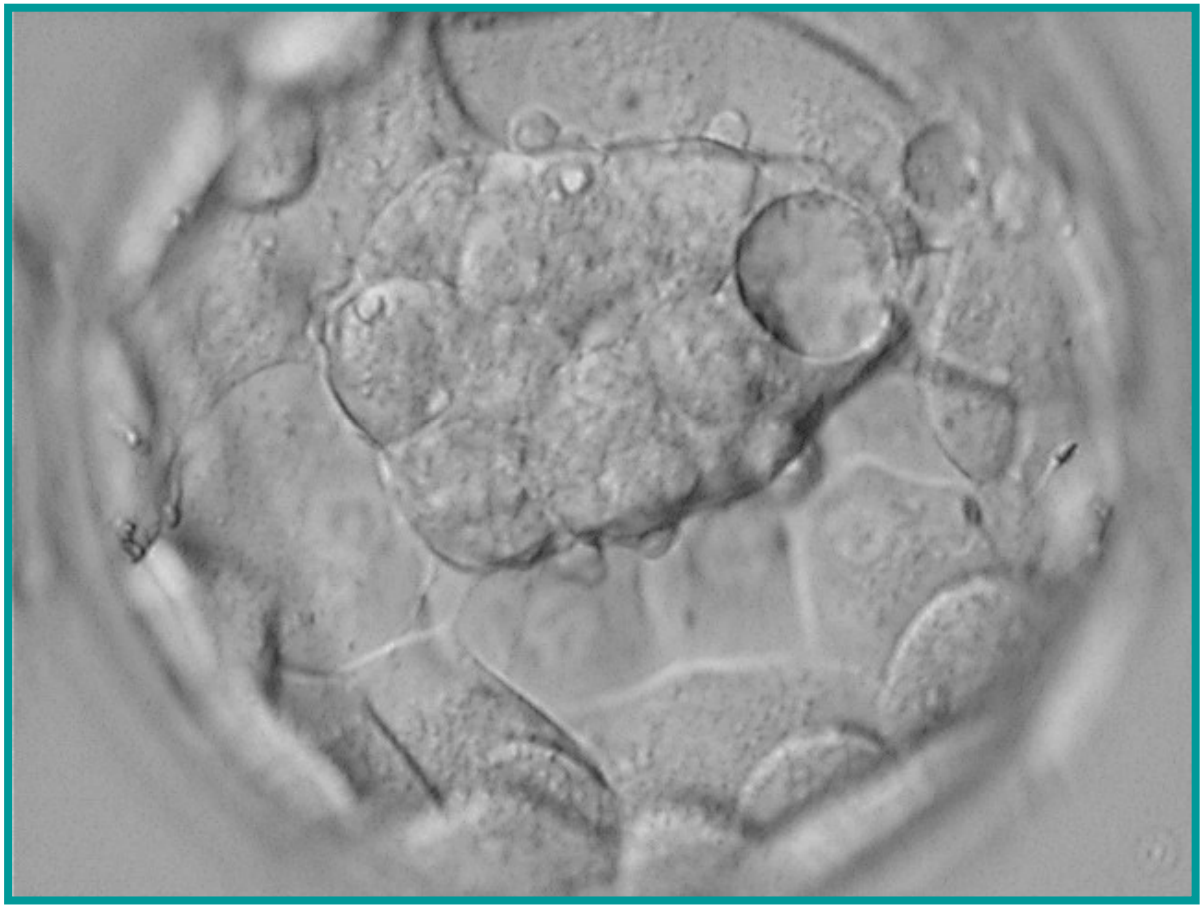




loss of cytoplasm, internalized excluded cell

Exclusion of fragments

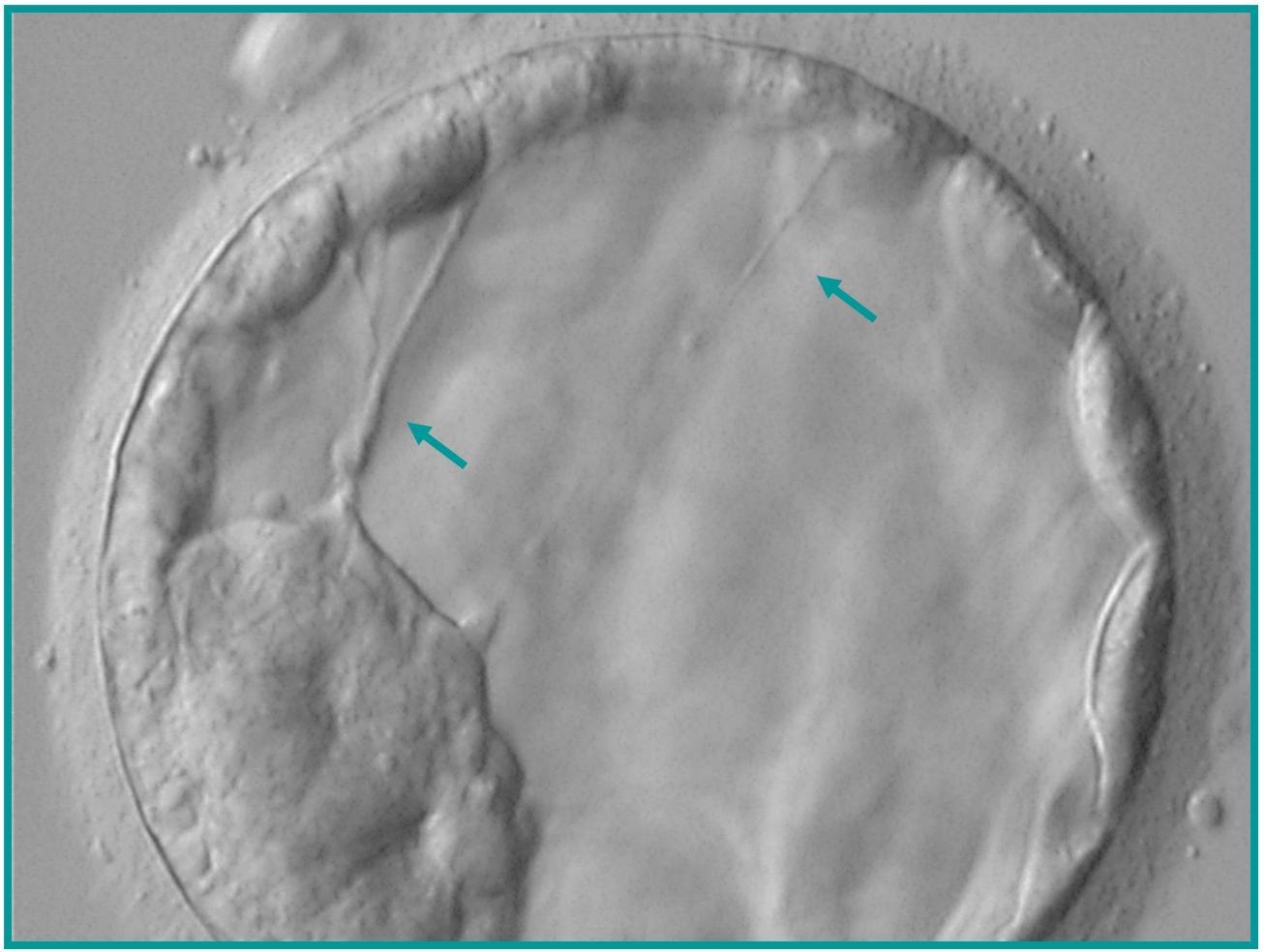
Vacuoles



critical: > 14 µm

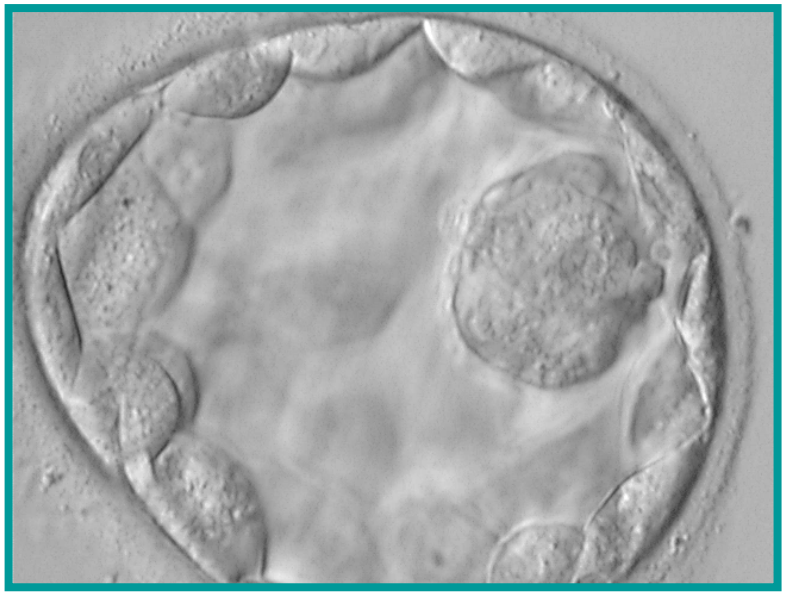
Ebner et al., 2005

Cytoplasmic strings



Scott, 2000

