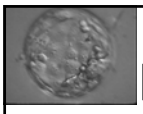

Morphological Markers of Blastocyst Quality

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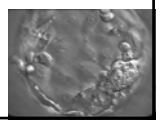

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Overview-I

- Why do we prefer to transfer embryos at the blastocyst stage?
- Outcome of blastocyst transfers
- Factors affecting the blastocyst formation & quality

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Overview-II

- What are the morphological markers of blastocyst quality?
- Blastocyst grading systems
- Clinical efficiency of current scoring systems
- Future aspects for an accurate grading system for the selection of the most implantation competent blastocyst

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Why do we prefer to transfer embryos at the blastocyst stage?

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Why an alternative for cleavage stage embryo transfer? ADVANTAGES

- 1. The embryo is transversing the fallopian tube at cleavage stage; it's in the uterus at blastocyst stage, so premature exposure of early stage embryos to the uterine environment may cause homeostatic stress on the embryo, resulting in a reduced implantation potential(Gardner 1996)
- 2. Selection of only the embryos that have demonstated the potential for continued development under embryonic genomic control (Braude 1988)
- 3. If a blastocysts is more viable than a cleavage-stage embryo than BT could result in higher IR, which gives the possibility of transferring fewer embryos that lowers the costly multiple birth rates (Jones 1999)

Johnson et al.,Best Practice&Research Clin.Obst.Gyanec. 2007

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Critics of blastocyst culture

- 1. Having no embryos to transfer(Marek 1999) (The day of patient recruitment into the BT is crucial to this argument)
- 2. Failure to have extra embryos that can be freeze-stored for future use(Tsirgotis 1998)(It's still not clear if this impacts on the final outcome of PR&LBR per started cycle)
- 3. MZ twinning(Only retrospective studies were able to show an increased frequency,Behr 2000, Da Costa 2001, Jain 2004)
- 4. Altered sex ratio in births(Menezo 1999)
- 5. Sensitivity of the system to suboptimal conditions

Johnson et al.,Best Practice&Research Clin.Obst.Gyanec. 2007

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■Clinical outcome of blastocyst transfers

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Blake et al.,Cochrane Database of Systematic Reviews 2007 CD002118

- 18 RCT comparing early stage ET (Day 2 to 3) with BT (Day 5 to 6) were included (14-published articles, 4 abstracts)
- Only patients undergoing IVF/ICSI for therapeutic reasons or for oocyte donation were included, whereas IVF/ICSI for IVM oocytes, PGD cases and co-culture methods were excluded
- 15 trials used sequential media, of which 9 used Vitrolife G1/G2 while the remaining media were combinations of brands or made in house. 3 did not state the media used
- Most studies recruited women aged <40 years with the exception of Gardner 1998 who had no age limit. The mean age across all studies varied from 29 to 34

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Cochrane Data

- Primary outcome: LBR per couple (no.of live-births per couple)
- Secondary outcome: CPR, MPR, high order MPR, miscarriage, embryo freezing, failure to have any ET rate per couple
- Outcomes not appropriate for statistical pooling: Live births per OPU and ET,CPR per OPU and ET, implantation rate

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Study groups

- **Patients with good prognosis selected (Gardner 1998, Coskun 2000, Levron 2002, Rienzi 2002, Bungum 2003, Frattarelli 2003, Papanikolaou 2005-2006)**
- **Unselected patients (Karaki 2002, Van der Auwera 2002, Emiliani 2003, Kolibianakis 2004)**
- **Poor prognosis factors with RIF, or poor response to ovulation induction (Levitas 2004)**

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Results of Meta-Analysis

- **SIGNIFICANT RESULTS**
- **LBR,CPR in favour of day BT**
- **Embryo freezing per couple, failure to transfer any embryos, cumulative pregnancy rates from fresh&frozen ET in favour of cleavage stage ET**
- **NON-SIGNIFICANT RESULTS**
- **MPR, high order multiplets (in favour of BT but not sig.), miscarriage rate, monozygotic twinning**

