

Optimized scoring at blastocyst stage

ESHRE SIG Embryology Course, Practical aspects of non-invasive selection of gametes, embryos and blastocysts in a modern IVF laboratory, Salzburg, 2011.

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History

- **Benefits of blastocyst culture and ET**
 - Improve synchronization of embryo and uterine development
 - Provide a mechanism of self-selection of the viable embryos
 - after activation of the embryonic genome
 - in human: between 4 – 8 cell stage

Introduction

ART success: birth of one healthy child

Transfer of one embryo: SET and sFRET

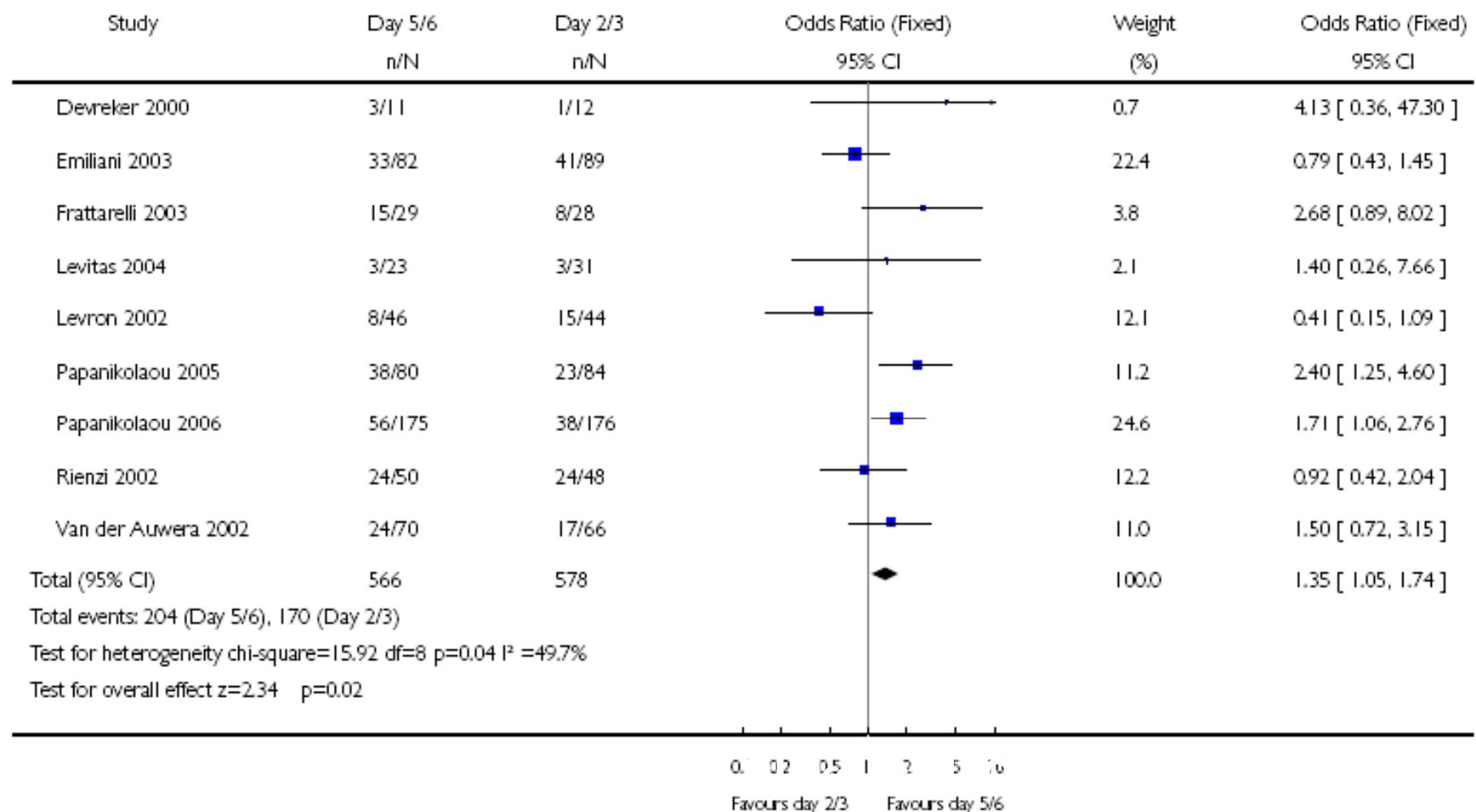
The Road for Single Embryo Transfer = blastocyst transfer?