Ovoid shape



Selection for Vitrification

Inclusion criteria

Morula, I, II

III – V:

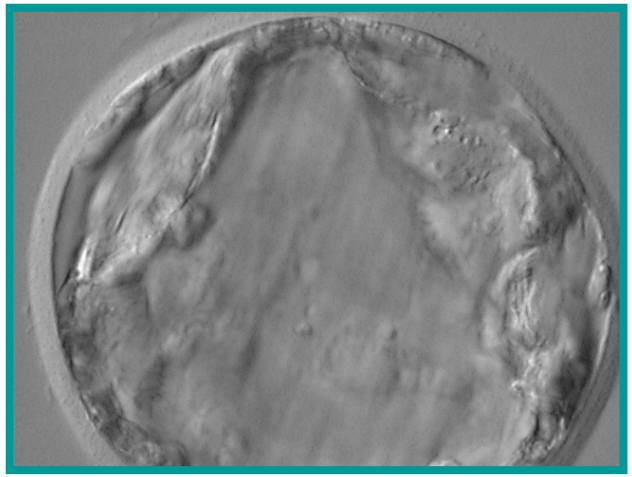
- ICM – grade a and b
- TE – grade a and b (also c, when ICM grade a)
- ovoid day 6 blastos with good quality

Exclusion criteria

