

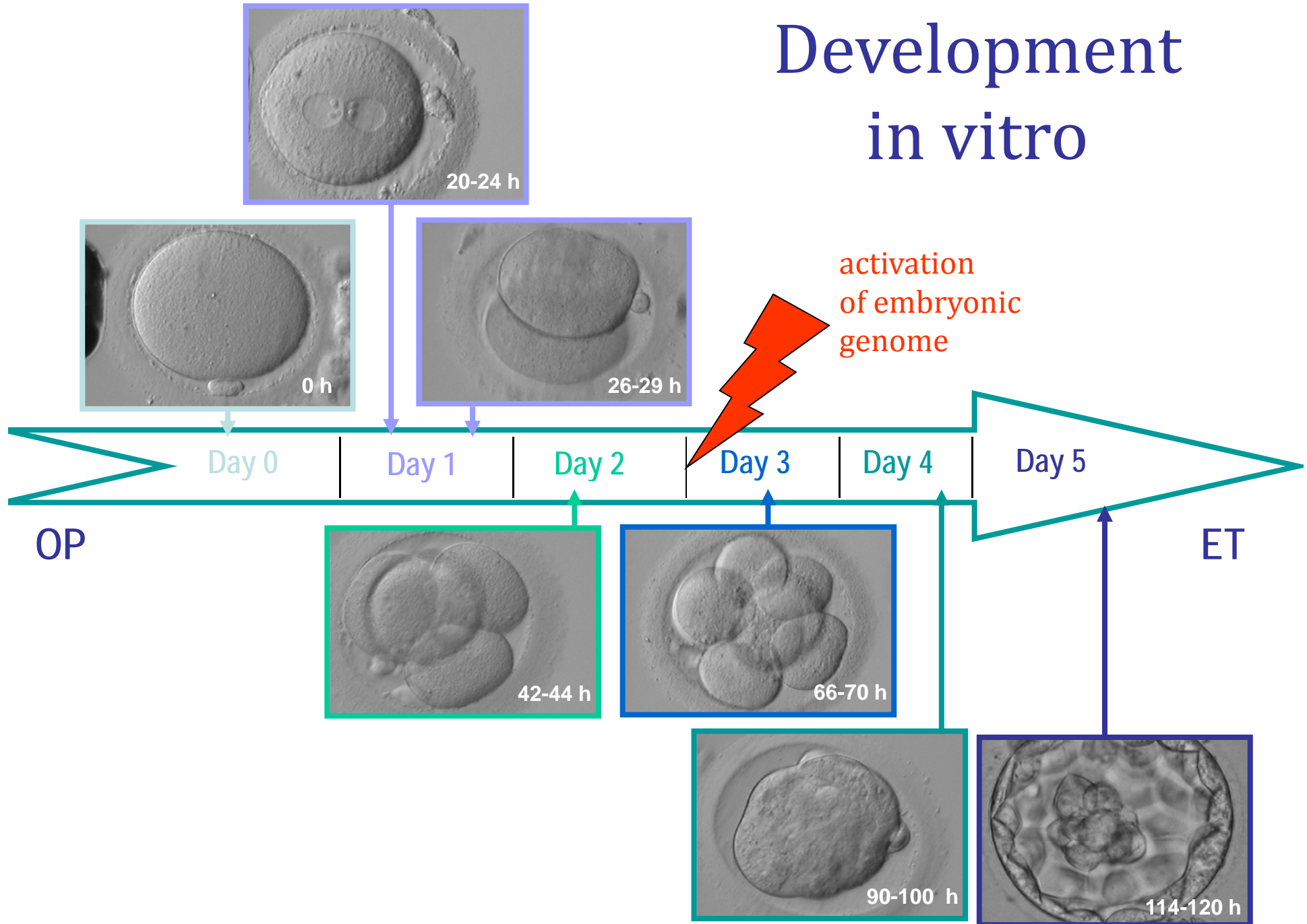
A grayscale microscopic image of a cell, likely a zygote, showing a distinct outer layer and internal structures. The cell is roughly circular and occupies the central portion of the frame.

# ***The neglected compaction stage***

Thomas Ebner

Kinderwunsch Zentrum, Linz, Austria

# Development in vitro



# Physiology of embryos



- Low level of biosynthesis
- Low respiratory rate
- Limited capacity to use glucose as an energy source



- Increasing biosynthetic rates
- Increased respiratory capacity
- Ability to utilize glucose
- Formation of intercellular junctions
  - capable of actively regulating ionic gradients
  - regulation of  $pH_i$

# COMPACTION WHEN?

EARLY

3-cells  
Day 2

LATE

16-cells  
Day 4

# COMPACTION

## WHO?



8-cell stage



16-32-cell stage



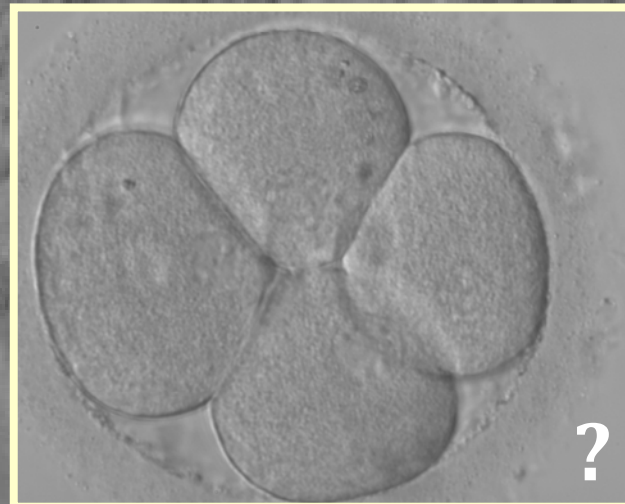
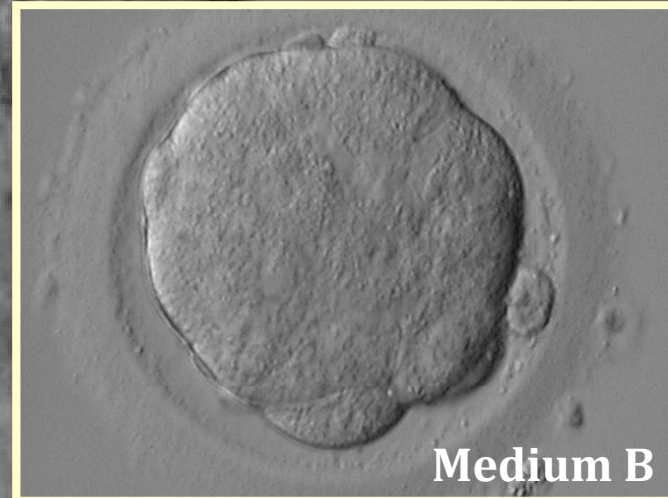
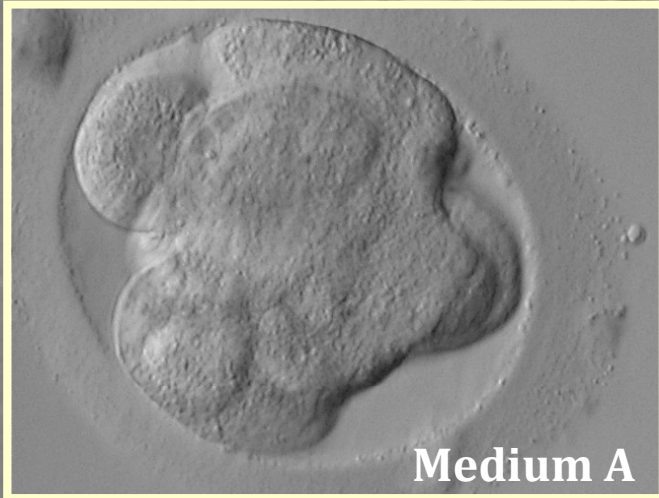
32-64-cell stage