Day of embryo transfer
Benefit of blastocyst transfer

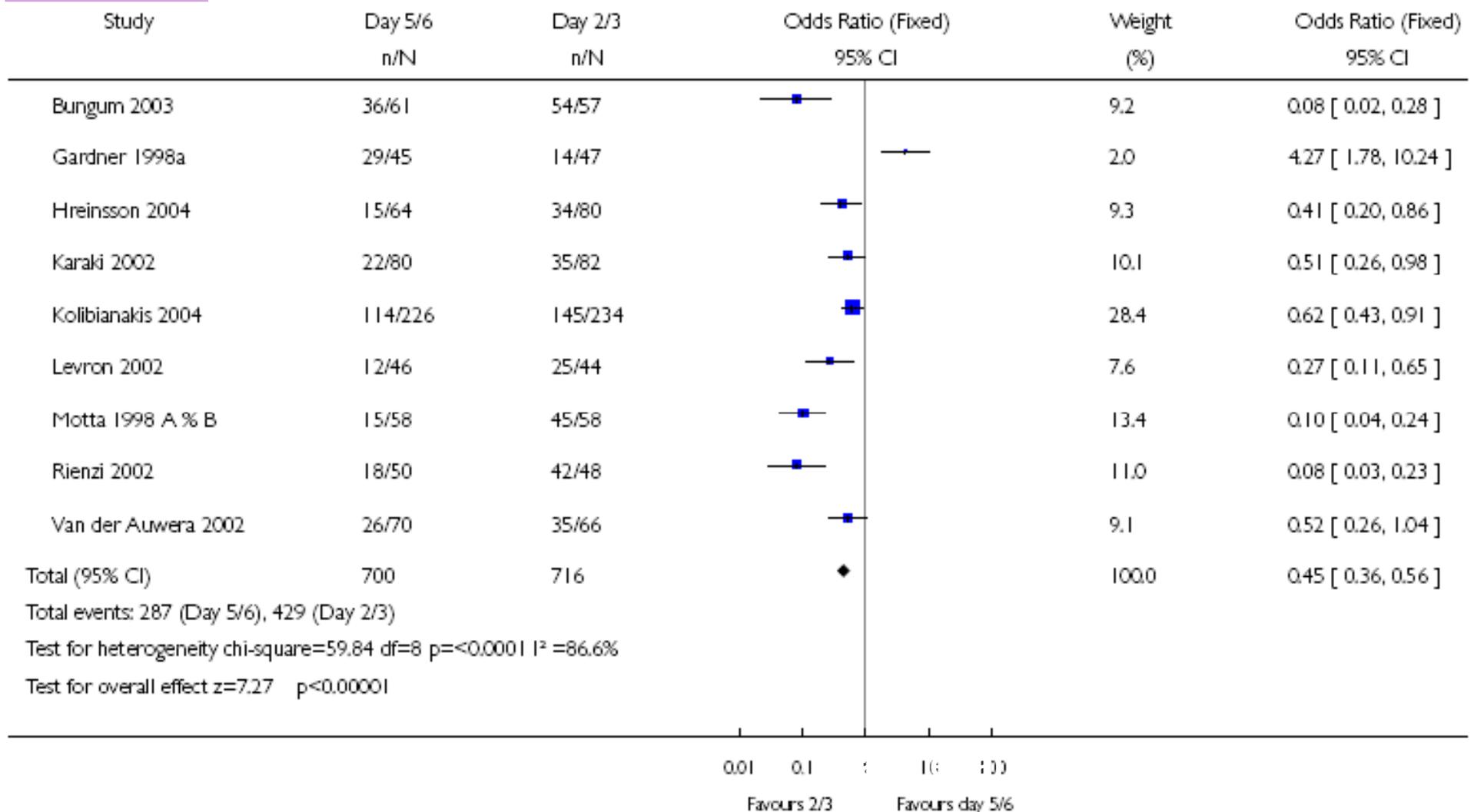
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Subject of debate

Live birth rate per couple: Cleavage & Blastocyst transfer



Embryo freezing per couple



Considerations

- ➔ Patient populations?
- ➔ Increased incidence of failed embryo transfer
- ➔ Reduced proportion of embryos for freezing
- ➔ Time of recruitment and allocation?
- ➔ Increased incidence of monozygotic twinning?
- ➔ Sensitivity of blastocyst culture to suboptimal conditions?

Considerations

- Different study variables may influence the success of day 2/3 versus day 5 embryo transfer
- In selected patient populations
 - Higher pregnancy and delivery rates per started cycle after day 5 ET
 - No lower transfer rate
 - Lower freezing rate
- The contribution of frozen ET cycles cannot be disregarded!
 - Mostly not included in literature reports

Considerations

- Higher cost **related to blastocyst culture – transfer**
 - Additional incubators
 - Additional culture media
 - More laboratory staff members
 - **Expertise in blastocyst quality assessment**
 - Expertise in blastocyst cryopreservation protocols
 - Optimal laboratory conditions

- Cost – benefit analysis ?

Assessment and grading blastocysts

- **Gardner scoring**
- Considerations with Gardner scoring
- Alternatives to Gardner scoring
- Evaluation of cryopreserved morulae/blastocysts
- In vitro culture and morulae/blastocysts
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- **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 degenerative foci in the ICM; cells appear dark and necrotic

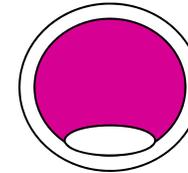
Docras, Hum Reprod 1993

BLASTOCYST MORPHOLOGY

✦ Expansion and hatching status

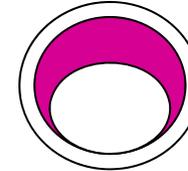
1 Blastocoel cavity less than half the volume of the embryo

1



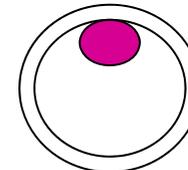
2 Blastocoel cavity more than half the volume of the embryo

2



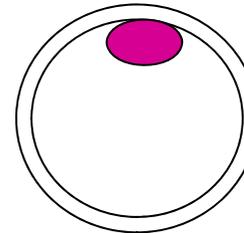
3 Full blastocyst, cavity completely filling the embryo

3



4 Expanded blastocyst, cavity larger than the embryo, with thinning of the shell

4



5 Hatching out of the shell

5 Hatching

6 Hatched out of the shell

6 Hatched

BLASTOCYST MORPHOLOGY

Inner cell mass (ICM) score

- A** Many cells, tightly packed
- B** Several cells, loosely grouped
- C** Very few cells



Trophectoderm (TE) score

- A** Many cells, forming a cohesive layer
- B** Few cells, forming a loose epithelium
- C** Very few large cells



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

| | 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 |

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Some morphologic characteristics of blastocysts with impact on implantation

- ➔ Location of herniation

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

| | Study group hatching 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 (11.8) |
| IR | 26/39 (66.7) ^{a,b} | 21/52 (40.4) ^b | 31/76 (40.8) ^a |
| ^a $p=0.009$; ^b $p=0.01$ | | | |

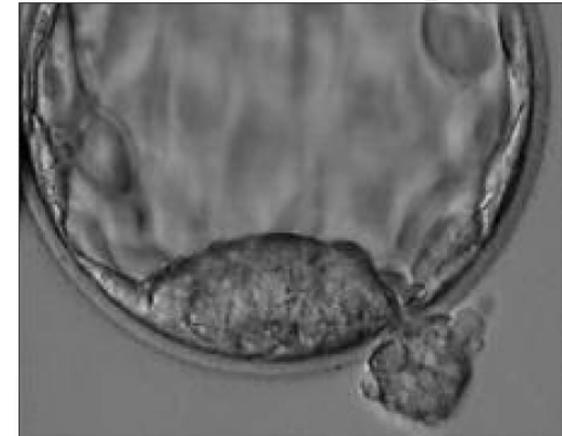


Figure 1. Expanded blastocyst hatching from the embryonic pole.