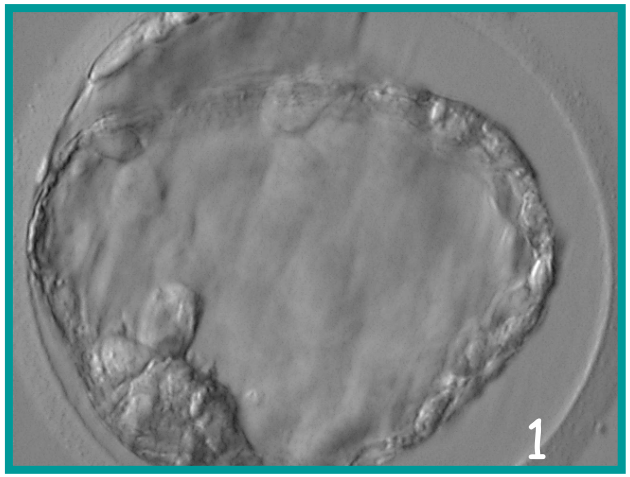
- many fragments, big vacuoles
- ICM - grade c or totally lacking

Incubation time

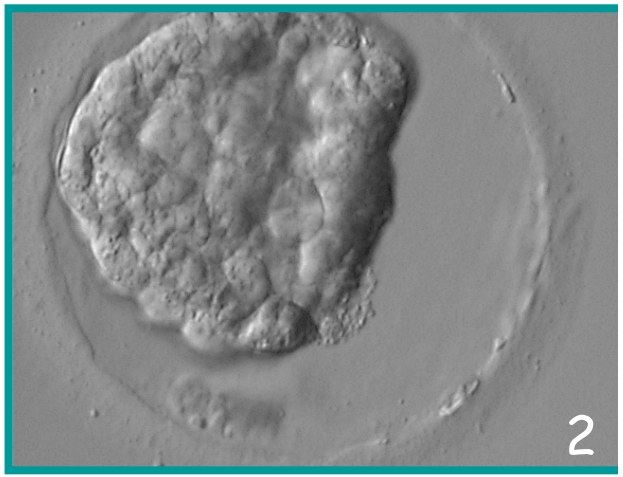
	PBS	medium 1	medium 2
compacting, early Bc:	1´	1.5´	20-30´´
full blastocyst:	1´	2´	20-30´´
expanded blastocyst:	1´	3´	20-30´´



