Pronuclear membrane breakdown and early cleavage

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Learning objective

- The first cell cycle of the human embryo
- The first results and importance on early cleavage assessment
- Correlation between early cleavage and further embryo development
- The optimal time of early cleavage assessment
- Assessment of early pronuclear breakdown
- The morphology of early cleaved zygotes

Declaration of interest

The author declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this presentation.

Human Reproduction vol.12 no.7 pp.1531–1536, 1997

Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability

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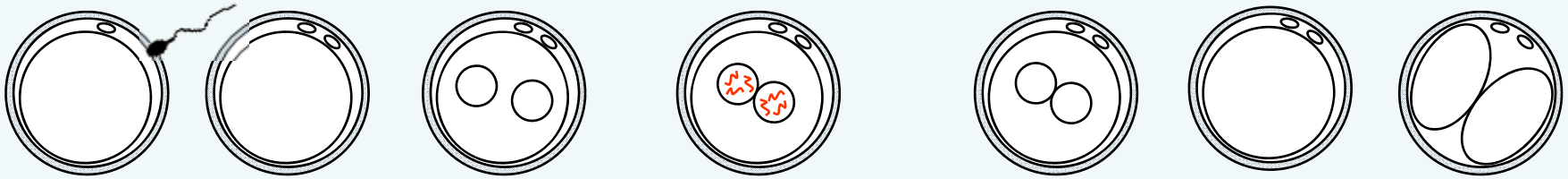
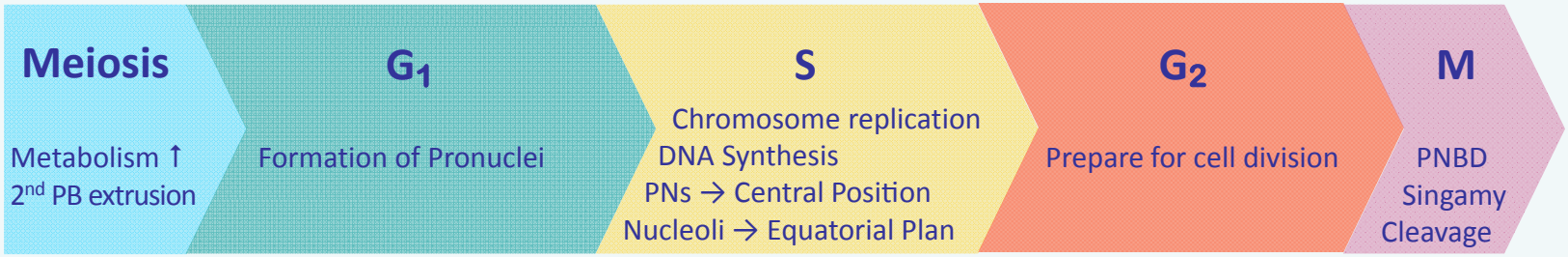
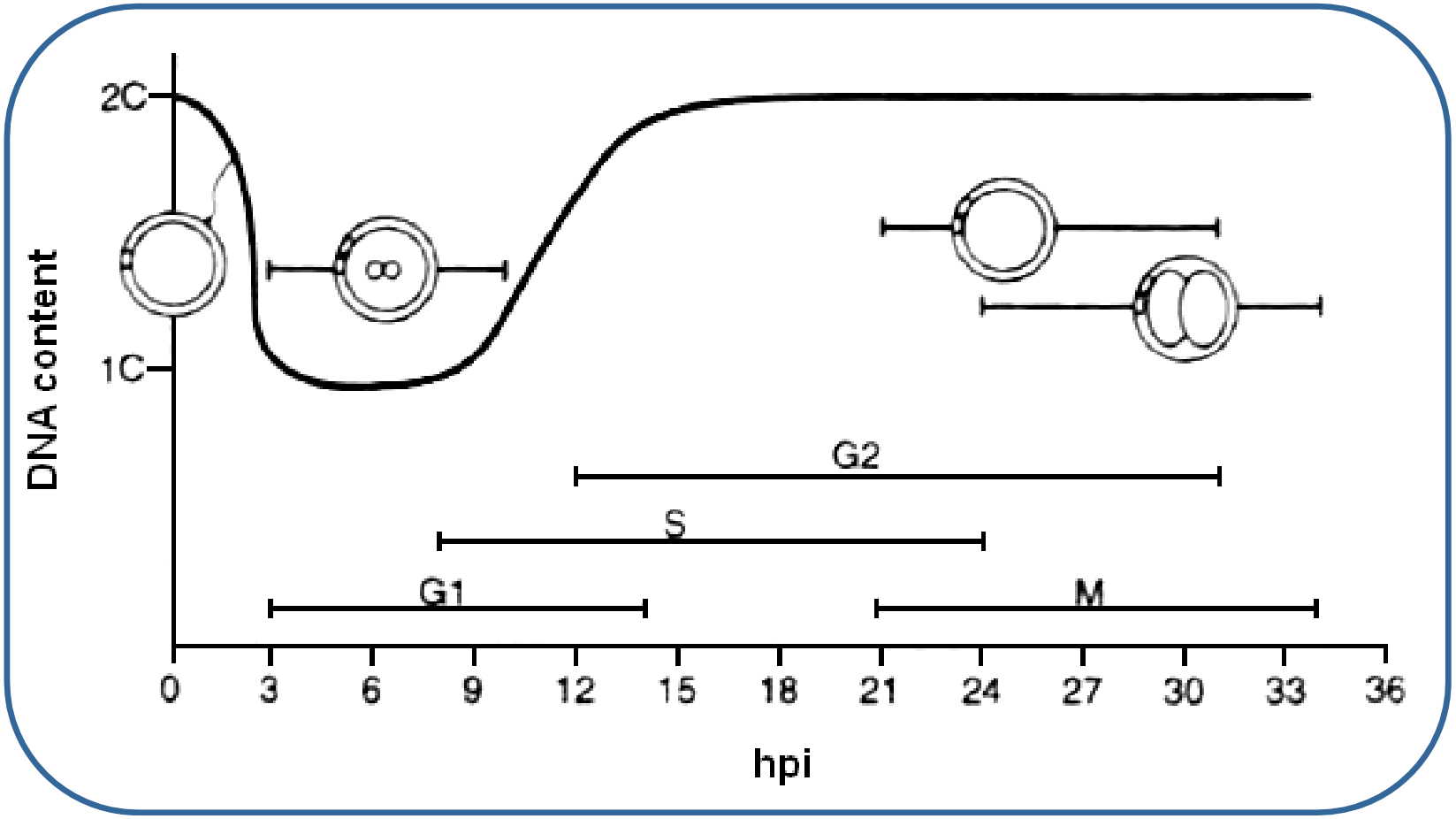
The first cell cycle of a human zygote I.