Early PR loss is sig. higher after day 3 single ET than day 5 single BT

	D3 (n = 603)	*D5 (n = 500)*	*OR (95%CI)*	*P-value*
Pregnancy rate (%)	209 (34.7)	221 (44.2)	1.49 (1.17–1.90)	0.001
Miscarriages <7 weeks	56	38		
Ectopic pregnancies	6	6		
Early pregnancy loss rate (%)	56/209 (26.8)	38/221 (17.2)	1.76 (1.10–2.81)	0.01
Clinical pregnancy rate (%)	148 (24.5)	177 (35.4)	1.68 (1.29–2.18)	<0.001
Twin pregnancies (%)	3 (2.0)	1 (0.6)		NS
Implantation rate (%)	151/603 (25.0)	178/500 (35.6)	1.68 (1.31–2.18)	<0.001
Only elective SET				
Pregnancy rate (%)	160/417 (38.4)	184/385 (47.8)	1.47 (1.11–1.94)	0.008
Clinical pregnancy rate (%)	116/417 (27.8)	146/385 (37.9)	1.58 (1.17–2.13)	0.002

D3 group: a single embryo was transferred on day 3 of culture. D5 group: a single blastocyst was transferred on day 5 of culture; SET = single embryo transfer; OR = odds ratio; CI = confidence interval; NS = not significant.

Patients < 36, ≤2rank trial, Equal no.of ET in each group, GnRH antogonist protocol

Papanikolaou et al.,RBM Online 2006

Single ET& BT: A prospective randomised trial

Day of ET	Day 3	Day 5	Day 3	Day 5
Embryo quality	Excellent	Excellent	Moderate	Moderate
No. of patients (%)	86 (86.9)	76 (59.4)	13 (13.1)	52 (40.6)
Age (y)	31.7 ± 2.5	31.7 ± 3.0	31.6 ± 4.3	31.9 ± 2.5
No. of IVF attempts	86	76	13	52
No. of of oocytes	902	942	131	578
No. of zygotes	625 (69.3%)	629 (66.8)	82 (62.6%)	398 (68.8%)
No. of embryos on day 3	607	610	79	368
Grades I and II (% of day 3 embryos)	299 (49.3%)	294 (48.2)	0	164 (44.6%)
Grades III and IV (% of day 3 embryos)	308 (50.7%)	316 (51.8)	79 (100%)	204 (55.4%)
No. of blastocysts (% of day 3 embryos)		279 (45.7)		91 (24.7%)
Grade A (% of day 3 embryos)		138 (22.6)		0
Grade B (% of day 3 embryos)		141 (23.1)		91 (24.7%)
No. of ETs	86	76	11	42
No. of pregnancies	28	37	3	15
No. of miscarriages	6 (21.4%)	5 (13.5%)	2 (66.6%)	4 (26.7%)
No. of ongoing pregnancies/cycles	22 (25.6%)	31 (40.8%)*	1 (7.7%)	11 (21.2%)
No. of ongoing pregnancies/ETs	22 (25.6%)	31 (40.8%)	1 (9.1%)**	11 (26.2%)**

* ; ** $P<.05$.

Patients ≤36, 1st./2nd. Attempt, ≥5 2PN
PR: 42.2% when ≥4G1, with 95.5% suitable for SBT,
PR: 27.8%when ≤3G1, with 88.5% suitable for SBT

Zech et al.,F&S 2007

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- **What are the factors affecting blastocyst formation & Quality?**

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Gamete related factors

1. Maternal factors

- **Maternal age**
- **Oocytes retrieved**

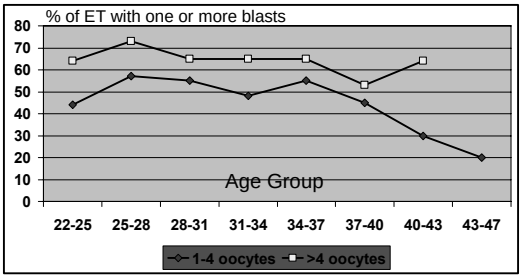
Results of blastocyst transfer in relation to age

	< 40	> 40
Prog to blast(%)	40.5	22.2*
Pregnancy/ET(%)	44.6	21.1**
Imp/emb(%)	19.9	8.9**
Cancelled ET(%)	11.6	38.7***

*p<.001,**p<.01,***p<.05

Pantos *et al.* Fertil Steril 1999

Blast formation according to patient age and retrieved oocytes



Scholtes and Zeilmaker Fertil Steril 1998

Effect of maternal age of recipient when receiving blastocysts

	< 35 years	35-39	>39
# recepients	8	27	78
# blasts txf	2.0	2.1	2.1
Implantation (fetal heart)	68.8%	64.3%	63.6%
CPR/ET	87.5	88.9	88.5