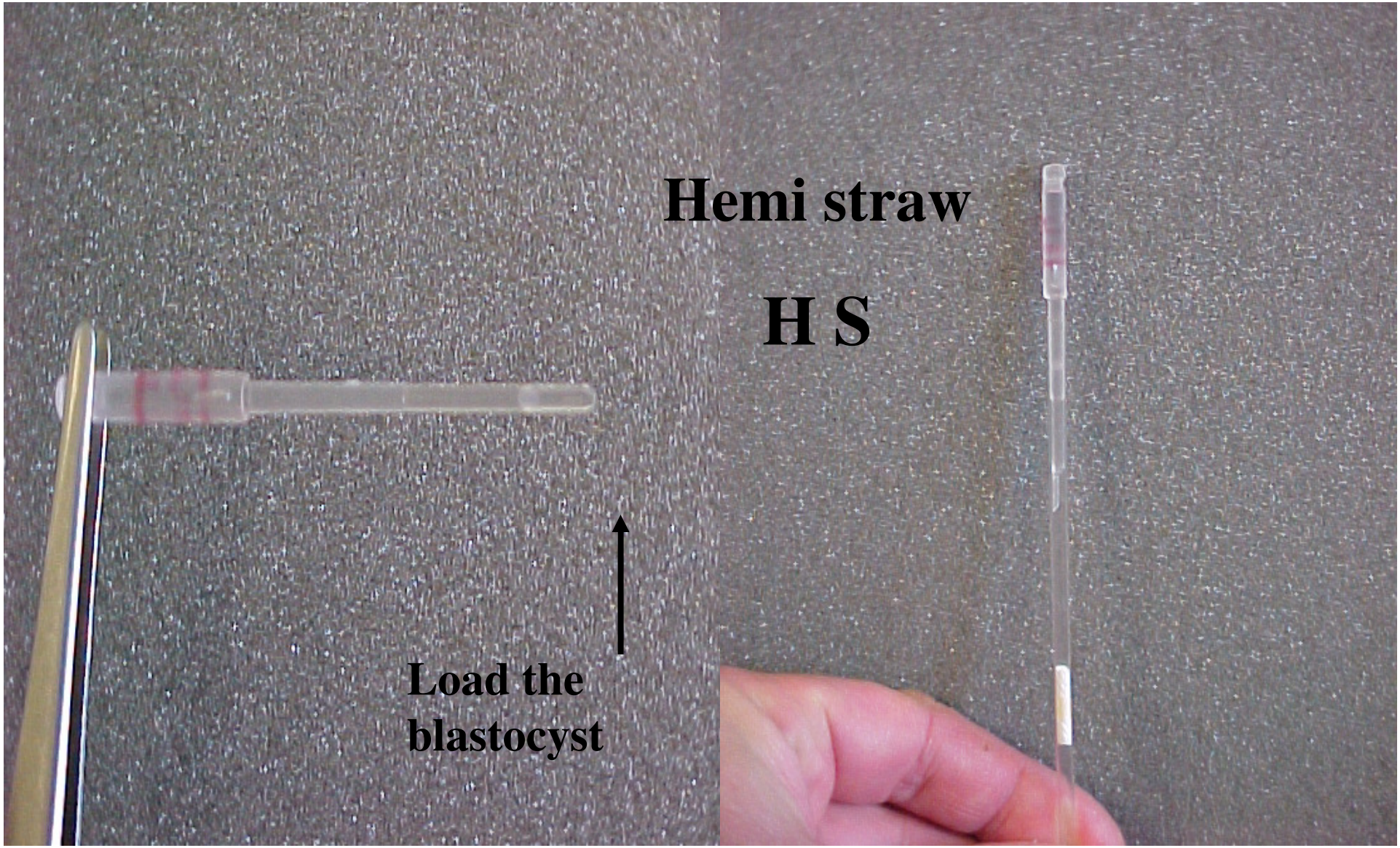
PBS/HSA (RT)