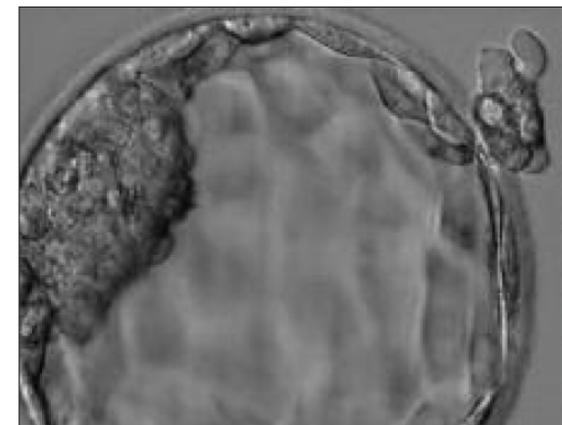
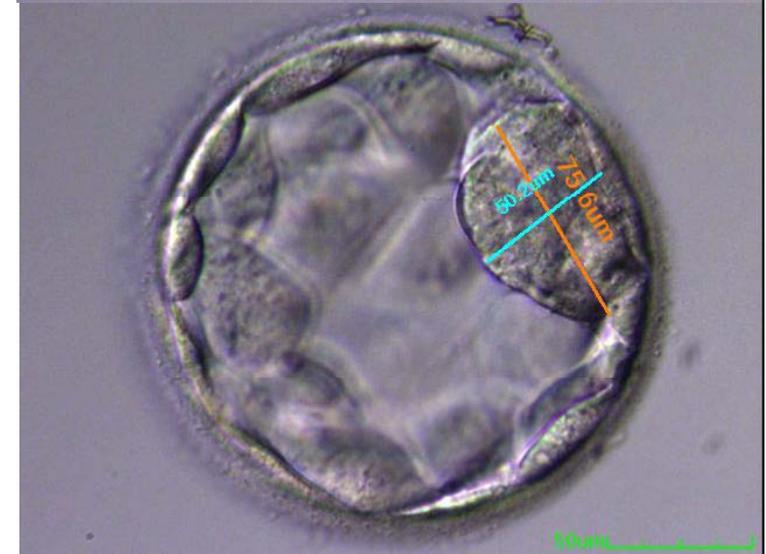
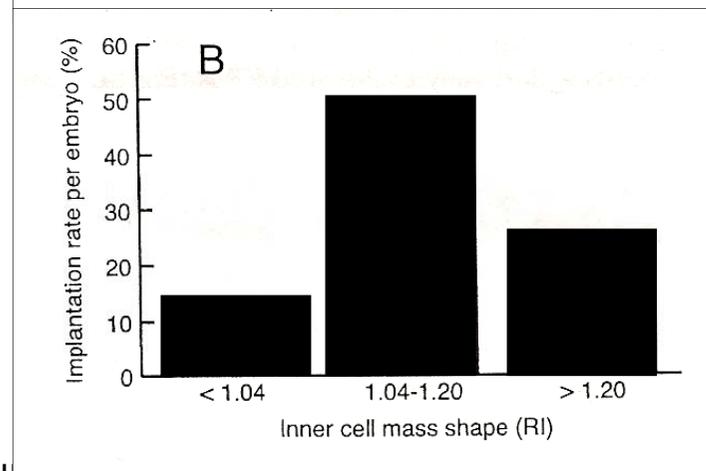
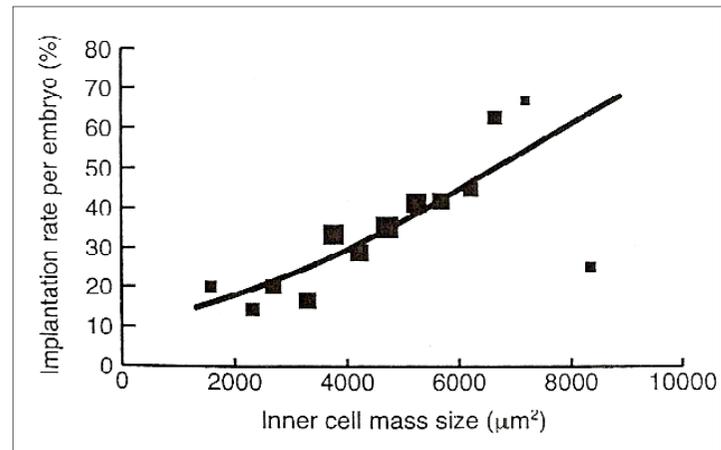
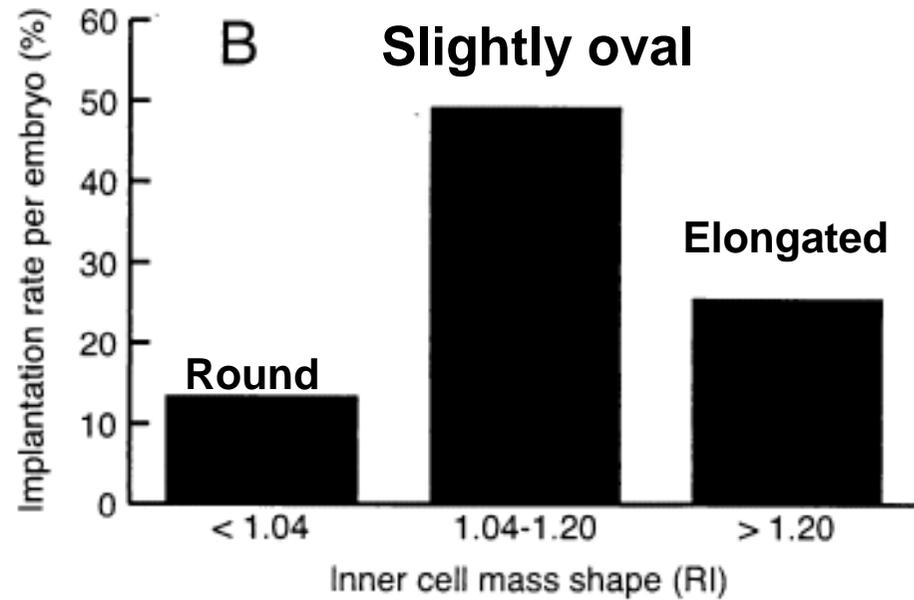
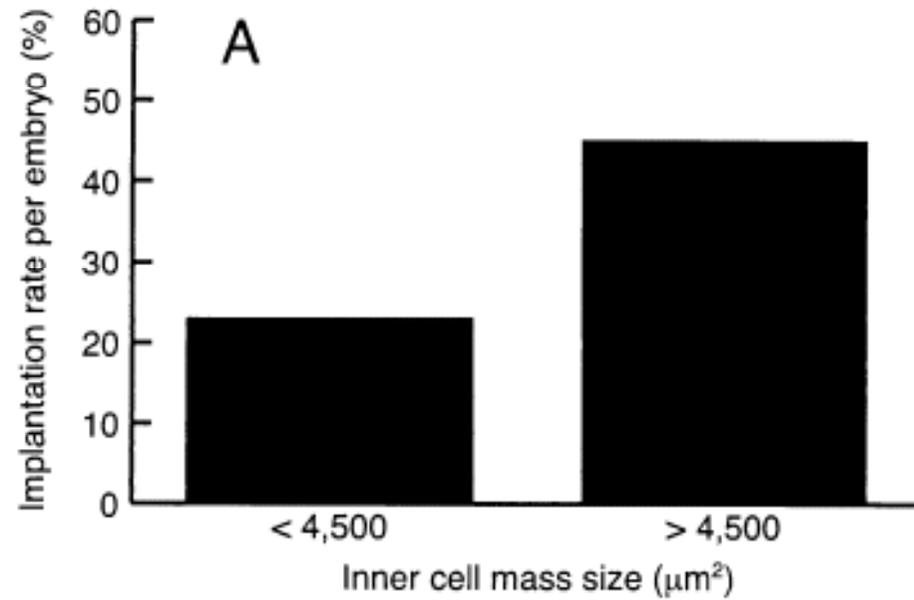
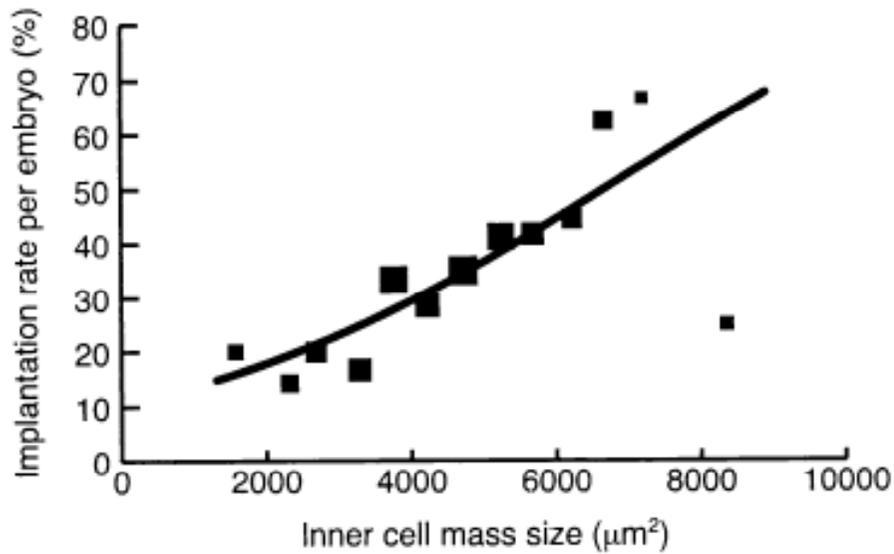