Schoolcraft and Gardner Fertil Steril 2000

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Gamete Related Factors

2. Paternal factors

- **Origin of the spermatozoa used for insemination (Ejaculated vs epididymal vs testicular spermatozoa**
- **Maturation stage of the sperm cell (Spermatids)**

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Blasts from ejaculated, epididymal, and testicular spermatozoa

	Ejaculated sperm	Epididymal sperm (OA)	Testicular sperm (OA)	Testicular sperm (NOA)
MII oocytes	4478	291	239	416
Fertilization(%)	76.8*	71.8	70.2	60.1*
Blasts %	62.5**	52.6	49	40**
Blasts on D5(%)	92*	76	77.9	61.7*
G1+G2 Blasts(%)	81	84	81.8	71.2

*p<0.05,**p<0.01

Balaban *et al.* Hum Reprod 2001

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Progression to blastocyst of ROSI embryos

	Testicular Sperm	Testicular RS
No of MII oocytes	556	356
Fertilization(%)	74.5*	56*
Embryos observed	153	141
Blastocysts (%)	51*	20*
BG1+BG2 blastocysts(%)	75.3	0
Blast formation on day 5(%)	58.4	0
Hatched blasts (%)	32	0

*P<0.05

Balaban *et al.* Hum Reprod 2000

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Procedural factors

- IVF/ICSI

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Blastocyst formation from spare IVF and ICSI embryos

	ICSI	IVF	P
Embryos cultured	446	748	
Mean spare emb/cycle	4.6	7.4	<0.001
Blastocysts %	8.9%	23.5%	<0.001
Hatched blastocysts	20%	39%	<0.05

Griffiths *et al.* Hum Reprod 2000

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Development to the blastocyst stage of ICSI vs IVF embryos

Treatment Procedure	IVF	ICSI
Cycles with at least one surplus emb cultured	274	429
Total number of embryos	1253	1622
Surplus embryo/cycle	4.57	3.88
Incidence of blast formation/cycle	31.8%	23.0%*

* p<0.001
Dumuolin *et al.* Hum Reprod 2000

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Indirect factors

- **Culture conditions**

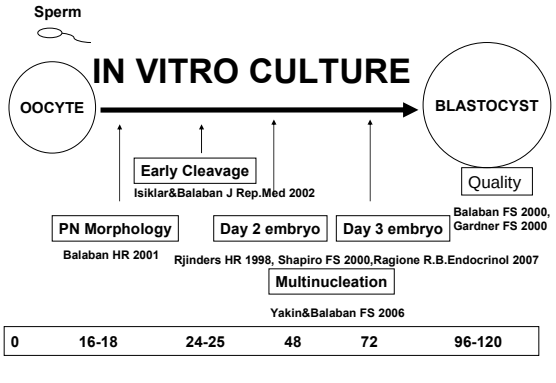
The effect of culture media on embryo quality

Table 2. Outcome of fresh embryo transfer cycles according to culture media and day of embryo transfer.

	Day 3 embryo transfer		*Day 5 embryo transfer*	
Culture media	GIII	G1.2–G2.2	GIII	G1.2–G2.2
No. transfer cycles	199	201	36	37
Mean no. oocytes (range)	9.8 (1–20)	10.4 (1–20)	10.7 (5–16)	10.8 (5–18)
2PN fertilization (%)	1432 (72.9)	1492 (71.1)	295 (74.4)	293 (73.0)
Grade 1 and 2 embryo on day 3 (%)[a]	885 (63)	746 (50.9)	185 (64)	149 (51.7)
8-cell embryo on day 3 (%)[a]	606 (43.1)	314 (21.4)	128 (44.2)	65 (22.5)
Blastocyst formation (%)[b]	–	–	189 (65.3)	137 (47.5)
Grade 1 and 2 blastocysts (%)	–	–	118 (62.4)	67 (48.9)
Hatching blastocysts (%)[b]	–	–	79 (42.7)	39 (28.4)
No. embryos transferred	607	664	82	93
Mean no. embryos transferred (range)	3.0 (1–4)	3.3 (1–4)	2.2 (1–3)	2.5 (1–3)
Clinical pregnancy per embryo transfer (%)[c]	100/199 (50.3)	76/201 (37.8)	25/36 (69.4)	19/37 (51.4)
Implantation per embryo transferred (%)[d]	156/607 (25.7)	96/664 (14.5)	37/82 (45.1)	27/93 (29.0)
Multiple pregnancies (%)	46/100 (46.0)	25/76 (32.9)	12/25 (48.0)	7/19 (36.8)
Singletons/twins/triplets	54/36/10	51/20/5	13/12/0	12/6/1

[a]GIII significantly different from G1.2–G2.2 for both day 3 and day 5 transfers ($P < 0.05$).
[b]GIII significantly different from G1.2–G2.2 for day 5 transfers ($P < 0.05$).
[c]GIII significantly different from G1.2–G2.2 for day 3 transfers ($P < 0.01$).
[d]GIII significantly different from G1.2–G2.2 for both day 3 ($P < 0.01$) and day 5 transfers ($P < 0.05$).