DMSO +
Ethylenglycol

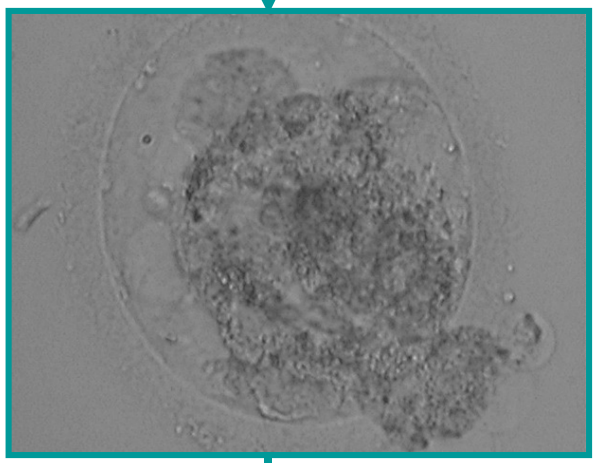


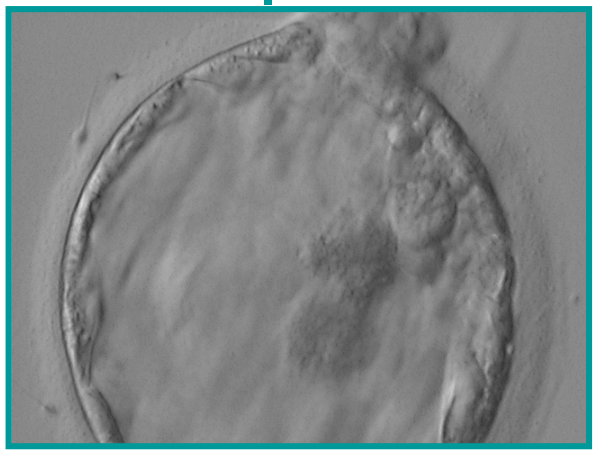
DMSO + Ethylenglycol
Sucrose + Ficoll

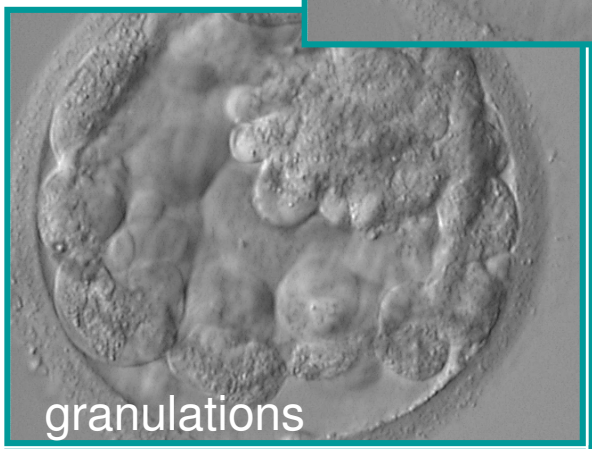


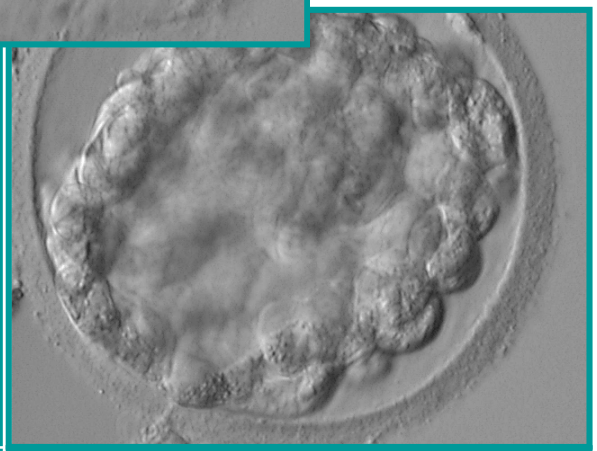
Vitrification of human blastocysts with the Hemi-Straw carrier: application of assisted hatching after thawing. Vanderzwalmen P Hum Reprod. Jul;18(7):1504-11, 2003