Figure 2. Blastocyst hatching opposite the inner cell mass.

Blastocyst evaluation by ICM morphometry

Richter et al., 2001





Other morphologic characteristics of blastocysts

- Cytoplasmic strings (*Scott, 2000*)
- Vacuoles (*Ebner et al., 2005*)
- Included blastomeres
- Developing blastocysts that appears to possess two separately cavitating segments

- *Correlates negatively with implantation*



Other morphologic characteristics of blastocysts

Early blastocysts



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Other morphologic characteristics of blastocysts



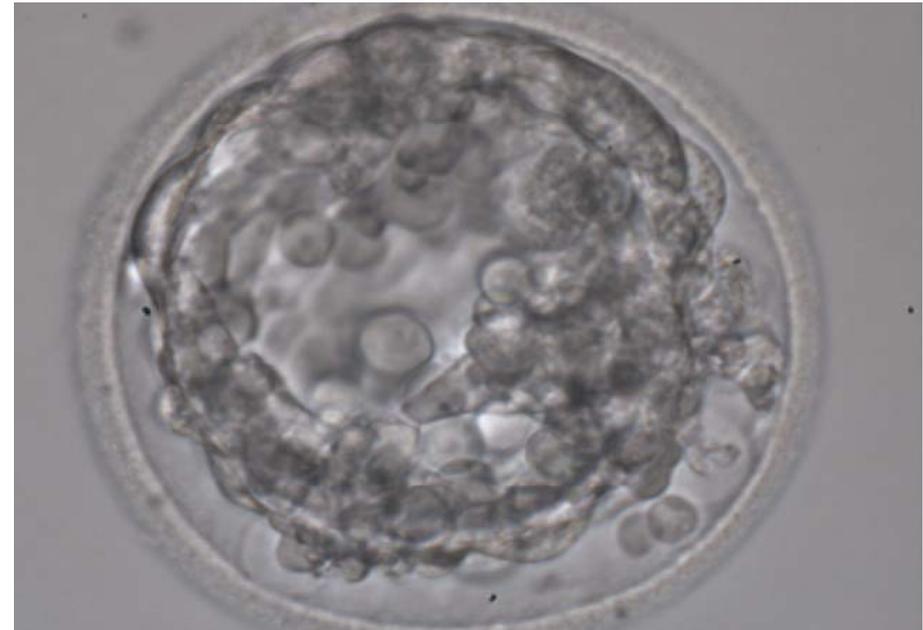
Other morphologic characteristics of blastocysts