Balaban et al.,RBM Online 2005



Blastocyst formation rates

Bungum 2003	55.2%	Levitas 2004	43%
Coskun 2000	28%	Levron 2002	34.2%
Devreker 2000	Not stated		
Emiliani 2003	48%	Livingstone 2002	Not stated
Frattarelli 2003	Not stated	Motta 1998	Not stated
Gardner 1998	46.5%	Papanikolaou 2005	Not stated
Hreinsson 2004	33%	Papanikolaou 2006	Not stated
Karaki 2002	33%	Rienzi 2002	44.8%
Kolibiankis 2004	50.7%	Schillaci 2002	60.3%
		Van der Auwera	44.7%

Blake et al.,Cochrane Review 2007

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- **What are the morphological markers of blastocyst quality?**
  - ** Cell charecteristics
  - ** Developmental speed
- **Blastocyst grading systems**

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Expansion & Cell charecteristics

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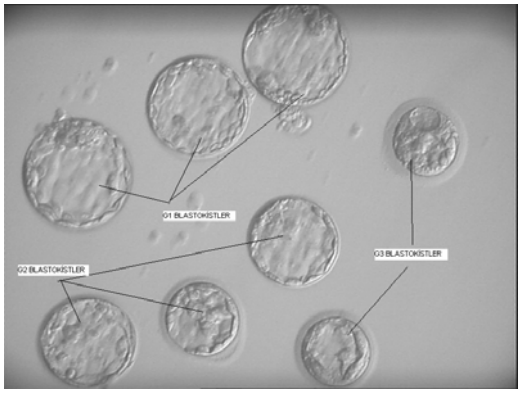
Blastocyst Grading

- **BG1**
  early cavitation resulting in an eccentric and then expanded cavity lined by a distinct ICM region and TE layer
- **BG2**
  delayed initial cavitation exhibiting a transitional phase between early cavitation and expansion
- **BG3**
  blastocysts with several degenative foci in the ICM; cells appear dark and necrotic

Docras, Hum Reprod 1993

Blastocyst quality



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Outcome of homogenous blastocyst transfers

Variable	Only G1 blastocysts	Only G2 blastocysts	Only G3 blastocysts
Cycles	32	47	98
Blasts txf Mean	96 (3)	155 (3.2)	392 (4)
CPR / ET	22/23 (68.7%)	29/47 (61.7%)	13/98 (13.3%)
Implantation / embryo	56.2%	46.4%	7.1%
Multiples / Total pregnancies	90.9%	68.9%	15.3%

Balaban, Fertil Steril, 2000

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3 Part Scoring system

- 1. Blastocyst expansion
- 2. ICM morphology
- 3. Trophoectoderm morphology

Gardner and Schoolcraft, 1999

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1. Blastocyst expansion

- Blastocysts scored(1-6) based on their degree of expansion and hatching status

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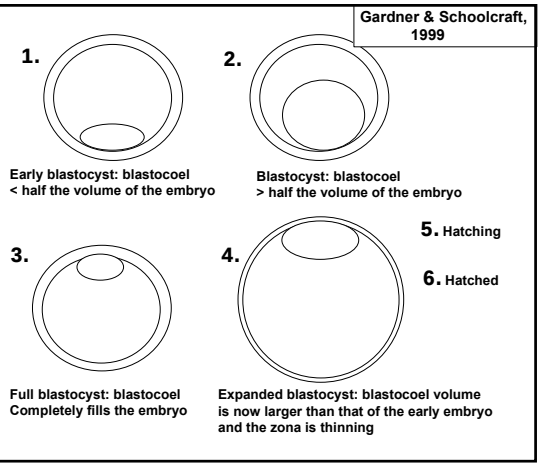

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2. ICM & Trophoectoderm Quality

For blastocysts graded as 3-6(full blastocyst onward)