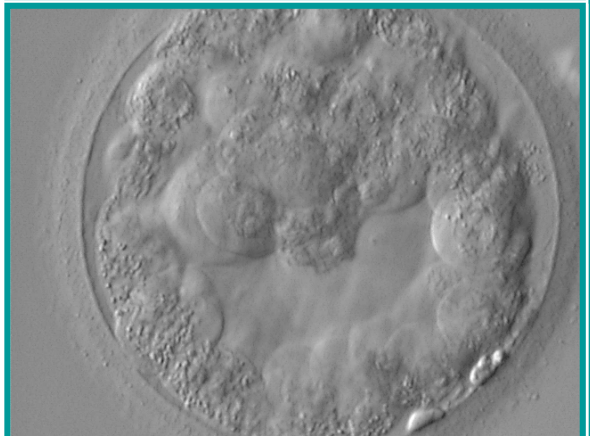
Morphology after warming

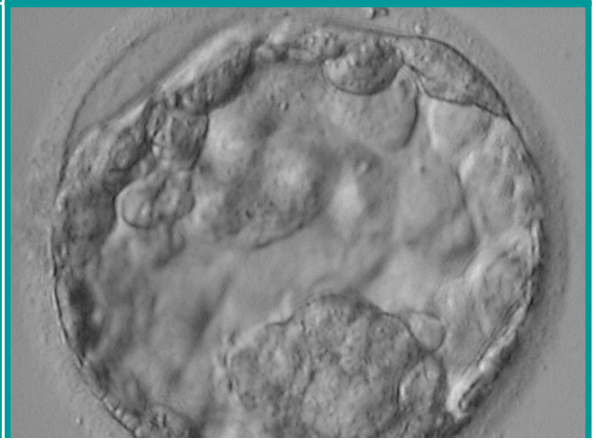






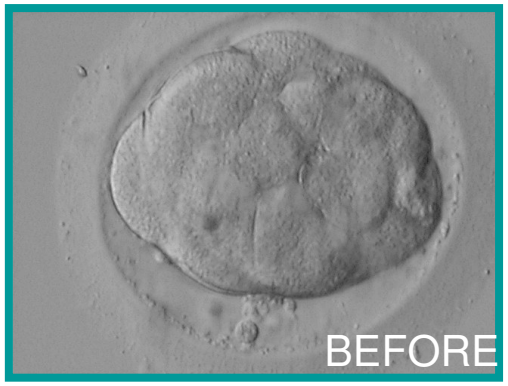


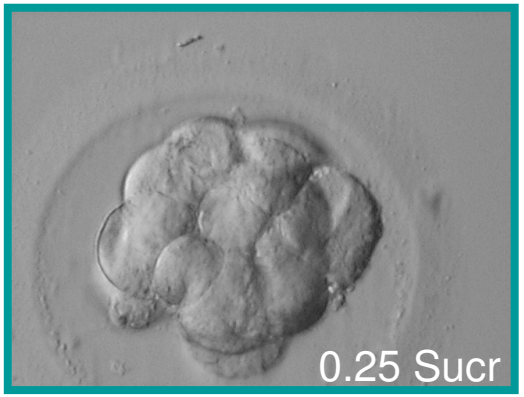


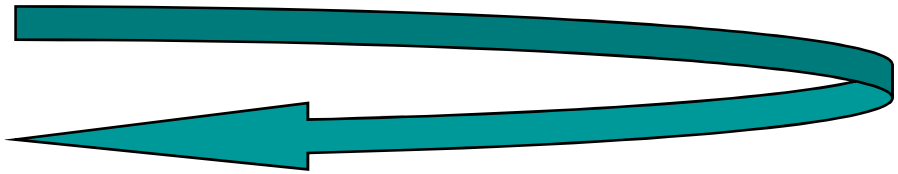


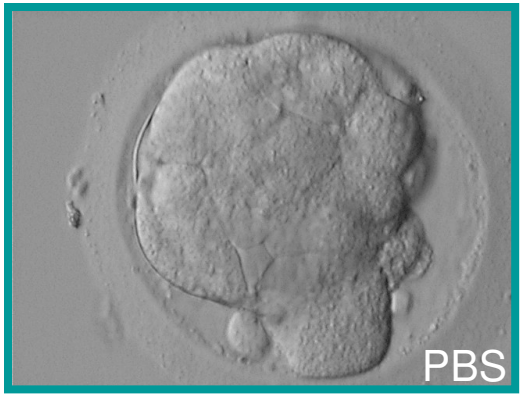
0.5 M Sucr.	3min
0.25M Sucr.	2min
0.125M Sucr.	2min
PBS/HSA	1 min
Transfermedium	until BT