Fancsovits et al. 2006

The first cell cycle of a human zygote II.



Timing of the main events taking place during the first cell cycle of human embryogenesis

Capmany, et al. 1996

The first cell cycle of a human zygote III.

Timing of first cell cycle events

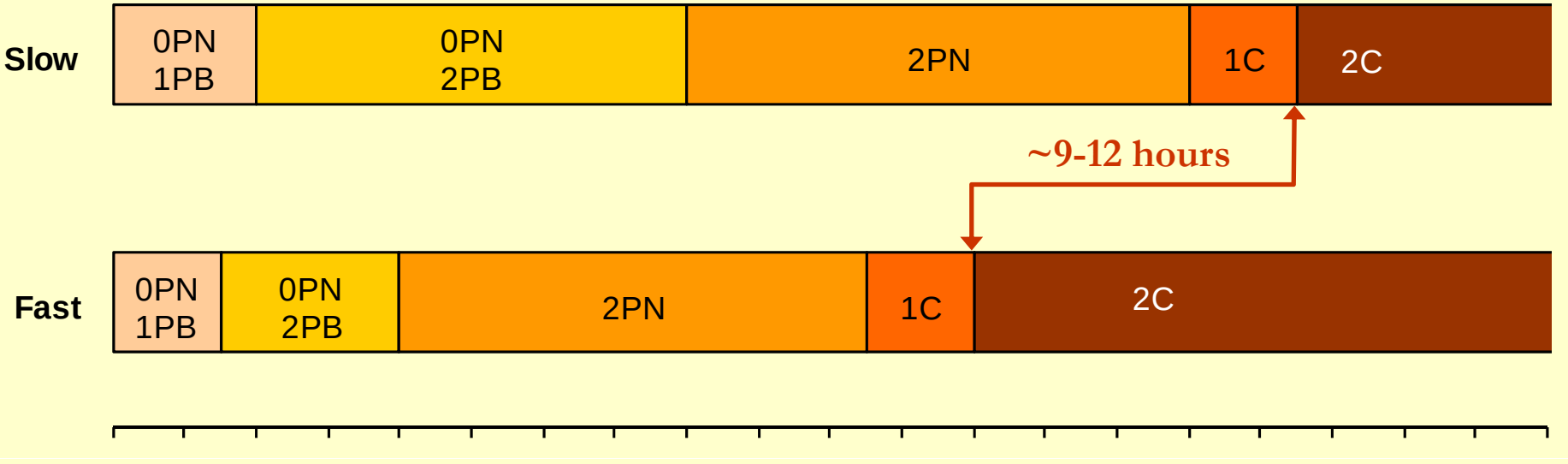
(conventional IVF)

Phase	Length of each phase (h)	Time of appearance (hpi*)	
		First	Last
Extrusion of 2ndPB	2-3	2-3	10
G_1 phase	5-6	2-3	14
S phase	4-5	8-14	20-24
G_2 phase	5-6	12-14	28-30
M phase	3-4	17-22	30-31
2-cell stage		20-25	33-34

*hpi: hours post-insemination

Plachot, 2000

The first cell cycle of a human zygote IV.



Fancsovits et al. 2006

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Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability

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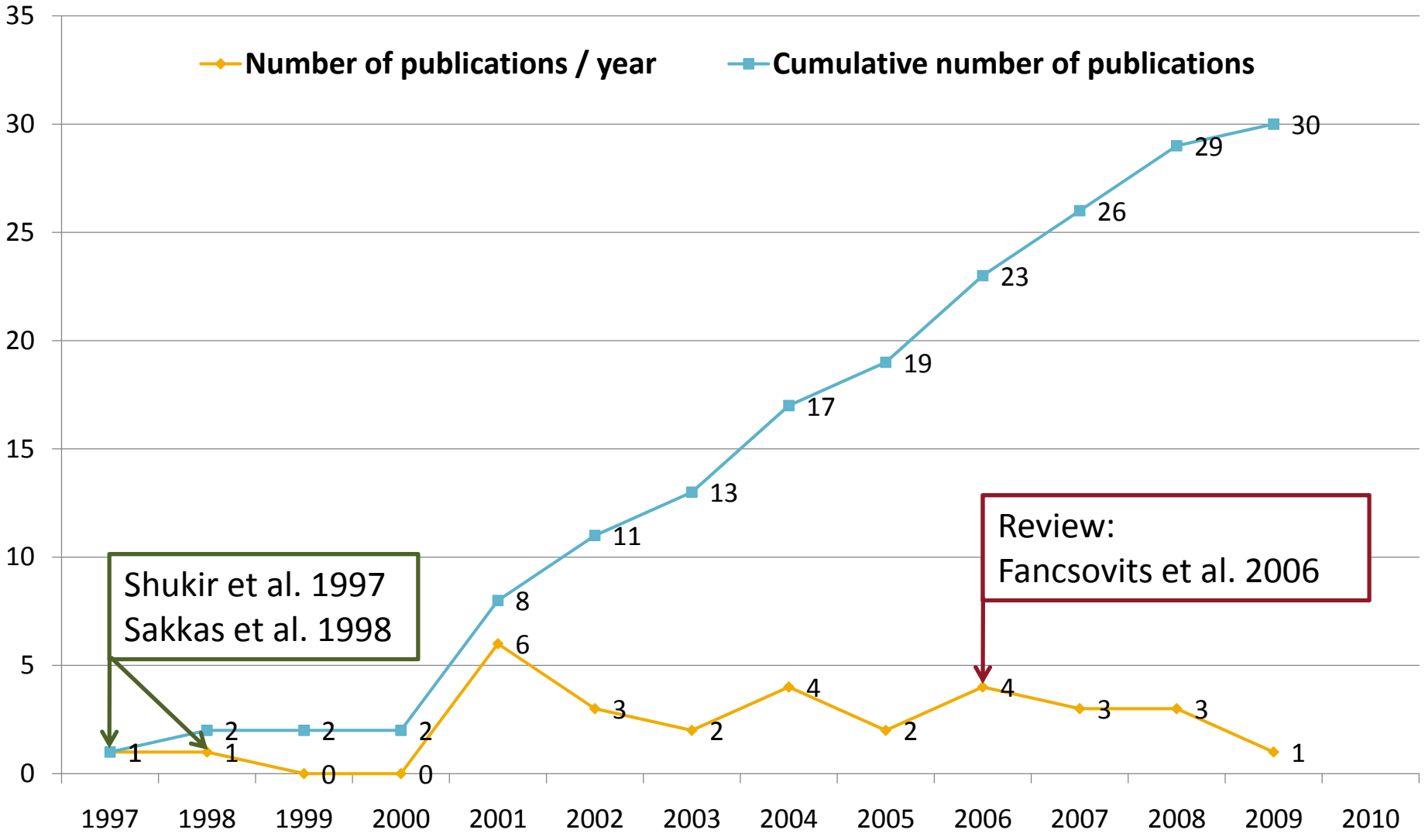
2PN