ICM Grading

A) Tightly packed, many cells
B) Loosely grouped, several cells
C) Very few cells

Trophectoderm Grading

A) Many cells forming a cohesive epithelium
B) Few cells forming a loose epithelium
c) Very few large cells

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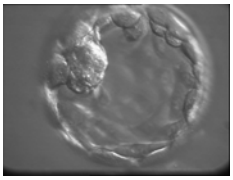
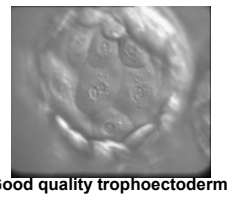
Blastocyst grading

2 x 4AA

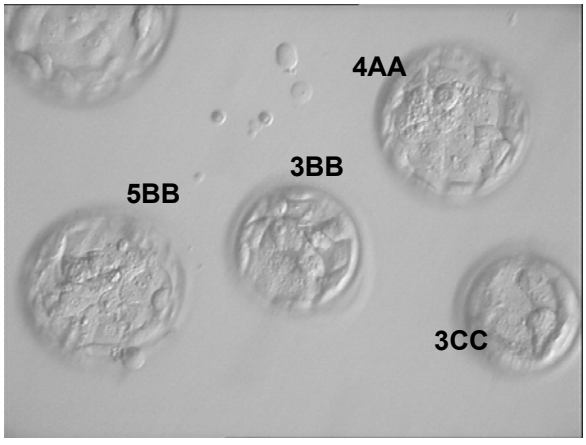


Good quality ICM



Good quality trophoectoderm

5BB



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Effect of blastocyst score on pregnancy

	2 blasts > 3 AA	1 blast > 3 AA	Blasts < 3AA
# embryos txf	2	2	2
Mean Age	32.9	33.3	33.3
# of transfers	68	23	16
Blast devel. From 2 PN(%)	57*	46.5**	33.3
Implantation/ embryo(%)	69.9*	50.0	28.1
Clinical PR(%)	86.8*	69.6	43.8

*p<.001,**p<.01

Gardner et al. Fertil Steril 2000

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Blastocyst TROPHOECTODERM QUALITY is the most important predictor of Implantation

	Blastocyst expansion & IR(%)	ICM Quality & IR(%)	Trophoectoderm Quality & IR(%)
Fresh cycles:156	3: 67 2: 58 1: 53	A: 68 B: 62 C: 61	A: 76** B: 56 C: 50
Correlation of cryosurvival of thawed blastocysts	NS	NS	A: 85 B: 63 C: 62

*p<.05,Gardner 3-part scoring system,NS-Not sig.

IR of a 3AA is 70%!!

Zaninovic et al.,F&S Vol:76,Suppl.1, 2001

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Blastocyst ICM QUALITY is the most important predictor of Implantation

	ICM Grade A	ICM Grade B	ICM Grade C
No.of cycles	72	83	32
Mean Age	35.0	35.4	32.8
CPR%*	41.7	24.1	6.3
IR%*	41.7	24.1	6.3

Gardner 3-part grading, Retrospective. SET

Marek et al., F&S Vol:82,Suppl.2 2004

Blastocyst Qualification

Developmental Event	Day 4	Day 5
Compaction		
Complete	N	S
Incomplete	N	S
Regional	S	S
Fusion-like	S	S
None	S	A
Cavitation		
Single cavity	*	N
Beginning	N	S
Large vacuoles	A	A
Multiple vacuoles	A	A
Inner cell mass formation		
Distinct/organized	*	N
Forming	*	S
Large cells	*	S
Small mass	*	S
None	*	A
Trophoectoderm organization		
Cohesive	*	N
Large cells	*	S
Irregular	*	A

* Not expected on this day, N=normal, S=suboptimal, A=abnormal

102 BT,CPR:67%, 49%IR **Alikani et al.,HR 2000**

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Quantative Grading of Human Blastocyst

- **Blastocyst diameter (400X-from outer zona to outer zona, ranged from 155-265µms)**
- **Trophoectoderm cell numbers (cross-sectional circumference of exp.blasts.ranged form 4-20)**
- **ICM SIZE (longest length and widest perpendicular width of each ICM-ranged from 1050-15.000 µm²)**
- **ICM SHAPE (Roundness index:-length divided by width. Round(1), slightly oval more elongated-ranged from 1-2.4)**

Richter et al.,F&S 2001

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Quantative Grading of Human Blastocyst

- **Blastocyst diameter (400X-from outer zona to outer zona, ranged from 155-265µms)**