> 8-cell stage

# COMPACTION

## HOW?



# Early compaction on day 3 may be associated with increased implantation potential

*Christine C. Skiadas, M.D., Katharine V. Jackson, B.S., and Catherine Racowsky, Ph.D.*

Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

**Objective:** To determine whether day 3 embryos exhibiting early compaction have an improved implantation potential compared to embryos without compaction.

**Design:** A retrospective cohort study.

**Setting:** Hospital-based academic medical center.

**Patient(s):** Women <38 years of age undergoing IVF cycles between November 2001 and December 2004 having a day 3 transfer of one or two embryos with >8 cells.

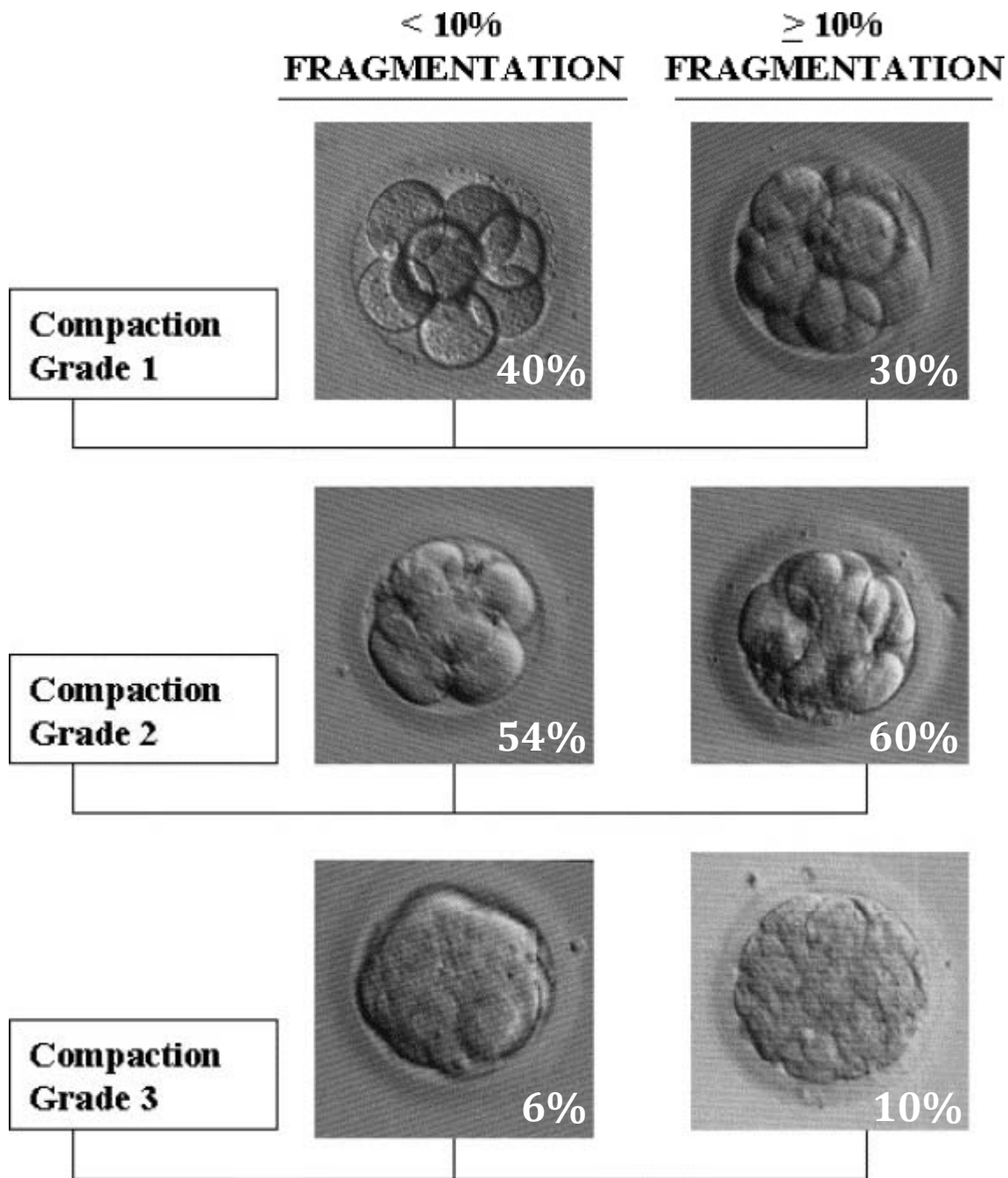
**Intervention(s):** Standard IVF protocol.

**Main Outcome Measure(s):** Compaction grading and implantation rates of 1,047 embryos as related to fragmentation of  $\geq$  8-cell embryos in patients with either 0% or 100% implantation.

**Result(s):** Compaction grading was strongly associated with implantation potential; however, the direction of this effect depended on the degree of fragmentation. In embryos with <10% fragmentation, implantation rates increased with the degree of compaction (grade 1, 25%; grade 2, 33%; and grade 3, 47%); in embryos with  $\geq$ 10% fragmentation, the effect was reversed (grade 1, 38%; grade 2, 20%; and grade 3, 9%).

**Conclusion(s):** Assessing the degree of compaction can be a valuable addition to traditional morphologic assessment in identifying optimal embryos for transfer on day 3. (*Fertil Steril*® 2006;86:1386–91. ©2006 by American Society for Reproductive Medicine.)

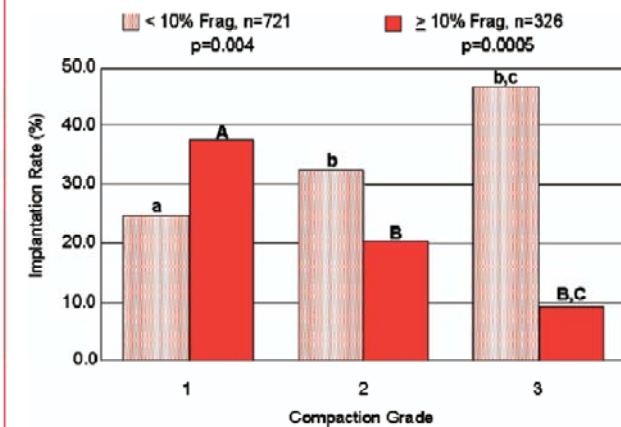
**Key Words:** Embryo compaction, embryo fragmentation, IVF, implantation rates



**PROBLEMS**  
**Day 3**  
 retrospective  
 Fragmentation and IR

**FIGURE 2**

Implantation rate by compaction grade. The stippled columns represent embryos exhibiting <10% fragmentation, the red columns represent embryos with ≥10% fragmentation for the entire study dataset. Columns denoted by different letters are statistically significant: a vs. b,  $P < .05$ ; a vs. c,  $P < .005$ ; A vs. B,  $P < .002$ ; A vs. C,  $P < .003$ .