2 cells

Number of publication on early cleavage



Advantages of early cleavage assessment

- Rapid
- Cheap
- Simple
- Non-invasive
- OBJECTIVE

2PN

2 cells

Correlation between early cleavage and further embryo development

- Number of blastomeres
- Amount of fragmentation
- Morphology score
- Good quality embryos
- Blastocyst formation rate
- Pregnancy and implantation rate

Correlation between early cleavage and number of blastomeres

Table V. Mean number of cells per embryo and mean quality per embryo of embryos transferred in patients with and without early cleavage to the two-cell stage (see Materials and methods for a description of the scoring system)

	Embryos of no early cleavage patients	Embryos of early cleavage patients		
	No early cleavage	All embryos	No early cleavage	Early cleavage
Number of embryos transferred	93	150	47	103
Mean number of cells ± SD	2.7 ± 1.4[a]	3.6 ± 1.1[a]	3.1 ± 1.3[b]	3.8 ± 1.0[b]
Mean quality of cells ± SD	1.8 ± 1.1[c]	2.5 ± 0.7[c]	2.2 ± 0.9[d]	2.7 ± 0.5[d]

Like letters a–a; b–b; c–c; d–d are significantly ($P < 0.01$) different.

Sakkas et al. 1998

Confirmed by:

- Fenwick et al. 2002
- Neuber et al. 2003
- Salumet et al. 2003
- Wharf et al. 2004
- Fancsovits et al. 2005
- Emmiliani et al. 2006
- Chen et al. 2006

Correlation between early cleavage and embryo quality

Table V. Mean number of cells per embryo and mean quality per embryo on day 2 of embryos transferred in patients with and without early cleavage to the 2-cell stage by 25 h post-insemination

	Embryos of no early cleavage patients: No early cleavage	Embryos of early cleavage patients		
		All embryos	No early cleavage	Early cleavage
No. of embryos transferred	322	72	21	51
No. of cells[a]	4.3 ± 1.7	4.5 ± 1.2	4.2 ± 1.7	4.8 ± 1.9
Quality of cells[a]	2.3 ± 0.8[b,c]	2.6 ± 0.6[b]	2.6 ± 0.5	2.7 ± 0.5[c]

[a]Means ± SD.
[b,c]Same letters differ significantly: [b]$P = 0.04$ and [c]$P = 0.02$.

Shukir et al. 1997

Confirmed by:

- Sakkas et al. 1998
- Sakkas et al. 2001
- Fenwick et al. 2002
- Neuber et al. 2003
- Salumet et al. 2003
- Van monfort et al. 2004
- Fancsovits et al. 2006
- Chen et al. 2006
- Hesters et al. 2008
- Fu et al. 2009

Correlation between early cleavage and blastocyst formation rate

Table II. Proportion of early cleaving (EC) and non-early cleaving (NEC) embryos developing to expanded or hatching blastocyst stage following extended culture to day 7

	EC	NEC
Embryos cultured	59	367
Blastocyst (%)	19 (32.2)[a]	61 (16.6)[a]
Hatched (%)	11 (18.6)[b]	26 (7.1)[b]

Same letters indicate significant differences, χ^2-test: [a]$P < 0.01$; [b]$P < 0.005$.

Confirmed by:

- Neuber et al. 2003
- Van Monfort et al. 2004
- Windt et al. 2004
- Guerif et al. 2007

Fenwick et al. 2002

Correlation between early cleavage and clinical pregnancy rate

Clinical pregnancy rate (%)				*P*	Time of assessment (hours)	Source
Early cleavage		Late cleavage				
9/27	(33.3)	17/119	(14.7)	0.045	25	*Shoukir et al.*1997
14/54	(25.9)	2/34	(5.9)	0.04	27	*Sakkas et al.*1998
124/306	(40.5)	163/521	(31.3)	0.005	25-27	*Lundin et al.* 2001
38/98	(38.8)	41/160	(25.6)	0.026	24-26	*Tsai et al.* 2002
36/72	(50.0)	28/106	(26.4)	0.001	25-27	*Salumets et al.* 2003
41/95	(43.2)	20/58	(17.2)	<0.001	25-27	*Wharf et al.* 2004
26/63	(41.3)	16/80	(20.0)	0.0092	25-27	*Windt et al.* 2004
28/58	(48.3)	38/139	(27.3)	0.0045	22-25*	*Fancsovits et al.* 2005
40/139	(28.8)	30/205	(14.6)	0.0014	25	*Hammoud et al.* 2008
372/962	(38.7)	35/133	(26.3)	<0.01	25-27	*Fu et al.* 2009