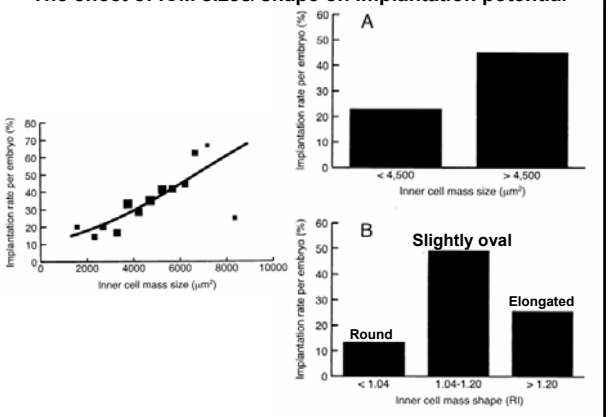
****NO EFFECT: The mean diameter of exp.blasts.identical between implanting and non-implanting blastocysts(195µm vs. 194µm,p:.81)**

- **Trophoectoderm cell numbers (cross-sectional circumference of exp.blasts.ranged form 4-20)**

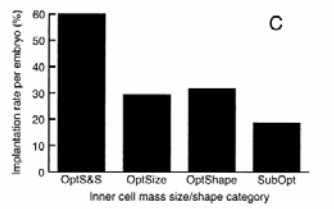
****NO EFFECT: The no.of cells in across-sectional circumference identical between implanting and non-implanting blastocysts(11.0 vs. 10.8,p:.64)**

Richter et al.,F&S 2001

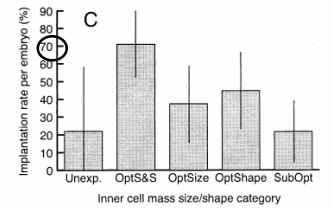
The effect of ICM size& shape on implantation potential



Richter et al.,F&S 2001



Day 5 & 6 blastocysts



DAY 5
TOP QUALITY
BLASTOCYST

Richter et al.,F&S 2001

Grading System for Optimal & Suboptimal Blastocysts

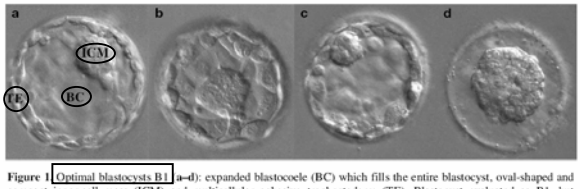


Figure 1 Optimal blastocysts B1 **(a–d)**: expanded blastocoele (BC) which fills the entire blastocyst, oval-shaped and compact inner-cell mass (ICM) and multicellular cohesive trophectoderm (TE). Blastocyst evaluated as B1, but collapsed during evaluation **(d)**.

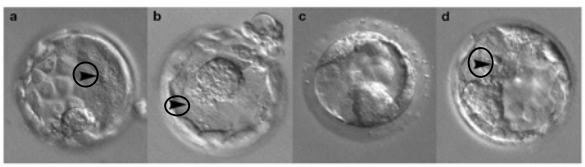


Figure 2. Blastocysts with non-optimal trophectoderm B2: expanded blastocysts with normal oval-shaped ICM but with necrotic foci (arrows) in TE **(a, b)**, or with completely necrotic TE **(c)**, or with fragments (arrow) among TE cells **(d)**.

Kovacic et al.,RBM Online 2004

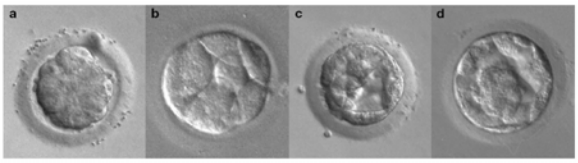


Figure 3. Unexpanded blastocysts and morulae B3: compact morulae (**a**) or unexpanded early blastocysts with beginning of cavitation (**b-d**).

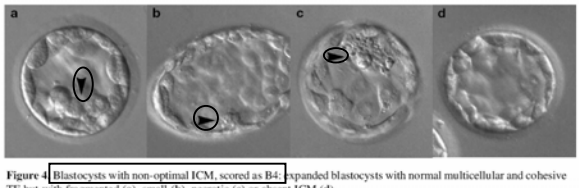


Figure 4. Blastocysts with non-optimal ICM, scored as B4: expanded blastocysts with normal multicellular and cohesive TE but with fragmented (**a**), small (**b**), necrotic (**c**) or absent ICM (**d**).