*Skiadas. Embryo compaction and implantation. Fertil Steril 2006.*



## The neglected morula/compact stage embryo transfer

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Jun Tao<sup>1,2,3</sup>, Robert Tamis<sup>1</sup>, Katharine Fink<sup>1</sup>, Brenda Williams<sup>2</sup>, Tresa Nelson-White<sup>2</sup> and Randall Craig<sup>2</sup>

<sup>1</sup>Arizona Institute of Reproductive Medicine, Phoenix, Arizona and <sup>2</sup>Fertility Treatment Centre, Chandler, Arizona, USA

<sup>3</sup>To whom correspondence should be addressed at: Fertility Treatment Centre, 3200 N Dobson Road, Chandler, AZ 85224, USA.  
E-mail: jtftc@aol.com

Analysing the patients who had transfers with all 'good' embryos, day 4 transfer achieved a significantly higher implantation rate compared with day 3 transfer (46.4 versus 21.4%,  $P < 0.01$ ), but the number of embryos transferred on day 4 was significantly lower than day 3 ( $2.1 \pm 0.5$  versus  $3.5 \pm 0.9$ ,  $P < 0.01$ ).

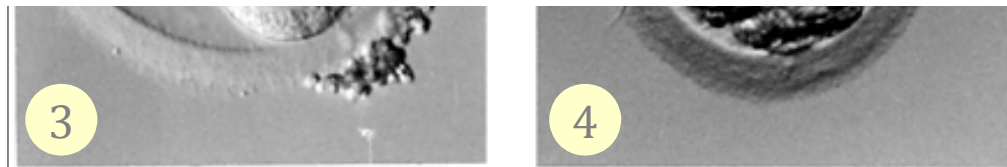
*Key words:* compact/embryo grading/implantation/morula/selection

Higher IR in day 4 transfer with less embryos transferred!



**Table I.** Grading criteria for morula/compact embryo

Score	Proportion of blastomere undergoing compaction	Morphology of compacted embryo	Blastomere on days 2 and 3	Fragments
4	All	Full size. Close to sphere with smooth profile	Even-sized blastomere	<5%
3	>75%	>3/4. Close to sphere with shallow indentation	} May have slight size difference	<25%
	65–75%	2/3–3/4. Close to sphere with smooth profile		
2	All or partial	Full or >3/4. Irregular with deep indentation.	} May have slight to moderate size difference and/or multinuclei	<50%
1	All or partial	1/2 or lobed to two 1/2		
		1/3 or lobed to two to three 1/3	} May have moderate to severe size difference and/or multinuclei	Varies



## PROBLEMS

complicated score / did not take into account embryos without compaction / retrospective

# Successful pregnancy rates achieved with day 4 embryo transfers

*Josh C. Skorupski, M.D., Daniel E. Stein, M.D., Uchenna Acholonu, M.D., Heather Field, B.A., and Martin Keltz, M.D.*

Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, St. Luke's-Roosevelt Hospital Center, Columbia University College of Physicians and Surgeons, New York, New York

**Objective:** To assess the success of day 4 embryo transfers (ETs) following IVF at one institution.

**Design:** Retrospective analysis.

**Setting:** A university hospital IVF program.

**Patient(s):** Two hundred nondonor, fresh IVF cycles.

**Intervention(s):** None.

**Main Outcome Measure:** Outcomes of IVF. Outcome assessments after day 4 ETs included rates of implantation, clinical pregnancy, and singleton and multiple live births.

**Result(s):** The overall live-birth rate was 54.4%. Implantation rates were highest in younger age groups, and similar in patients 35–40 years of age. Pregnancy and live-birth rates were similar across all age groups up to age 40 years. Multiple gestations were highest in women  $\leq 40$  years of age.

**Conclusion:** Acceptable pregnancy rates can be achieved with day 4 ETs. (Fertil Steril® 2007;87:788–91. ©2007 by American Society for Reproductive Medicine.)

**Key Words:** Embryo transfer, blastocyst, morula

## Comparable and acceptable pregnancy rates in day 4 transfers irrespective of age

**TABLE 2**

**Comparison of day 4 IVF transfers by age.**

Variable	Age				Mean $\pm$ SD (all age groups) (%)
	$\leq 34$ (%)	35–37 (%)	38–40 (%)	$\geq 41$ (%)	
No. of cycles	96	47	36	21	
Mean no. of embryos per cycle ( $\pm$ SD)	2.6 $\pm$ 0.72	3.2 $\pm$ 0.96	3.4 $\pm$ 1.11	4.2 $\pm$ 1.62	3.04 $\pm$ 1.09
Implantation rate	113/250 (45.2)	47/149 (31.5)	42/123 (34.1)	9/87 (10.3)	211/609 (34.6)
Pregnancy rate	66/96 (68.8)	34/47 (72.3)	26/36 (72.2)	8/21 (38.1)	134/200 (67.0)
Live birth rate	53/96 (55.2)	28/47 (59.6)	21/36 (58.3)	7/21 (33.3)	109/200 (54.4)
Live birth rate of twins	18/53 (34.0)	4/28 (14.3)	7/21 (33.3)	1/7 (14.3)	30/109 (27.5%)
Live birth rate of triplets	1/53 (1.8)	1/28 (3.6)	0/21 (0%)	0/7 (0%)	2/109 (1.8%)

*Skorupski. Success with day 4 embryo transfers. Fertil Steril 2007.*

**PROBLEMS:** retrospective / no scoring at all / AH in more than half of the embryos

# Day 4 versus day 3 embryo transfer: a prospective study of clinical outcomes

*Konstantinos Pantos, M.D., Evangelos Makrakis, M.D., Margaret Chronopoulou, B.Sc., Maria Biba, M.Sc., Anastasios Perdikaris, M.D., and Alex Dafereras, M.D.*

Center for Human Reproduction, 'Genesis Athens' Clinic, Athens, Greece

**Objective:** To compare the clinical outcomes after day 4 or day 3 embryo transfers in controlled ovarian hyperstimulation-IVF/intracytoplasmic sperm injection (ICSI) cycles.

**Design:** Prospective randomized study.

**Setting:** Center for Human Reproduction, 'Genesis Athens' Clinic (private gynecological and surgical clinic), Athens, Greece.

**Patient(s):** Three hundred fifty women with primary infertility and indication for IVF/ICSI treatment. Patients were randomly allocated to day 4 or day 3 embryo transfer.