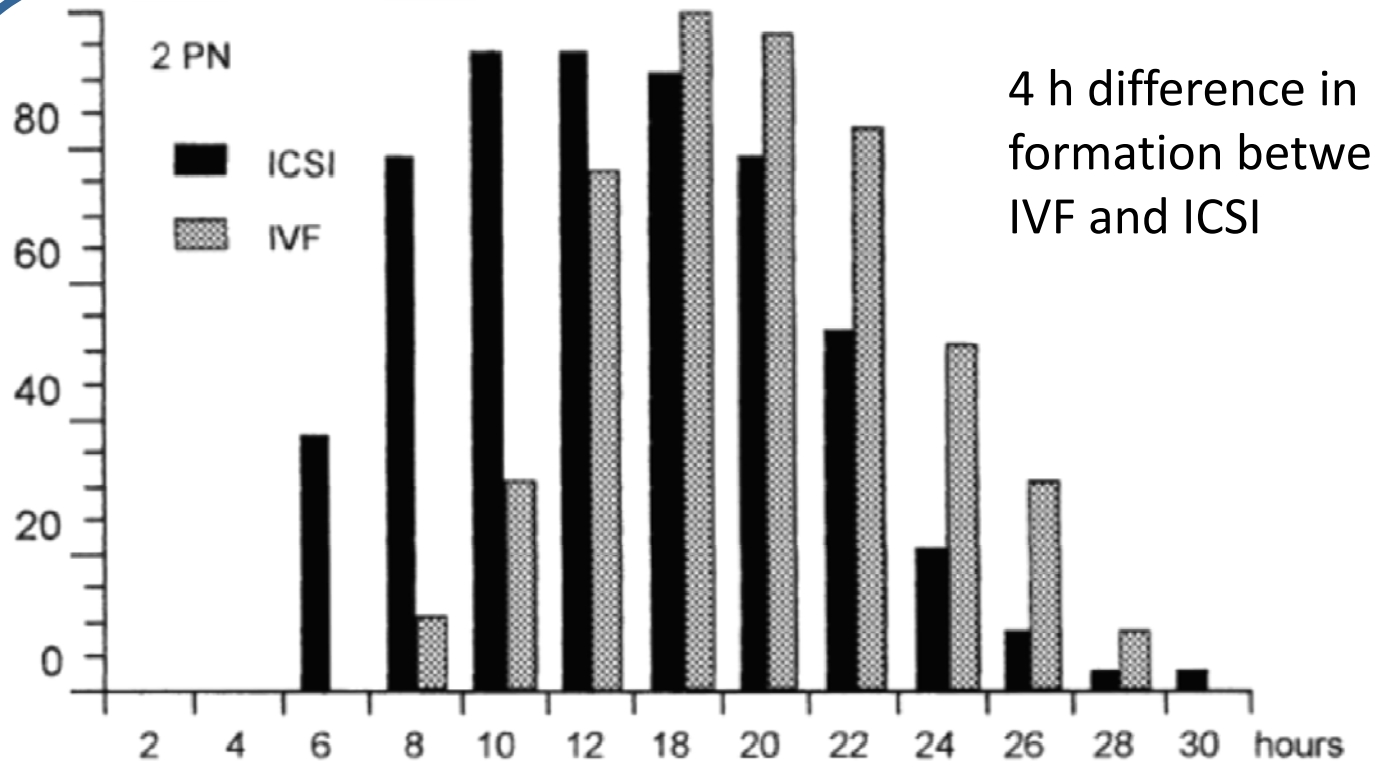
Correlation between early cleavage and implantation

Implantation rate (%)				*P*	Time of assessment (hours)	Sorce
Early cleavage		Non early cleavage				
27/72	(23.6)	24/322	(7.5)	0.0001	25	*Shoukir et al.1997*
21/150	(14.0)	3/93	(3.2)	0.01	27	*Sakkas et al.1998*
28/152	(18.0)	9/115	(8.0)	0.024	25-27-29	*Bos-Mikich et al.* 2001
58/219	(25.5)	43/290	(14.8)	<0.01	25-28	*Sakkas et al.* 2001
165/589	(28.0)	200/1057	(19.5)	<0.0001	25-27	*Lundin et al.* 2001
15/70	(21.5)	5/83	(6.0)	<0.005	24.5-25.5	*Fenwick et al.* 2002
40/183	(21.9)	53/371	(14.3)	0.03	23-28	*Neuber et al.* 2003
41/155	(26.5)	61/404	(15.1)	0.0019	22-25*	*Fancsovits et al.* 2005
105/250	(42.0)	42/152	(27.6)	0.0067	25-27	*Hesters et al.* 2008
41/270	(15.2)	30/394	(7.6)	0.0014	25	*Hammoud et al.* 2008

The optimal time of early cleavage assessment

- 23 – 29 hours post insemination
- Same time for IVF and ICSI?
- Assessing early pronuclear breakdown or early cleavage?

Same time for IVF and ICSI?

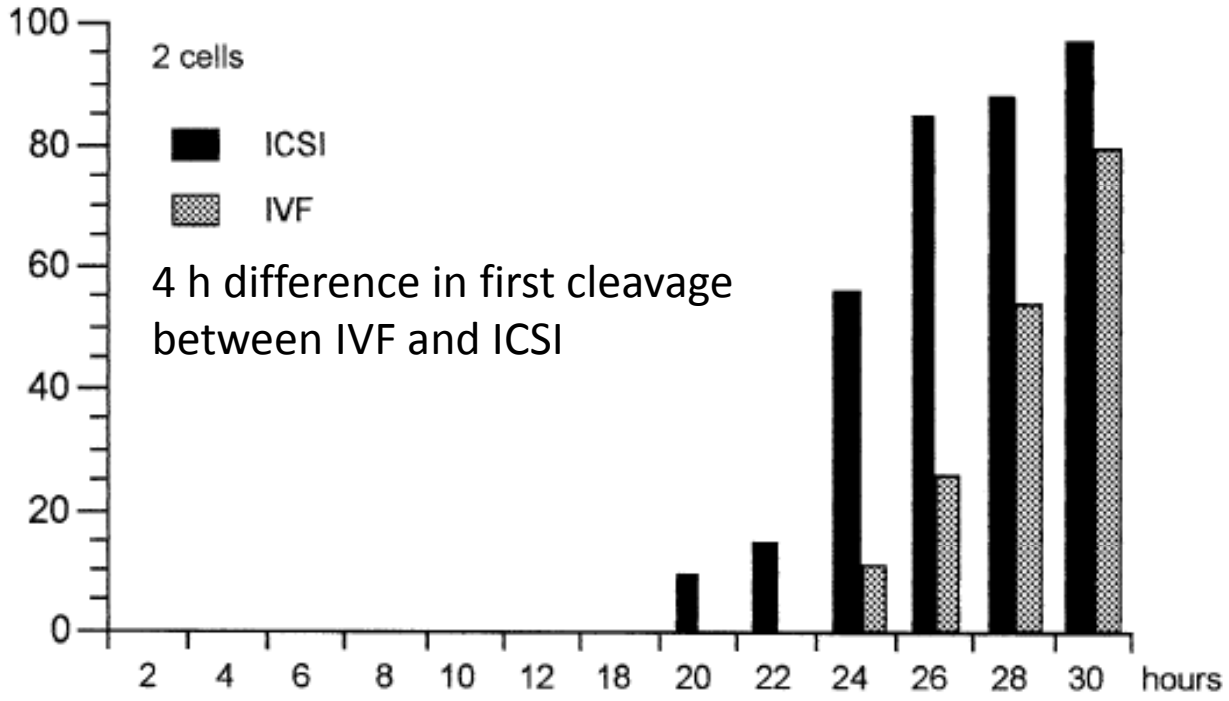


4 h difference in PN formation between IVF and ICSI

Figure 2. Timing of pronucleus (PN) formation and disappearance in the normally fertilized oocyte groups after intracytoplasmic sperm injection (ICSI, $n = 34$) and after in-vitro fertilization (IVF, $n = 35$) using ejaculated spermatozoa on sibling oocytes, expressed in percentages.