No ICM



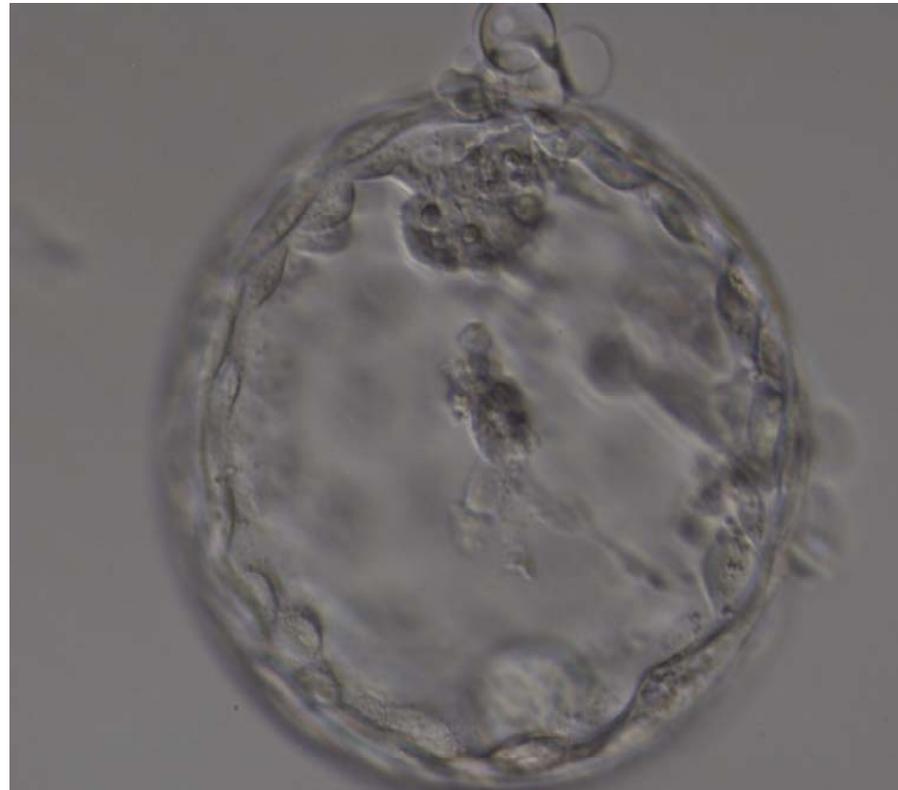
Other morphologic characteristics of blastocysts

**Collaps(ing)ed
expanded
blastocysts**



Other morphologic characteristics of blastocysts

Hatching?