**Intervention(s):** Controlled ovarian hyperstimulation, oocyte retrieval, IVF/ICSI, embryo transfer.

**Main Outcome Measure(s):** Implantation rate; clinical, ongoing, and multiple pregnancy rates.

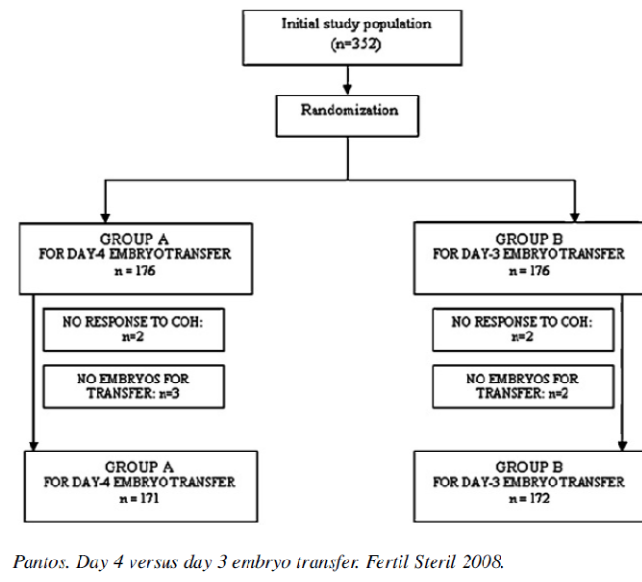
**Result(s):** A trend toward higher clinical and ongoing pregnancy rates was noted after day 4 embryo transfers. Implantation and multiple pregnancy rates were comparable after day 4 or day 3 embryo transfers.

**Conclusion(s):** The transfer of day 4 embryos in IVF/ICSI cycles may result in favorable implantation and pregnancy rates which are at least comparable to those after the transfer of day 3 embryos. (Fertil Steril® 2008;89:573–7. ©2008 by American Society for Reproductive Medicine.)

**Key Words:** Day 4 embryo transfer, day 3 embryo transfer, in vitro fertilization, assisted reproductive technologies

**FIGURE 1**

Flow chart of the randomized subjects.  
COH = controlled ovarian hyperstimulation.

**TABLE 2**
**Characteristics and outcomes of embryo transfers (ETs).**

	Group A: day 4 ET	Group B: day 3 ET	P value
Number of ETs	171	172	
Number of embryos per transfer (mean ± SD)	2.67 ± 0.76	2.53 ± 0.72	.07
ETs with all embryos of grade 2	12.28%	7.55%	.14
Implantation rate	22.05%	21.33%	.83
Clinical pregnancy rate	49.7%	45.34%	.41
Multiple pregnancy rate	15.29%	15.38%	.98
Viable pregnancy rate	43.27%	40.69%	.62

*Pantos. Day 4 versus day 3 embryo transfer. Fertil Steril 2008.*

Day 4 as least as good as day 3 transfer

## Day 4 embryo selection is equal to Day 5 using a new embryo scoring system validated in **single embryo transfers**

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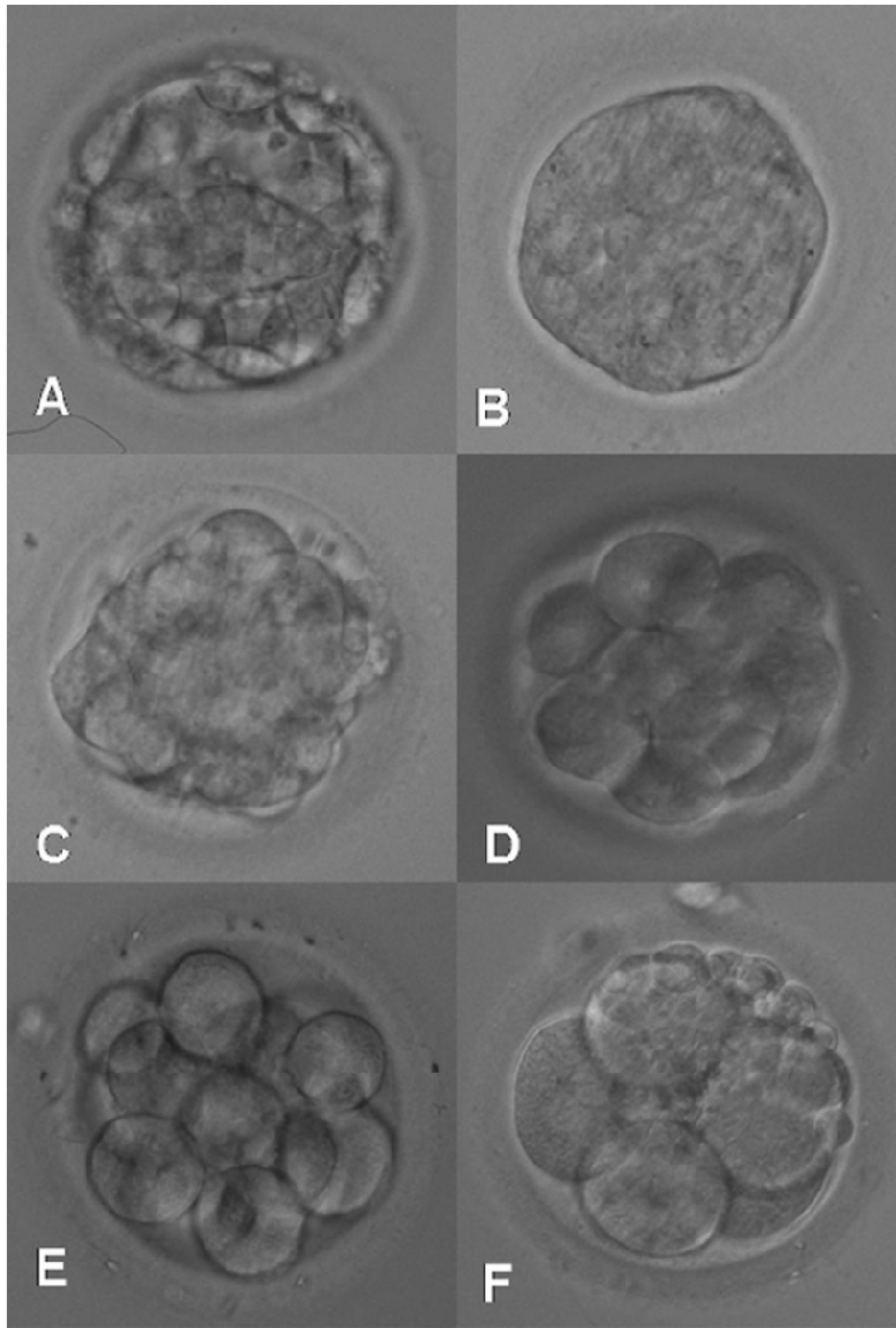
Deanne Feil<sup>1,2,3</sup>, Richard C. Henshaw<sup>2</sup> and Michelle Lane<sup>1,2</sup>

<sup>1</sup>*Department of Obstetrics and Gynaecology, Research Centre for Reproductive Health, The University of Adelaide, Adelaide, Australia;*