Nagy et al. 1998

Same time for IVF and ICSI?



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Nagy et al. 1998

Same time for IVF and ICSI?

Fertilization steps need time in conventional IVF treatments

- Penetrating the cumulus and corona radiata cells
- Binding to the zona pellucida
- Penetrating the zona pellucida

EC from IVF	EC from ICSI	P-value	Time of assessment	Author
21.5%	34.7%	<0.0001	25-27 h	Lundin et al. 2001
22.8%	28.5%	NS	25-27 h	Wharf et al. 2004
37.1%	41.1%	NS	22-25 h	Fancsovits et al. 2005
44.6%	37.5%	NS	25 h	Hammoud et al. 2008
19%	36%	<0.001	26 h	Terriou et al. 2007

Contradictory data

More studies and / or metaanalysis needed

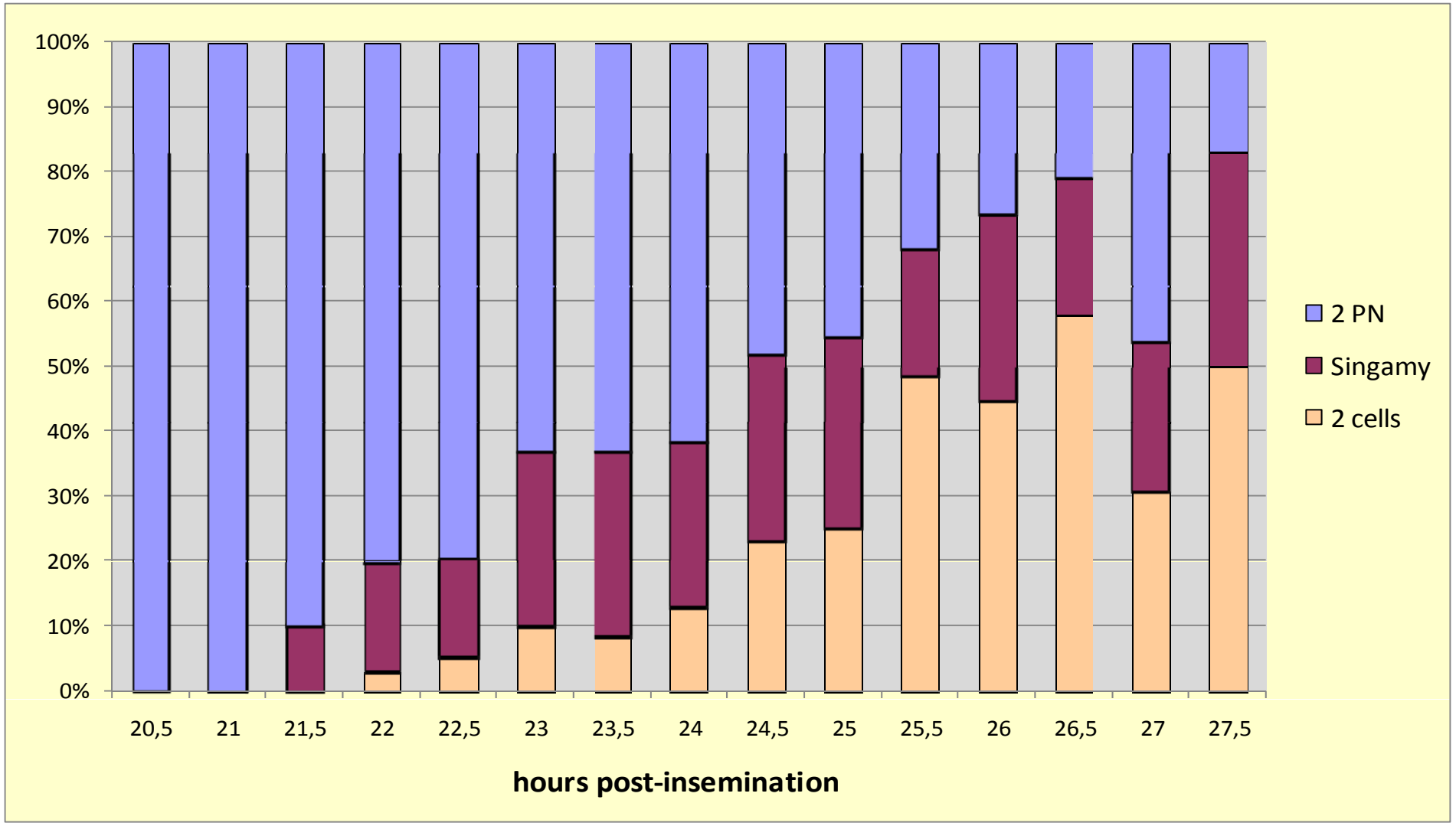
Timing of observations

- **Standardized timing relative to insemination time**

Observation	Timing (hours post-insemination)	Expected stage of development
Fertilization check	17 ± 1 h	Pronuclear stage
Syngamy check	23 ± 1 h	Up to 50% in syngamy (up to 20% at the 2-cell stage)
Early cleavage check	26 ± 1 h post-ICSI 28 ± 1 h post-IVF	2-cell stage
Day 2 assessment	44 ± 1 h	4-cell stage
Day 3 assessment	68 ± 1 h	8-cell stage
Day 4 assessment	92 ± 2 h	Morula
Day 5 assessment	116 ± 2 h	Blastocyst

Balaban B. & Magli C: The Istanbul Consensus Workshop on Embryo Assessment: Proceedings of an Expert Meeting. Human Reproduction 2011. ***in press***

Early cleavage and early pronuclear breakdown according to the time of assessment



Assessment of early pronuclear breakdown

Author	Time of assessment
Neuber et al. 2003	23 – 28 h
Wharf et al. 2004	25 – 27 h
Fancsovits et al. 2005	22 – 25 h
Chen and Kattera 2006	26 h
Terriou et al. 2007	26 h
Hesters et al. 2008	25 – 27 h
Hammoud et al. 2008	25 h

Assessment of early pronuclear breakdown

Early pronuclear breakdown is a good indicator of embryo quality and viability

Peter Fancsovits, M.Sc., Laszlone Toth, Zoltan F. Takacs, M.D., Akos Murber, M.D., Zoltan Papp, D.Sc., and Janos Urbancsek, Ph.D.