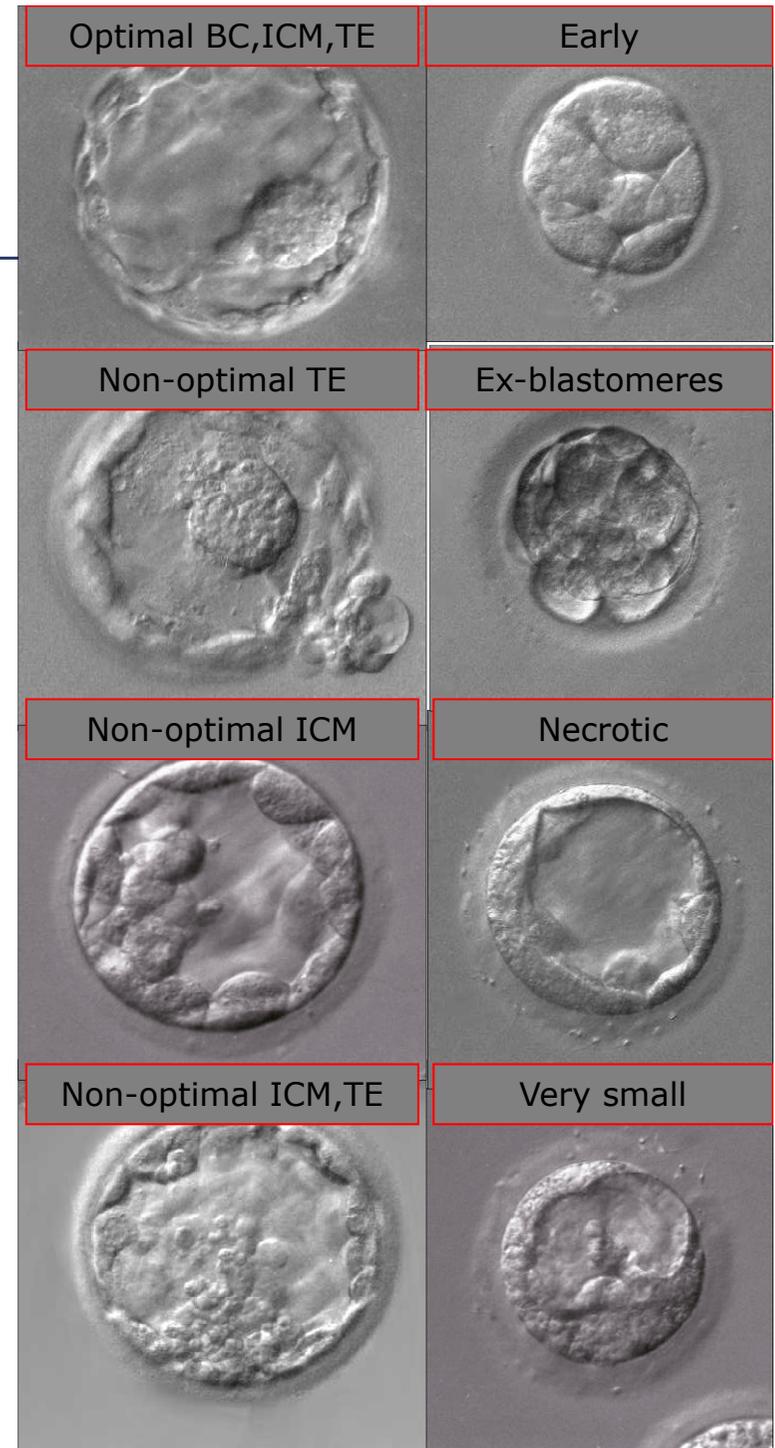
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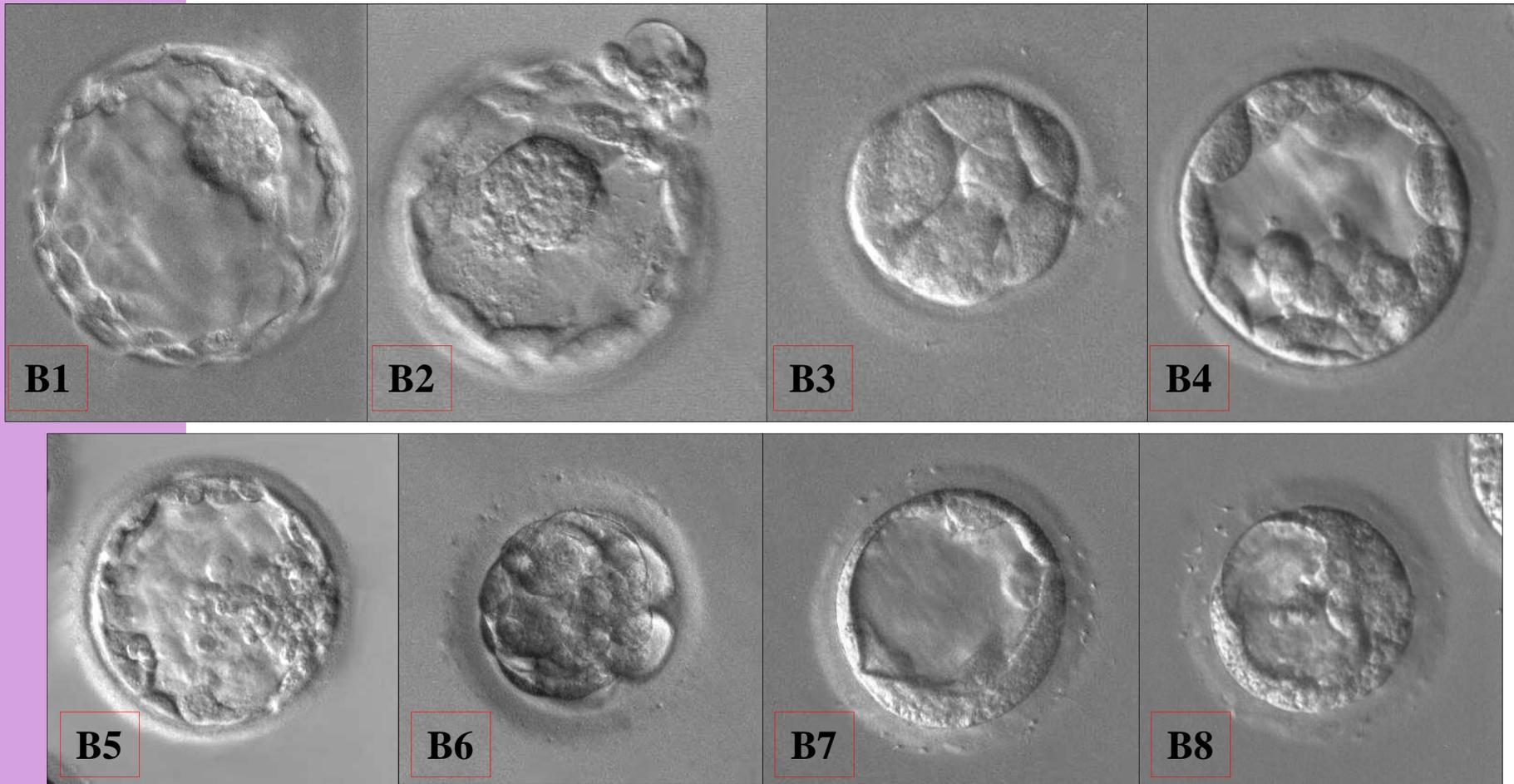
Blastocyst grading system

Kovačič et al., 2004:

- Definition of 8 morphology types of morulae and blastocysts, most frequently observed on day 5.
- Blastocoel expansion,
- ICM shape,
- TE cohesiveness,
- Amount of excluded blastomeres from blastocyst



Blastocyst grading system



Blastocyst grading system

| <i>Morphologic type of blastocyst</i> | <i>Transferred blastocysts</i> | <i>Transferred blastocysts with known outcome</i> | <i>Implantations (%)^a</i> | <i>Miscarriages (%)</i> | <i>Births (%)^b</i> |
|---------------------------------------|--------------------------------|---|--------------------------------------|-------------------------|-------------------------------|
| 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; b P<0.0001

CONCLUSIONS: How to evaluate day-5 embryos ?