<sup>2</sup>*Repromed, Adelaide, Australia*

<sup>3</sup>Correspondence address. E-mail: deanne.feil@adelaide.edu.au

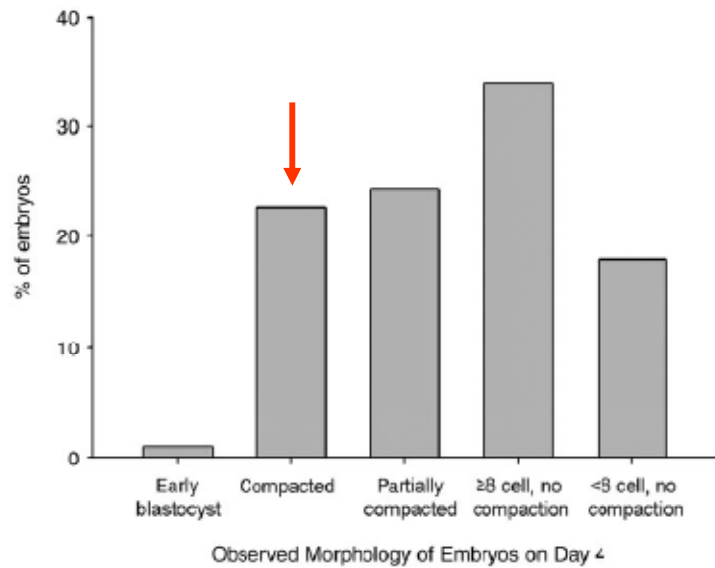
**BACKGROUND:** Single embryo transfers (SETs) require the most viable embryo from a cohort to be selected. Day 4 embryos may provide a selection advantage similar to blastocyst transfer as embryos are replaced post-embryonic genome activation and into the uterus where they would normally reside. However, there is currently no adequate morphological system to assess Day 4 embryos. Therefore, we developed an assessment system for Day 4 embryos and retrospectively assessed Day 4 SET success rates compared with Day 5 SETs. **METHODS:** Embryos ( $n = 996$  after IVF or ICSI) were assessed on Day 4 of development, prior to implementing Day 4 embryo transfers, to obtain a descriptive range of embryo morphologies observed at this time point. A morphological scoring system was developed from this. All patients having an SET after extended culture (Day 4,  $n = 124$  or Day 5,  $n = 193$ ), at Repromed, Adelaide, Australia, during June 2006–January 2007 were analysed for pregnancy outcome. Ongoing pregnancy was determined by fetal cardiac activity at 6–8 weeks after oocyte collection. **RESULTS:** Day 4 and 5 SETs resulted in similar ongoing pregnancy rates of 38.7% and 32.1%, respectively. **CONCLUSIONS:** A Day 4 scoring system was successfully developed and implemented. Day 4 SETs were found to be a viable option or alternative to Day 5 SETs with no difference in pregnancy rates.



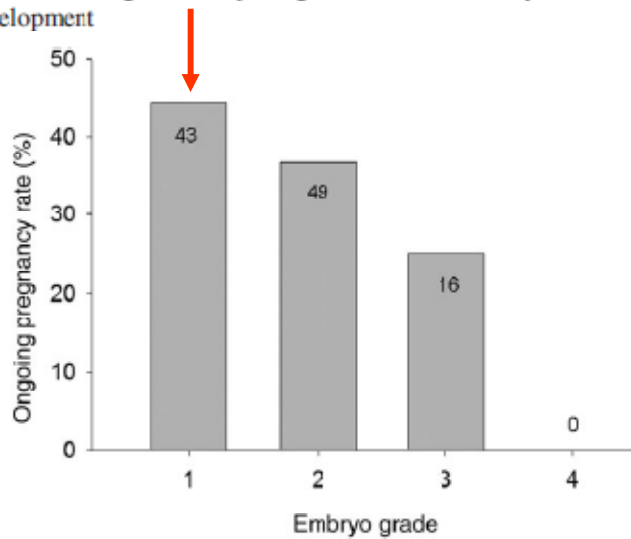
**Figure 1:** Scoring system developed for Day 4 embryos. Grade 1: early blastocyst, visible signs of cavitation occurring (A) or completely compacted embryo, lacking negative morphological anomalies (B), e.g. vacuolation, excessive fragmentation, large number of excluded cells and self-cavitation of cells. Grade 2: Grade 1 compacted morula, with some morphological anomalies, e.g. one of the following present: vacuolation, excessive fragmentation, excluded cells, self-cavitation of cells or at least an eight-cell embryo, with partial compaction present or the majority of cells at least showing signs of compaction (C and D). Grade 3: partially compacted embryo with vacuoles or excessive fragmentation present or eight-cell embryo or greater, with no signs of compaction evident (E). Grade 4: embryos with eight cells or greater, with no signs of compaction and vacuoles or excess fragments or embryos with less than eight cells, and no signs of compaction (F)

**PROBLEM**  
**Mixture of degree and morphology**  
**retrospective**

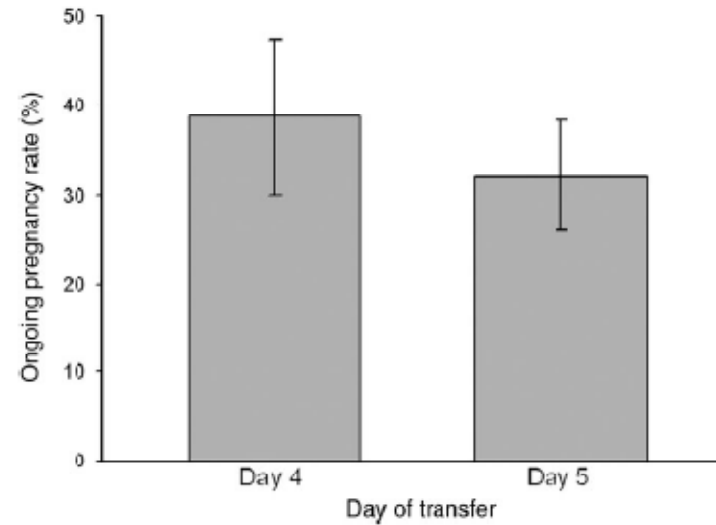




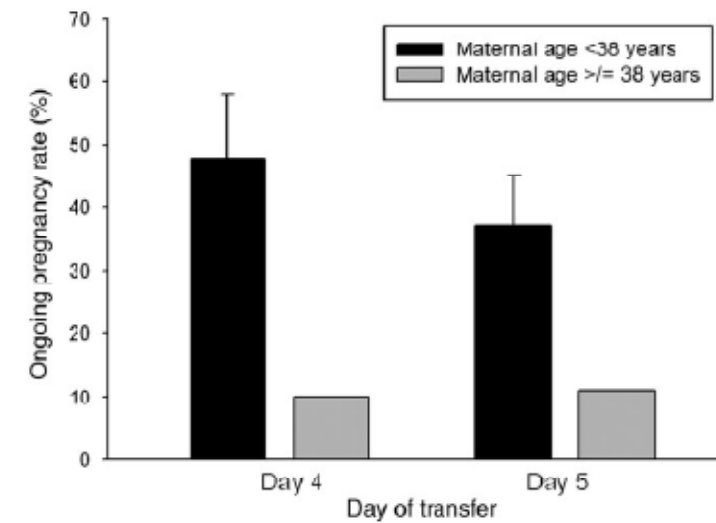
**Figure 2:** Range of morphologies observed on Day 4 of embryo development