First Department of Obstetrics and Gynaecology, Semmelweis University School of Medicine, Budapest, Hungary

Objective: To examine whether the timing of pronuclear breakdown can be a predictor of embryo quality and viability.
Design: Retrospective comparison of the development and quality of early and late developing zygotes.
Setting: Infertility and endocrinology unit in a university hospital.
Patient(s): One thousand seven hundred eighty-two zygotes obtained in 383 consecutive IVF cycles.
Intervention(s): Culture of all fertilized embryos.
Main Outcome Measure(s): Number of fertilized zygotes showing early pronuclear breakdown at 22–25 hours postinsemination, embryo quality, pregnancy rates (PR), implantation rates.
Result(s): Early pronuclear breakdown embryos had a significantly higher cell number (4.4 ± 1.2) compared with the late pronuclear breakdown embryos (3.6 ± 1.4). When comparing the frequency of the early pronuclear breakdown embryos according to the method of fertilization, we failed to find any significant difference between the IVF (37.1%) and the intracytoplasmic sperm injection (ICSI) (41.1%) groups. The transfer of early pronuclear breakdown embryos resulted in a significantly higher clinical pregnancy rate than those with late pronuclear breakdown (48.3% vs. 27.3%). The implantation rate was higher in the early pronuclear breakdown group than in the late pronuclear breakdown group (26.5% vs. 15.1%).
Conclusion(s): Early pronuclear breakdown is a strong indicator of embryo viability, and may be used as an additional criterion in the selection of embryos for transfer. (Fertil Steril® 2005;84:881–7. ©2005 by American Society for Reproductive Medicine.)

Key Words: Early pronuclear breakdown, early cleavage, embryo quality, pregnancy rates, implantation rate

Fertility and Sterility® Vol. 84, No. 4, October 2005

Assessment of early pronuclear breakdown

- Both in IVF and ICSI treatments
- 22-25 hours after insemination
- Presence of pronuclei or passing the first cleavage