Kovacic et al.,RBM Online 2004

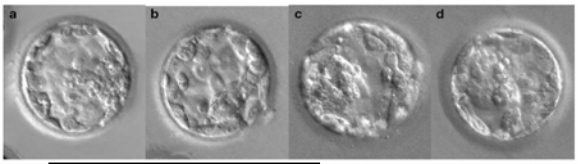


Figure 5. Blastocysts with non-optimal ICM and TE, scored as B5: expanded blastocysts with fragmented ICM and with fragments in TE (**a-d**).

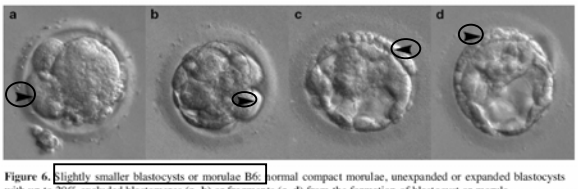


Figure 6. Slightly smaller blastocysts or morulae B6: normal compact morulae, unexpanded or expanded blastocysts with up to 20% excluded blastomeres (**a**, **b**) or fragments (**c**, **d**) from the formation of blastocyst or morula.

Kovacic et al.,RBM Online 2004

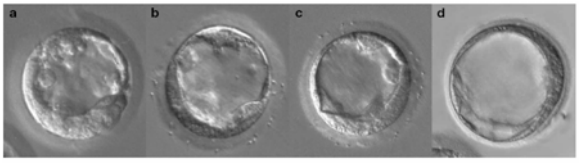


Figure 7. Necrotic blastocysts B7: unexpanded or expanded blastocysts without ICM represent a large necrotic area (**a–d**). The central fluid-filled structure can be either a blastocoele or a vacuole.

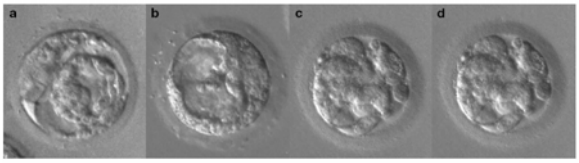


Figure 8. Small blastocysts B8: less than 80% of embryonic mass transformed into compact morulae or blastocysts, mostly with vacuoles, fragmentations or necrosis (**a–d**).

Kovacic et al.,RBM Online 2004

Table 2. Implantation rate, miscarriage rate and live birth rate per transferred blastocyst of specific morphological type. For explanation of morphological types of blastocysts, see text.

Morphological type of blastocyst	*Transferred blastocysts*	*Transferred blastocysts with known outcome (%)*	*Implantations (%)[a]*	*Miscarriages (%)*	*Births (%)[b]*
Optimal					
B1	766	706	366 (51.8)	47 (12.8)	319 (45.2)[c]
Suboptimal					
B2	71	61	22 (36.1)	2 (9.1)	20 (32.8)
B3	178	145	44 (30.3)	5 (11.4)	39 (26.9)
B4	111	87	25 (28.7)	5 (20)	20 (23)
B5	73	62	16 (25.8)	5 (31.3)	11 (17.7)
B6	87	72	17 (23.6)	5 (29.4)	12 (16.7)
B7	26	26	3 (11.5)	1 (33.3)	2 (7.7)
B8	84	82	6 (7.3)	5 (83.3)	1 (1.2)
Total	1396	1241 (88.9)	527 (41.3)	75 (14.2)	424 (34.2)

[a] $P < 0.0001$: logistic regression for evaluation of the relationship between blastocyst morphology and implantation.
[b] $P < 0.0001$: logistic regression for evaluation of the relationship between blastocyst morphology and live birth rate.
[c] $P < 0.0001$: optimal (B1) versus suboptimal (B2–B8) blastocysts.

Kovacic et al.,RBM Online 2004

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Comparison of two blastocyst grading systems

	Docras system			Gardner system		
	2 Blasts BG1/BG2	1 Blast BG1/BG2	All blasts BG3	2 blasts >3AA	1 blast >3AA	All blasts < 3AA
Blastocyst%	54.1	50.0	45.3	58.4	46.2	38.7
No. ET	2.2	2.2	3.3	2.1	2.4	3.5
CPR (%)	70.0	60.8	18.8	86.4	72.5	37.5
IR (%)	43.2	30.2	5.8	64.5	44.2	12.8
MPR (%)	35.8	29.5	0	52.7	42.9	16.7