**Figure 3:** Pregnancy rates using developed Day 4 scoring system with signal embryo transfers. Numbers in columns indicate the number of single embryo transfers performed in each group



**Figure 4:** Day 4 and Day 5 SET Ongoing Pregnancy Rates. Error Bars indicates 95% Confidence intervals



**Figure 5:** Effect of Maternal Age and Day of Transfer on Pregnancy Rates with single Embryo Transfers. Error bars indicates 95% Confidence intervals

# Morphological analysis at compacting stage is a valuable prognostic tool for ICSI patients



Thomas Ebner, PhD, graduated with honours from the Paris Lodron University of Salzburg, Austria, in 1992. His doctorate (1994) on cancer research was taken at the Institute for Pathology, General Hospital, Salzburg. After 2 years research at Salzburg University, he started in IVF in Linz. Completing his post-doctoral thesis, he became a university lecturer in Salzburg. He has published more than 60 papers. Research interests include non-invasive IVF selection processes, laser application, apoptosis, cryopreservation and culture media. He was certified as a senior clinical embryologist in 2008. Currently he is scientific director of the European School of ART in Linz.

*Dr Thomas Ebner*

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Landes- Frauen- und Kinderklinik, IVF-Unit, Linz, Upper Austria, Austria

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## Abstract

In terms of treatment outcome, little prognostic power is attributed to day-4 morphology. A day-4 score was applied to 56 patients separating non-compacting embryos from compacting (some areas of compaction) and fully compacted embryos. The latter were further subdivided according to the morphology of compaction. Grade C1 embryos represented optimal quality, while grades C2 (exclusion of fragments) and C3 (exclusion of blastomeres) were characterized by a loss of cytoplasm. Grade 4 embryos (C4) showed incomplete compaction with several blastomeres not yet incorporated into cell mass. Pooled embryos without compaction showed a reduced ( $P < 0.001$ ) blastulation (28.8%) as compared with compacting embryos with the same cell number (68.8%), which, in turn, revealed lower ( $P < 0.05$ ) rates of blastulation as compared with concepti that completed compaction process (84.6%). Among fully compacted embryos grade C1 had a better ( $P < 0.01$ ) blastocyst formation rate (94.4%) as compared with grade C3 (68.2%). Grade C1 embryos showed significantly higher rates of top-quality blastocysts as compared with grade C2 ( $P < 0.05$ ) and C3 ( $P < 0.01$ ). Blastocysts deriving from grade C1/C4 embryos led to a higher pregnancy rate as compared with the C2/C3 counterparts ( $P < 0.05$ ). This modified score allows for adequate prediction of both blastocyst formation/quality and pregnancy.

**Keywords:** *blastocyst formation, compaction, ICSI, multinucleation, oocyte quality*

DAY 4



no  
compaction

46%



beginning  
compaction

20%



complete  
compaction

34%

# DAY 4

no  
COMPACTION

n	BFR
17	0%
61	13%
34	35%
21	52%
13	85%



# cells

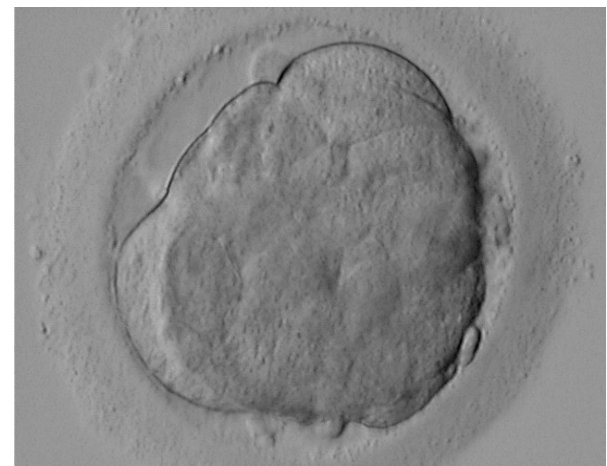
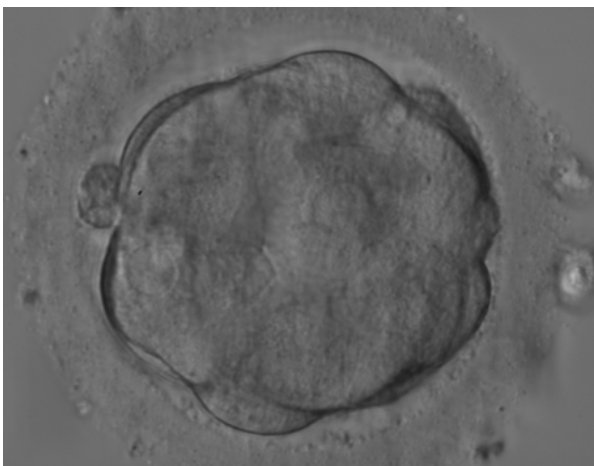
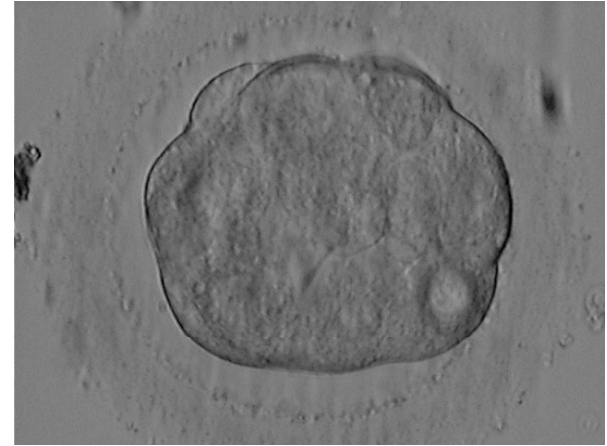
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8
10
12
14