- Turn the embryos during evaluation.
- Pay regard that ICM morphology is the most predictable parameter for the live birth.
- Ascertain that the structure within the blastocyst is really an ICM (not a blastomere).
- Take into account that blastocyst can be collapsed during the observation.
- Suboptimal blastocysts or morulae should be evaluated by taking into account the dynamics of development, before the final selection decision is done.
- Allow suboptimal embryos on day 5 to be cultured for an additional day.

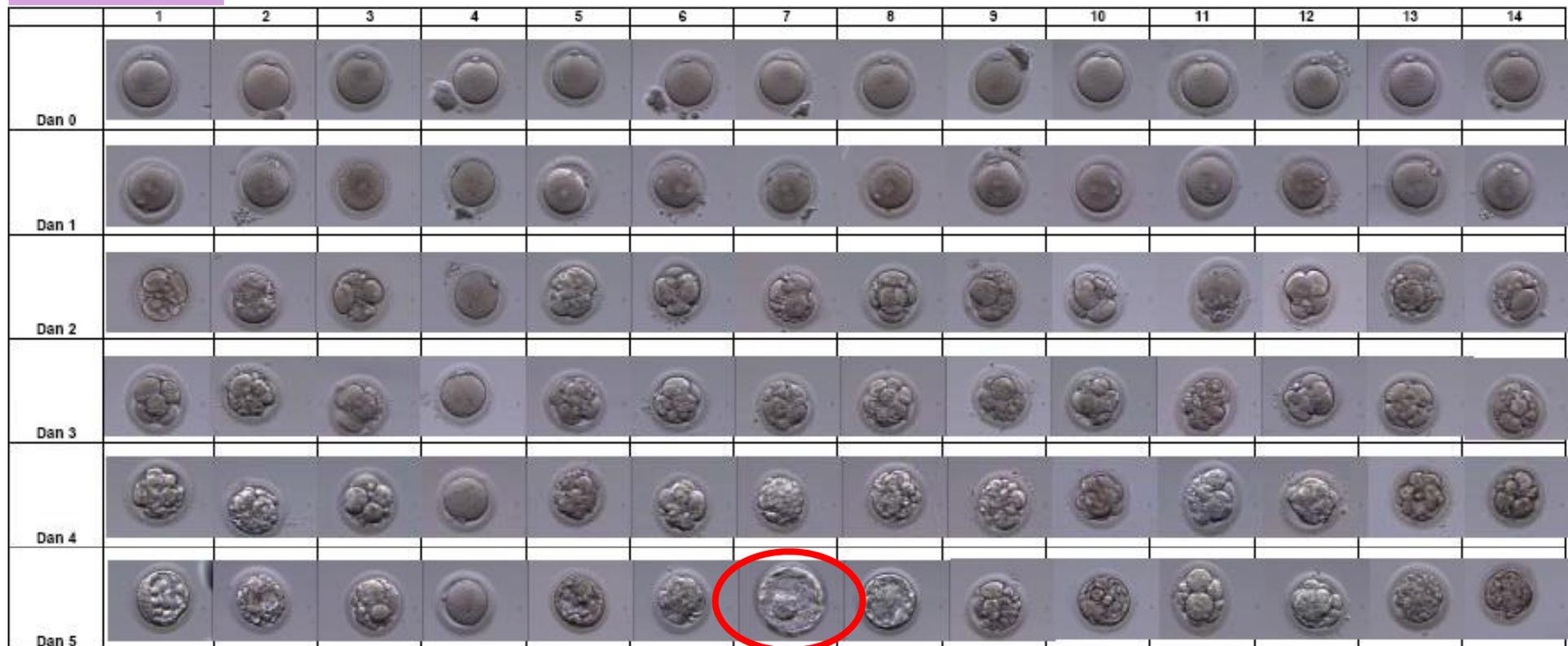
How to evaluate day-5 embryos ?

→ Short culture

- precise sequential embryo evaluation required (each day at the same time).

→ Prolonged culture

- sequential evaluation is recommended but selection only on day-5 is enough for clinical use.



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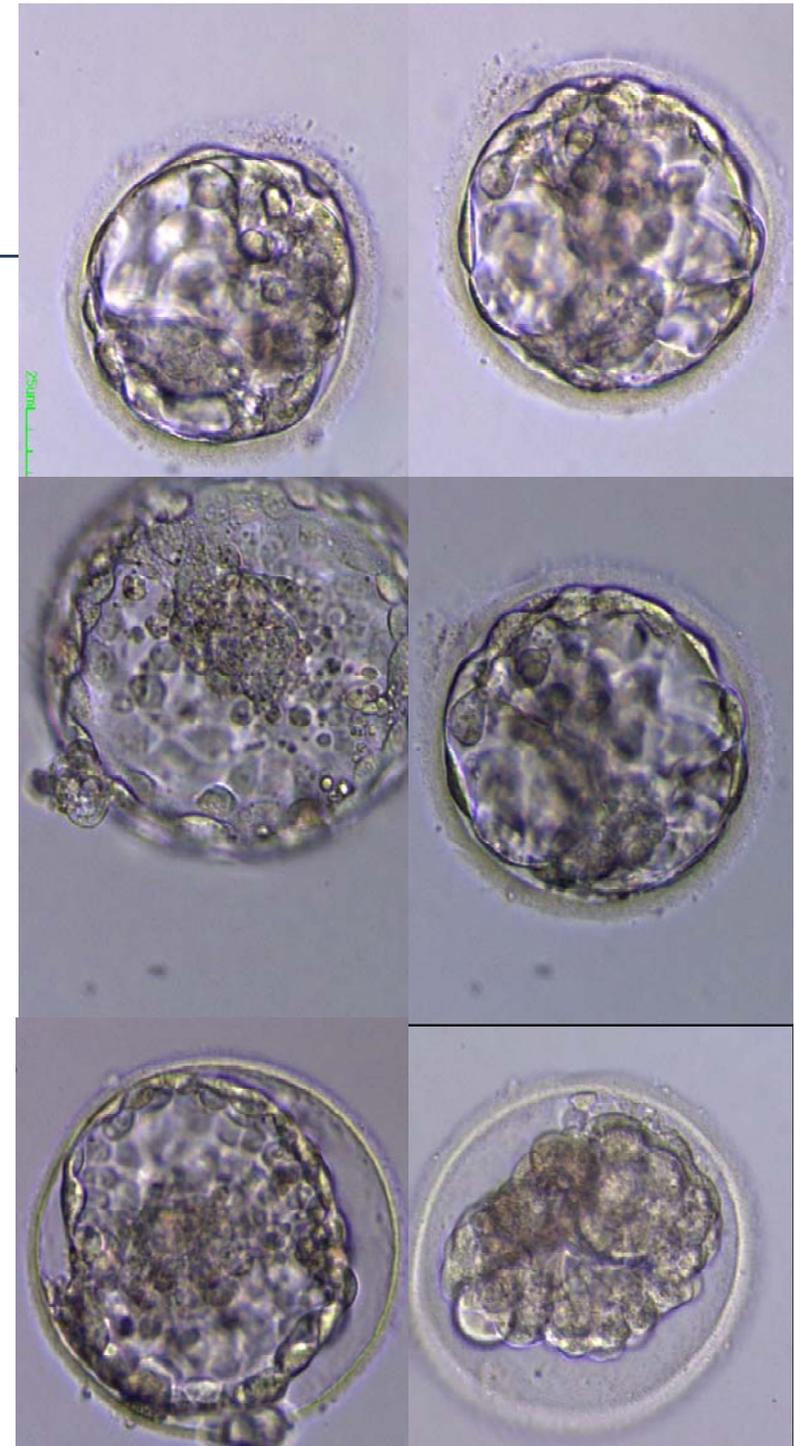
Morphological characteristics of frozen / thawed blastocysts