Balaban et al.,F&S 2006

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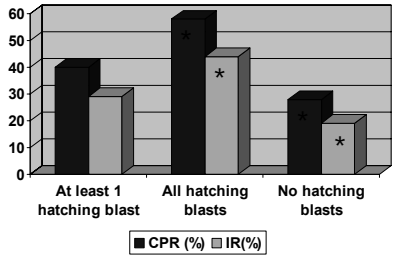
Developmental speed of the blastocyst

V K V A M E R I C A N

Outcome of blast transfer according to blast hatching



Balaban et al. Fertil Steril 2000

Lower PR&IR is obtained with late developing blastocysts on day 6

	Day 5 transfer		Day 6 transfer	
	All expanded[a]	Mixed (56% exp.)[b]	All expanded[a]	Mixed (58% exp.)[b]
Number of cycles	96	22	55	10
Number of embryos[c]	2.4 ± 0.9	3.1 ± 0.7	2.1 ± 1.0	3.1 ± 0.7
Implantation (%)	37.4	31.4	20.6	10.0
Pregnancy[d]	56/96 (58.3)	14/22 (63.6)	19/55 (34.5)	2/10 (20.0)
Multiple pregnancy[d]	31/56 (55.4)	6/14 (42.8)	4/19 (21.0)	1/2 (50.0)
Triplet pregnancy[d]	9/56 (16.1)	2/14 (14.3)	0/19 (0.0)	0/2 (0.0)

[a] Day 5 transfers of all expanded blastocysts resulted in significantly higher implantation ($P = .0038$) and pregnancy rates ($P = .0049$) compared with day 6 transfers of all expanded embryos.
[b] Day 5 transfers of mixed cohorts resulted in significantly higher implantation ($P = .042$) and pregnancy rates ($P = .022$) compared to day 6 transfers of mixed cohorts.
[c] Values are means ± one standard deviation. Fewer embryos were transferred in all expanded cohorts compared to mixed cohorts for both day 5 ($P = .017$) and day 6 ($P = .0038$) transfers.
[d] Values are proportions, with percentages in parentheses.

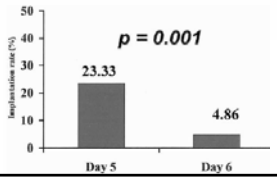
Shapiro et al.,F&S 2001

Higher IR with day 5 blastocysts

Variable	Day 5 transfer group	Day 6 transfer group	*P* value
Number of patients	73	63	
Age (y)[a]	34.1 ± 3.4	33.5 ± 3.9	NS
No. of oocytes[a]	14.3 ± 5.4	14.0 ± 7.0	NS
No of cleavage embryos[a]	8.71 ± 4.62	7.89 ± 4.38	NS
Fertilization (%)[a]	61.9 ± 18.2	56.3 ± 15.5	NS
Blastocyst number[a]	3.0 ± 1.8	2.1 ± 1.1	.003
Blastocyst formation (%)[a]	46.3 ± 19.1	32.9 ± 20.7	.002
Number transferred[a]	3.0 ± 1.1	1.9 ± 1.0	.002
Implantation rate (%)[b]	23.33 [22.1]	4.86 [3.6]	.001
Pregnancy rate (%)[c]	38.36 (28/73)	11.11 (7/63)	.001
Twinning rate (%)	17.86 (5/28)	0 (0/7)	

[a]Means ± one standard deviation.
[b]Means (medians in parentheses).
[c]Proportions (percentages in parentheses).

Retrospective clinical study,
Day 5 CPR triple as high as day 6,
Day 5 IR five times higher than day 6



Barrenetxea et al.,F&S 2005

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Future aspects for an accurate grading system for the selection of the <u>most implantation competent blastocyst</u>

- **Other markers with/without using morphological evaluation??**

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- **Metabolic markers**

** Measurement of glucose consumption&lactate production(Gardner&Lane 1996)
** BhCG,HLA-G, SP1 measurements(Saith 1996, Jurisicova et al,1999)
** sHLA-G in culture media(Fuzzi 2002, Sher 2004,2005, Noci 2005)

- **Genetical Markers**

** PGD for aneoploidy screening-Trophoectoderm biopsy. High levels of mosaicism and long duration of an in depth chromosome analysis(Magli 2000, DerHaag 2003)
** PGD for specific gene mutations(Kokkali 2005)

- **Epigenetic Markers**

** Gene expression profile in the ICM and trophoblast(Dreesen 2002,)