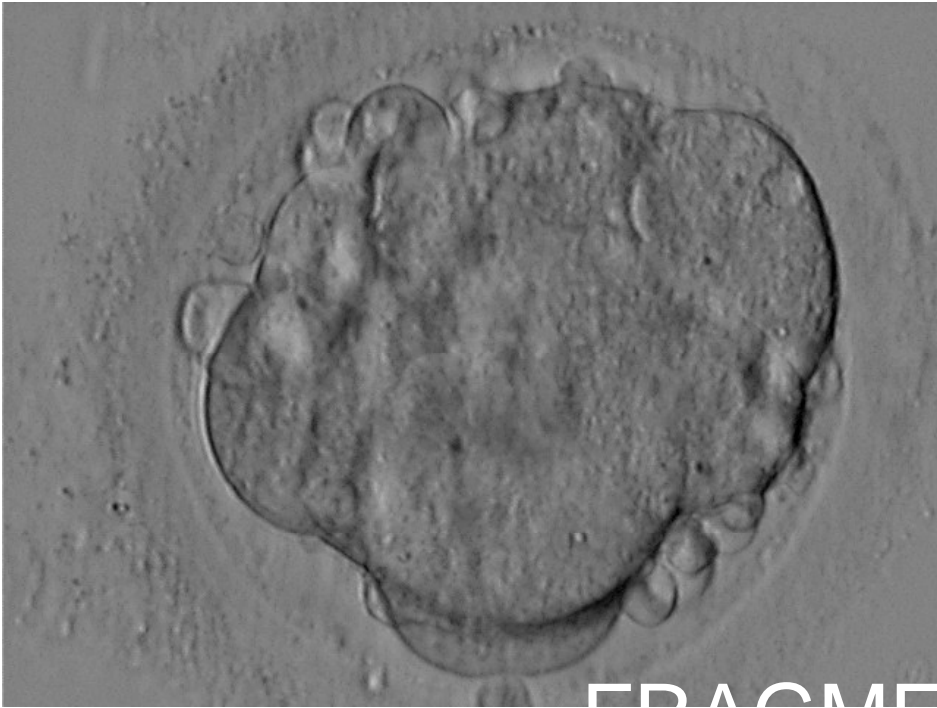
beginning  
COMPACTION

n	BFR
2	0%
10	40%
23	74%
17	82%
9	82%

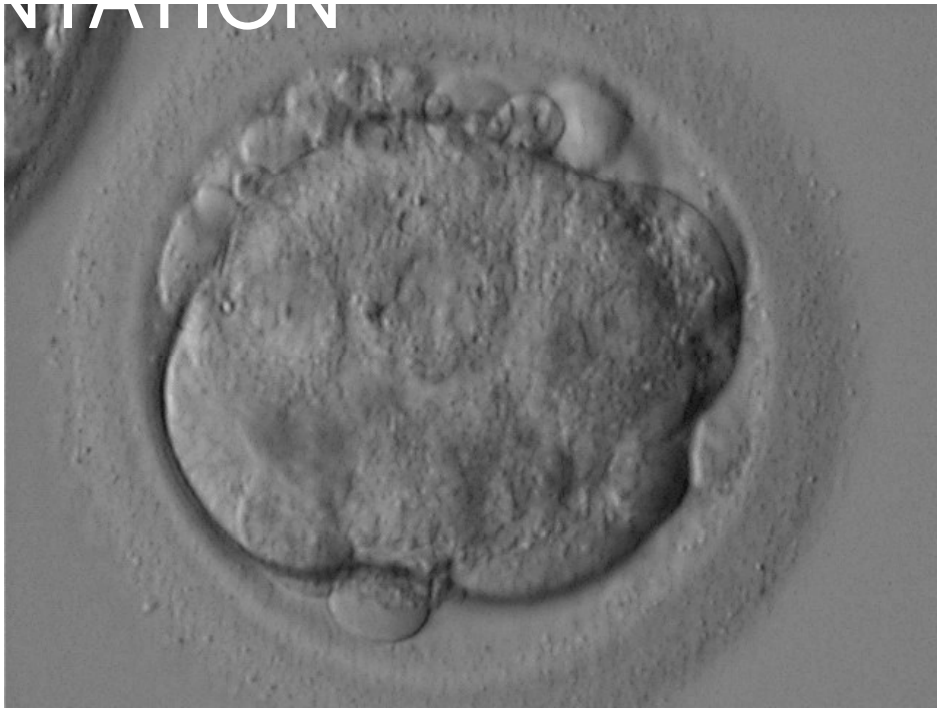


- ① The more cells, the higher BFR
- ② The more compacting cells, the higher BFR
- ③ Without signs of compaction BFR is lower as compared to compacting embryos with the same mitotic activity





FRAGMENTATION





BLASTOMERES

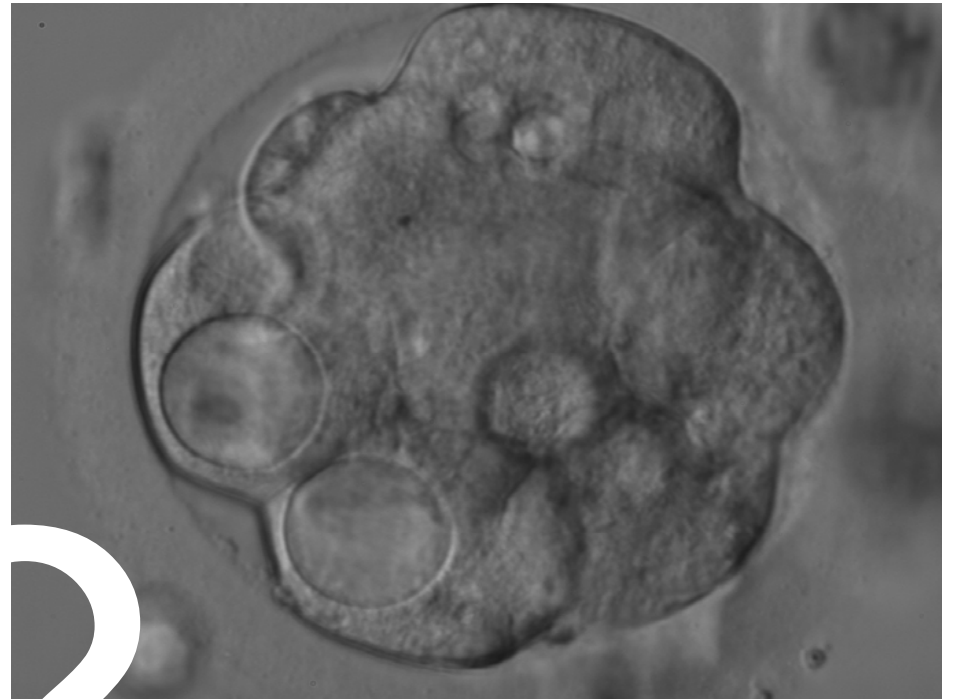
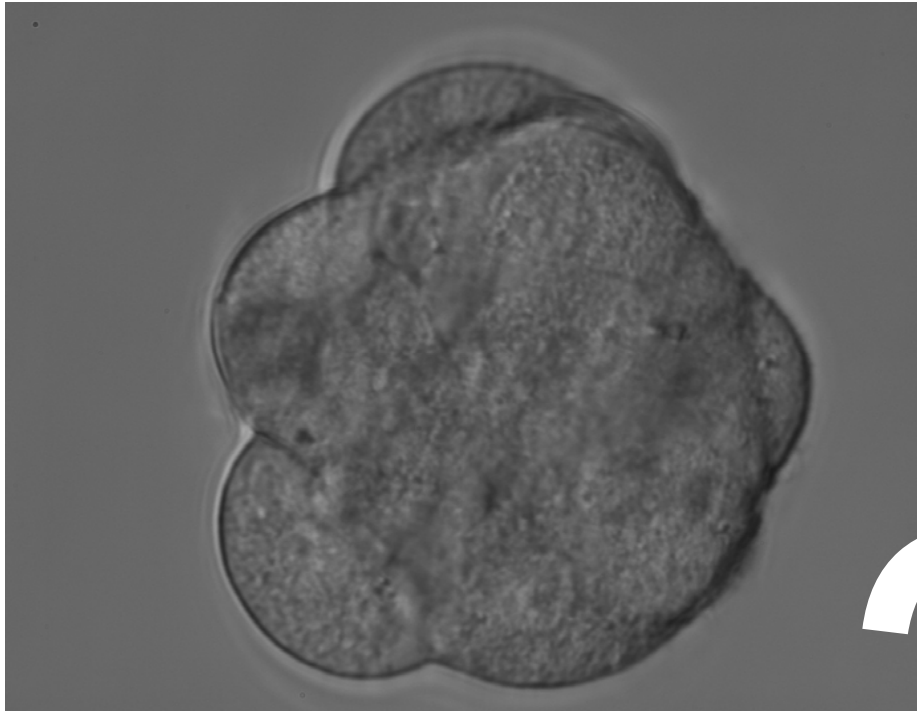