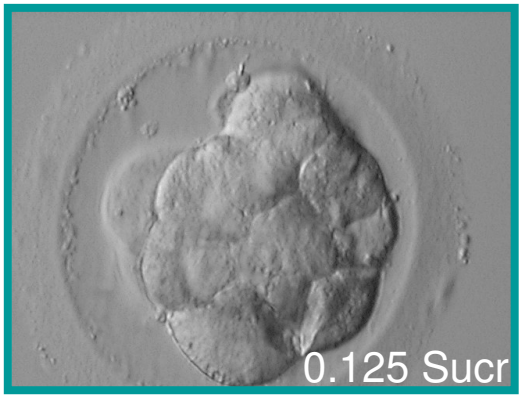
Warming



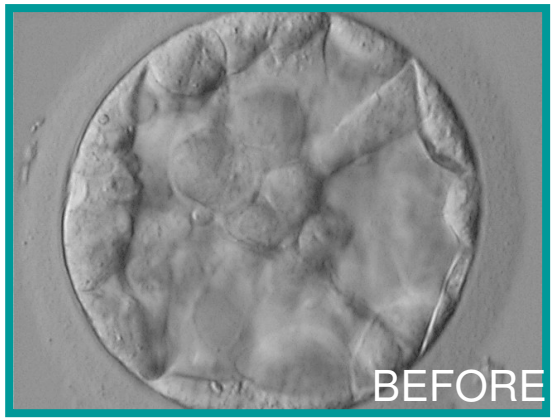




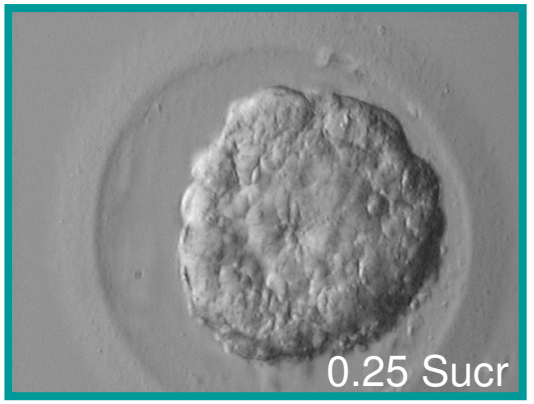


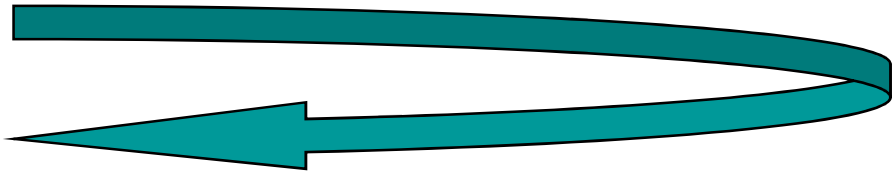


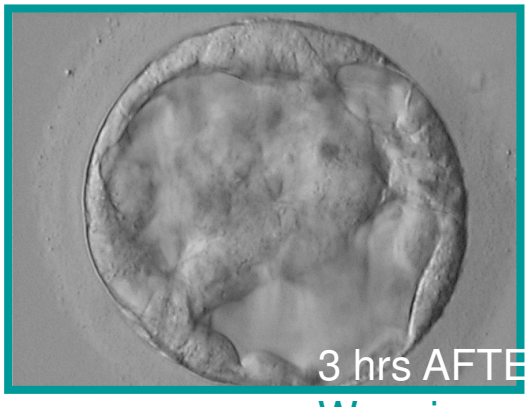
Warming

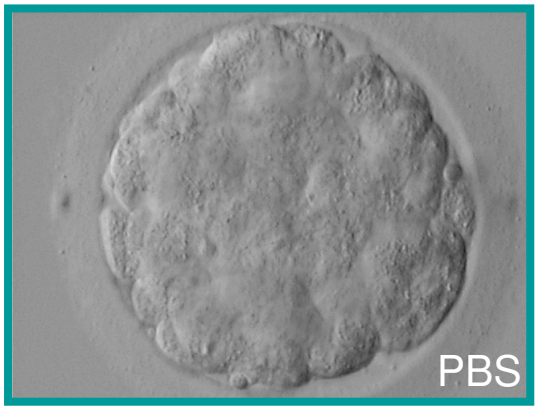

BEFORE
Vitrification


0.5 Sucr


0.25 Sucr




3 hrs AFTER
Warming


PBS


0.125 Sucr

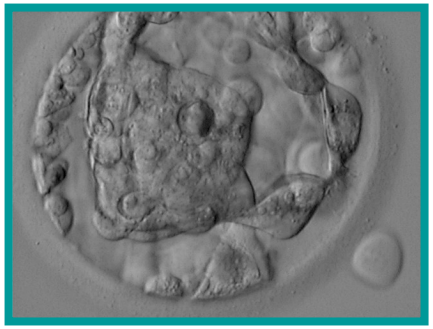
Exclusion of fragments

before removing the fragments

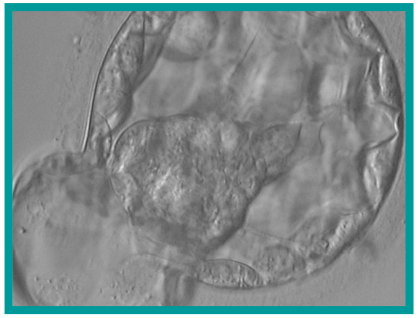
after removing the fragments

no removal of fragments

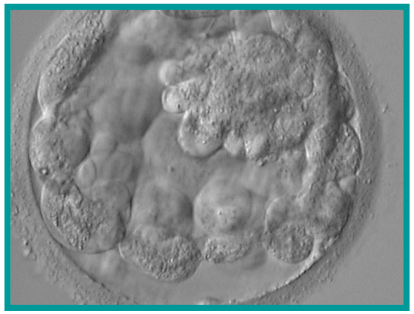
Prognostic criteria after warming



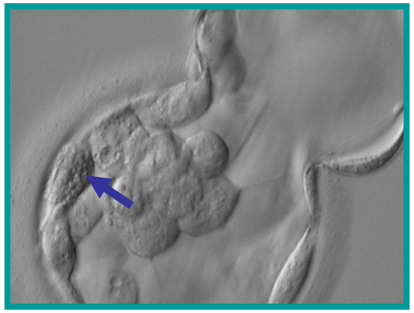
→ Re-Expansion



→ Hatching



→ Granulation



→ Necrotic foci

Ebner et al., RBM Online, 2009

Table 3. Morphological aspects of vitrified blastocysts after thawing and 2-h culture. Only single blastocyst transfers and homogeneous double blastocyst transfers were considered.

	n	β-*HCG*	*Clinical PR*	*Live birth*	*Implantation rate*
Re-expansion					
All[a]	81	45 (55.6)	39 (48.2)	38 (46.9)	53/121 (43.8)
None[a]	21	6 (28.6)	5 (23.8)	4 (19.0)	6/29 (20.7)
Precocious hatching					