2PN

1 cell

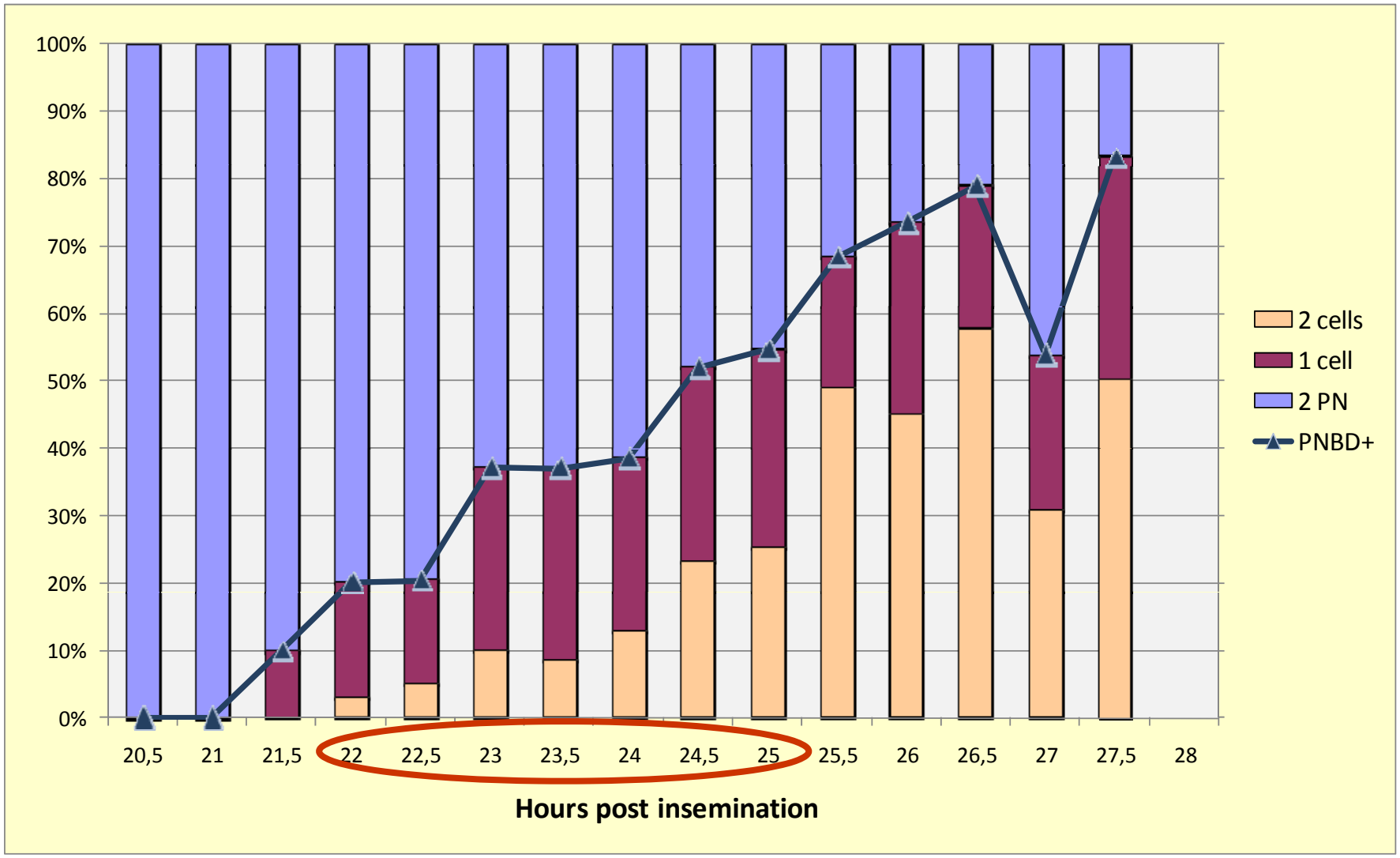
2 cells

Correlation between early pronuclear breakdown and further embryo development

Outcomes measures

- Speed of development
- Embryo morphology
- Pregnancy and implantation rates

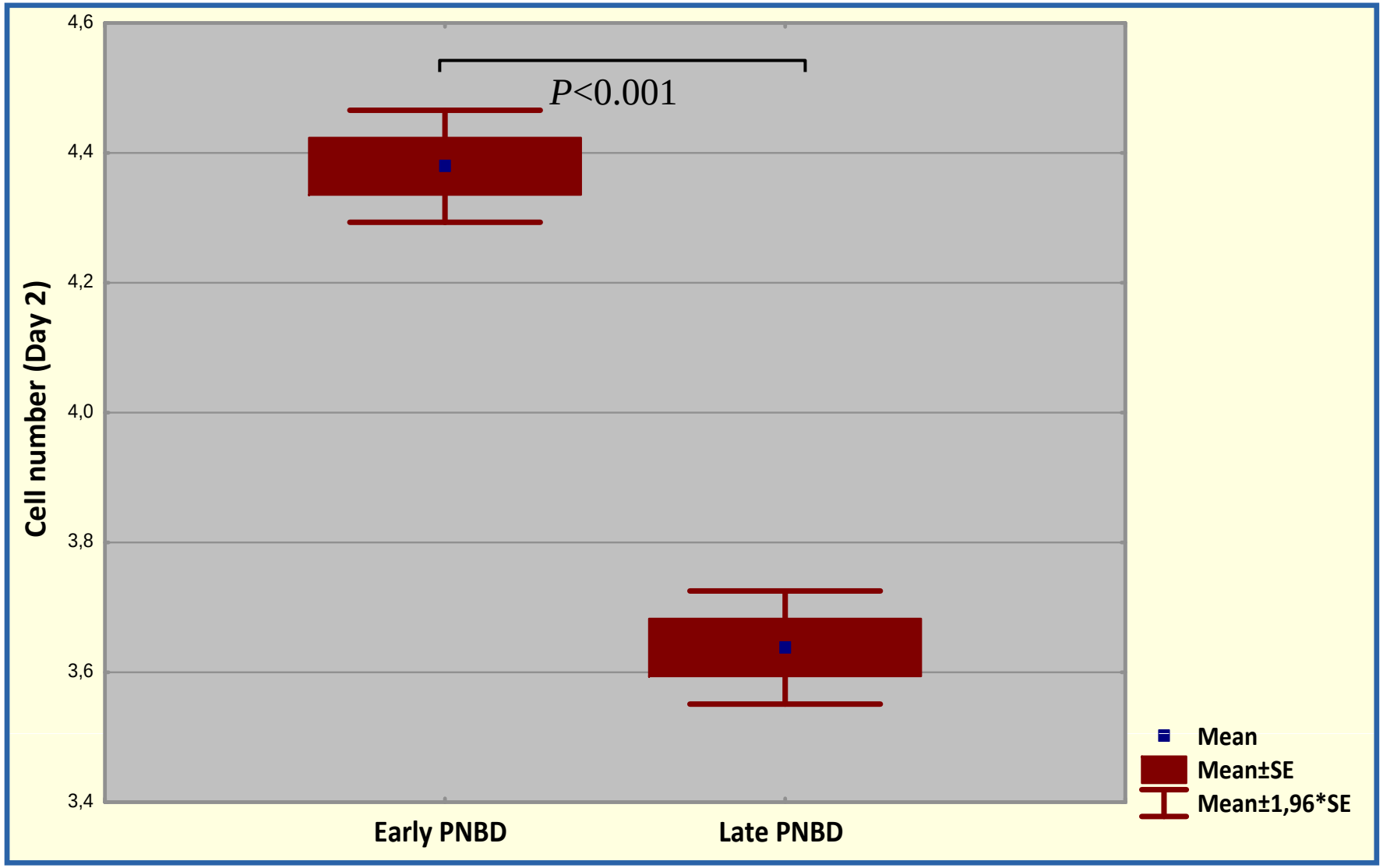
Determining the optimal time of the assessment of pronuclear breakdown



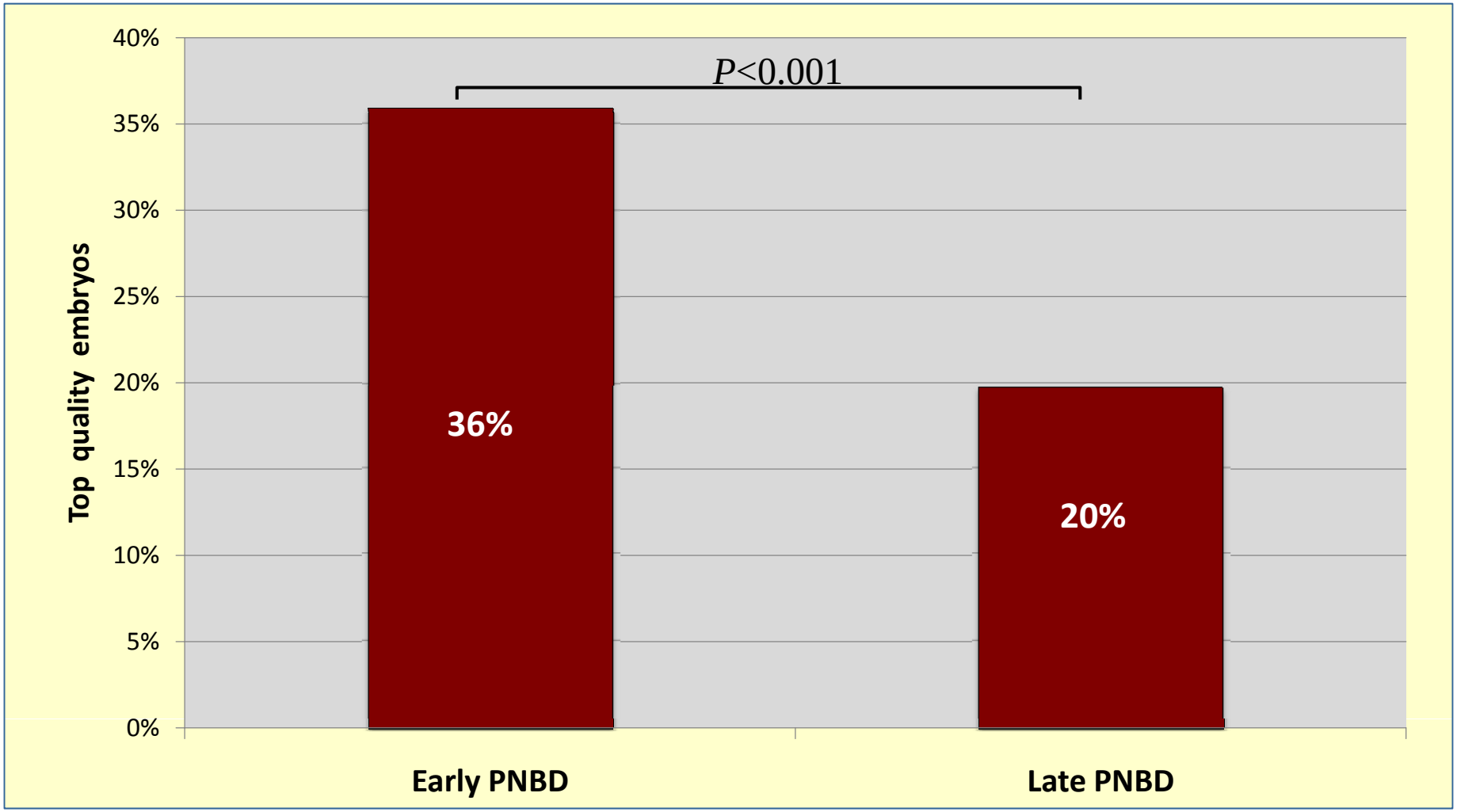
Morphological characteristics of early and late PNBD embryos