□ Effect of freezing on morphology:

- Collapsed blastocoel
- Necrosis
- Damaged cells

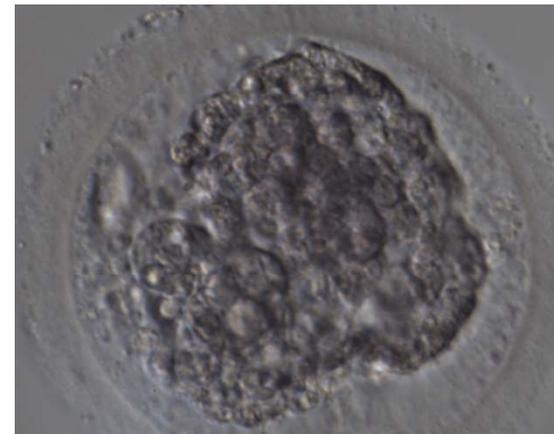
□ Selection criteria for suitability for transfer:

- Reexpansion
- ICM normality



Assessment and grading morulae and blastocysts: cryopreserved/warmed blastocysts

- Immediate morphological survival
 - Literature:
 - > 50% of initial number of cells intact?
 - Scoring:
ICM/TE
 - fully intact
 - moderately damaged
 - Severely damaged
 - degenerated



Assessment and grading morulae and blastocysts: cryopreserved/warmed blastocysts

Developmental potential in-vitro of thawed/warmed blastocysts

➔ Early blastocysts

- Capability of expansion
 - ➔ Van den Abbeel et al 2005) (Hum Reprod 20, 2939-2945)
 - ➔ Guerif et al (2003) (Theriogenology 60, 1457-1466)

➔ Expanded blastocysts

- Capability of re-expansion
 - ➔ Van den Abbeel et al 2005) (Hum Reprod 20, 2939-2945)
 - ➔ Guerif et al (2003) (Theriogenology 60, 1457-1466)
 - ➔ Shu et al 2008 (Fertil Steril)

Is it necessary to culture thawed/warmed blastocysts overnight?

- ➔ **Yes:** 4 h culture versus 24 h culture
Guerif et al (2003) (Theriogenology 60, 1457-1466)
- ➔ **No:** 4 h culture - degree of re-expansion
Shu et al 2008 (Fertil Steril)

Blastocyst vitrification and warming

Assessment of expansion/re-expansion after warming

Immediately after warming

1 hour post

4 hours post (transfer)

BI2



BI1



BI2



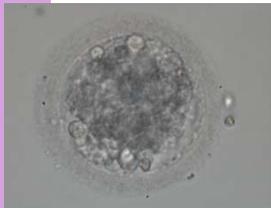
➤ **Assessment of expansion/re-expansion after warming**

Immediately after warming

1 hour post

Transfer (4h post)

BL1



BI4AA



BI4AA



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Effect of culture media

- Several types of commercially available media for prolonged embryo culture.
- The quality/morphology of human, bovine and mouse embryo growth is improved when embryos are cultured in groups rather than separately (Moessner and Dodson, 1995, Salahuddin et al, 1995, Ahern et al, 1998, Ebner et al 2010)
- Embryo density
- **Comparative studies:**
 - Differences in dynamics of embryo development.
 - Differences in blastocyst quality/morphology.
 - Differences in pregnancy rates? (cumulative ?).
- **Lack of robust multicentre randomized clinical trials for comparing the influence of different culture media on human embryo development and clinical outcomes (*Biggers and Summers, F&S, 2008; Blake et al., Cochrane database, 2007*).**

General conclusions

1. **Assessing human blastocysts is a challenge**
2. **Grading and scoring blastocysts is difficult**
 - **An optimal blastocyst (116 h): a fully expanded through to hatched blastocyst with an ICM that is prominent, easy discernable and consisting of many cells, with the cells compacted and tightly adhered together, and with a TE that is comprised of many cells forming a cohesive epithelium (Istanbul consensus document)**
 - **Variants with unknown/doubtfull significance: “strings”, cellular or a-cellular structures within the PVS or blastocoel cavity**
 - **Scoring: use a combination of stage and score and use a numerical score to facilitate statistical analysis (Matsuura et al, 2010)**