All	30	21 (70.0)[b]	19 (63.3)[c]	19 (63.3)[d]	23/39 (59.0)[e]
None	77	27 (35.1)[b]	22 (28.6)[c]	22 (28.6)[d]	31/125 (24.8)[e]
Granulation					
All[f]	18	4 (22.2)	3 (16.7)	3 (16.7)	4/26 (15.4)
None[f]	102	50 (49.0)	43 (42.2)	42 (41.2)	59/156 (37.8)
Necrotic foci					
All	19	8 (42.1)	7 (36.8)	7 (36.8)	8/25 (32.0)
None	93	42 (45.2)	35 (37.6)	35 (37.6)	47/143 (32.9)

Values in parentheses are percentages.
Values with the same superscript letter are significantly different: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$; [d,e]$P < 0.0001$.
All = patients with transfer of day-5 embryos that were exclusively positive for the given phenomenon. None = patients with transfer of day-5 embryos that were exclusively negative for the given phenomenon.
HCG = human chorionic gonadotrophin; PR = pregnancy rate.

Morphology after warming
Conclusion

positive predictors

- reexpansion
- hatching
- absence of extensive cytoplasmic granulation, large necrotic areas

negative predictors

- cytoplasmic granulation, halo-like structure in the periphery of the cells,
- extensive exclusion of fragments
- large necrotic foci, > than half of the ICM

thank you for your attention