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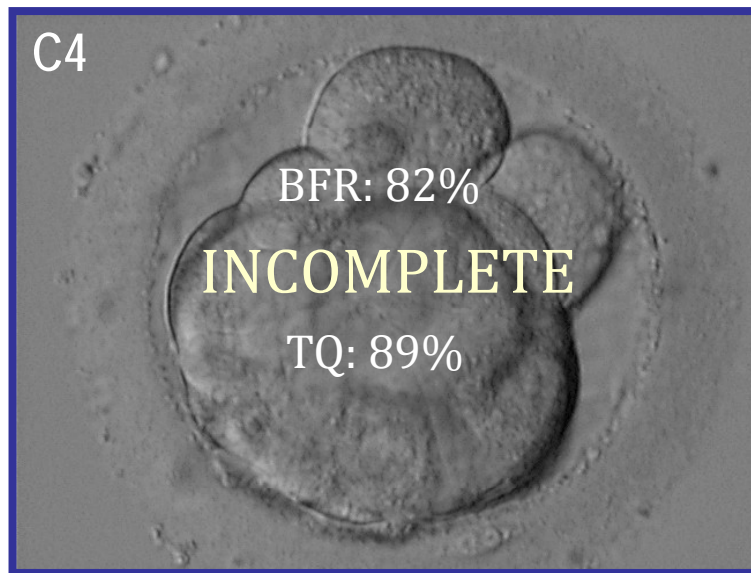
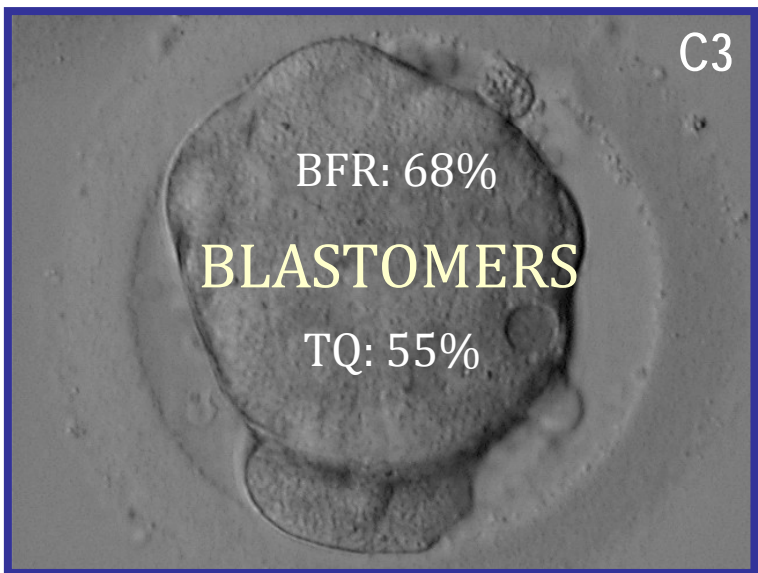
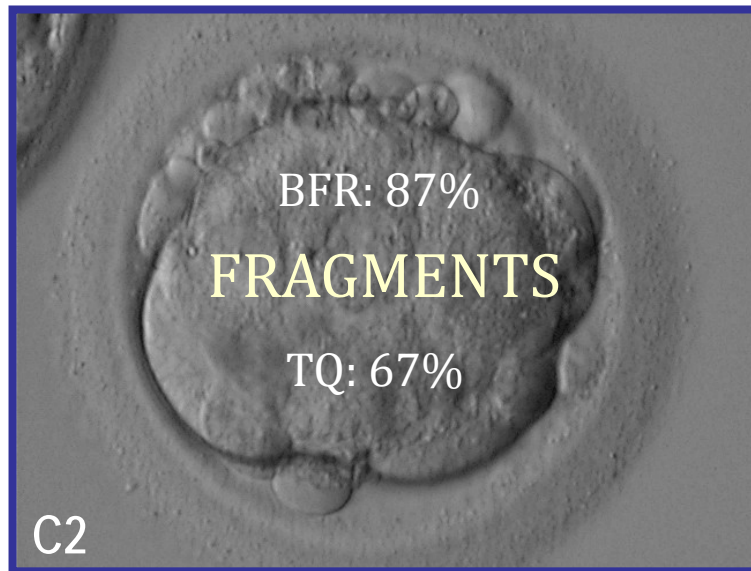
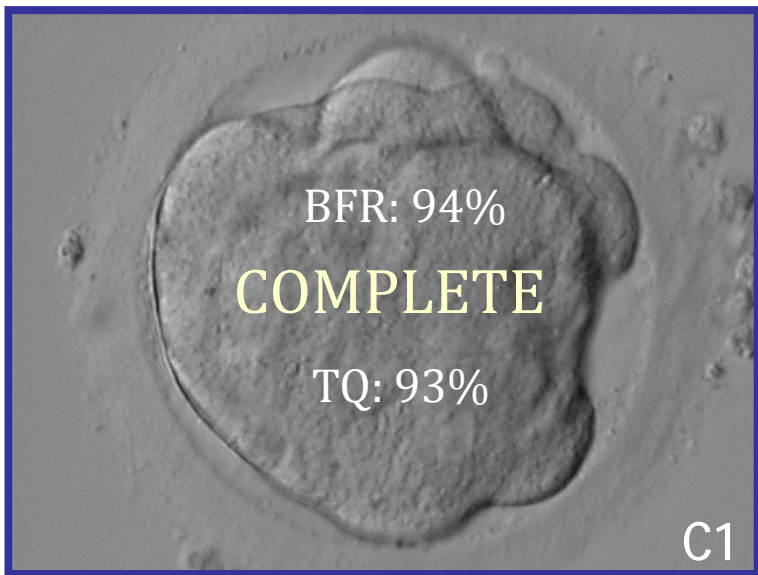






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# Don't be afraid of day 4 transfer

- adequate prognosis in terms of blastocyst development
  - shorter in vitro culture
  - flexible schedule (weekend)
- better survival after cryopreservation
  - facilitates „assisted hatching“

# Thanks for your



G. Tews  
M. Moser  
R. Mayer  
O. Shebl  
R. Wiesinger  
M. Puchner

# attention

 Kinderwunsch  
Zentrum **Linz**  
an der Landes- Frauen- und Kinderklinik