	Early PNBD	Late PNBD	*P*-Value
Number of embryos	688	1048	—
Number of blastomeres	4.4 ±1.2	3.6 ±1.4	<0.001
Amount of fragmetnation (%)	16.6 ±13.1	16.9 ±13.4	NS
Morphology scoore	2.4 ±0.6	2.3 ±0.6	NS
Top quality embryos (%)	35.9	19.7	<0.001
Embryos with multinucleation (%)	4.8	9.3	<0.001

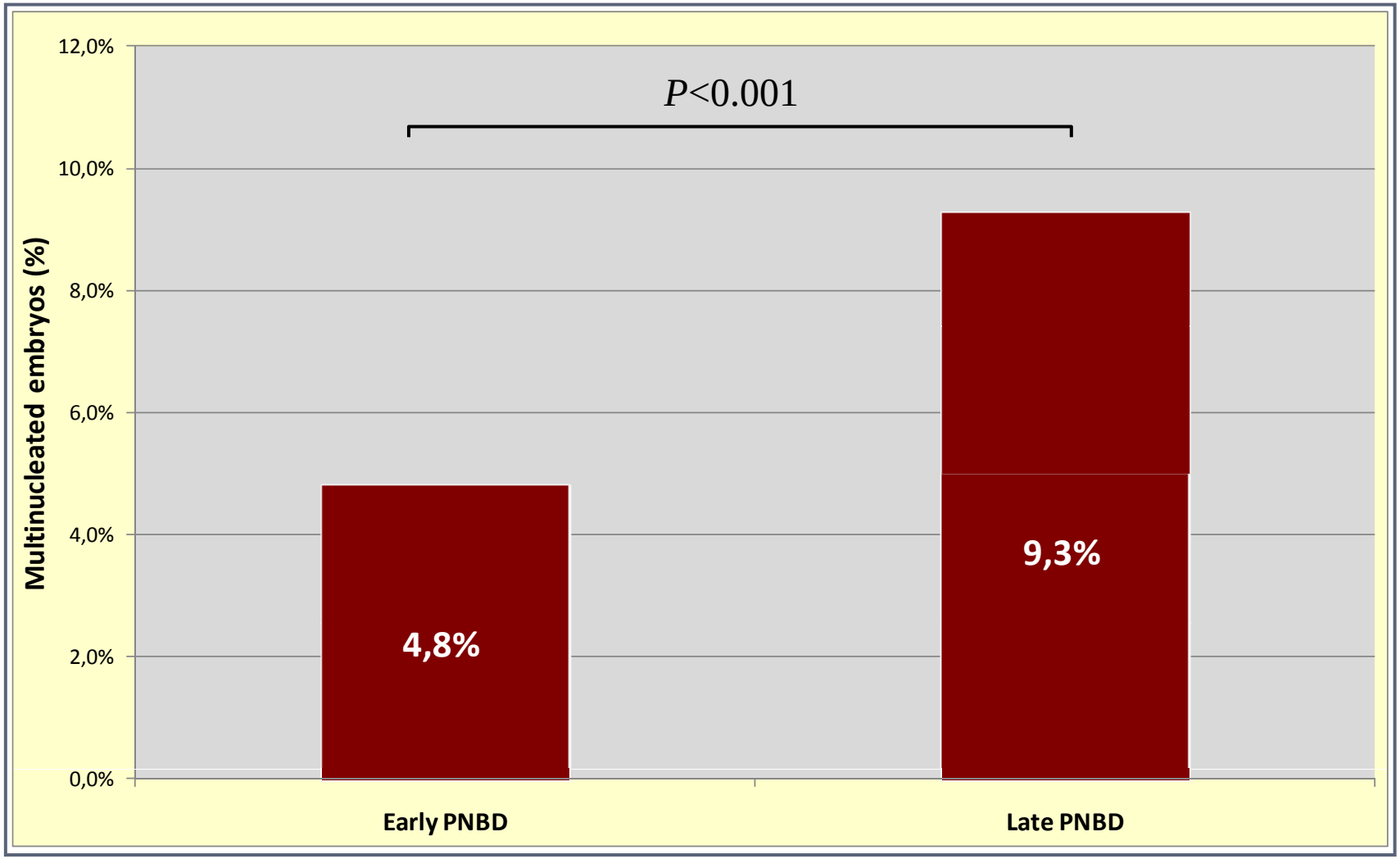
Numbers of blastomeres in early and late PNBD embryos



Frequency of top quality embryos



Frequency of multinucleated embryos

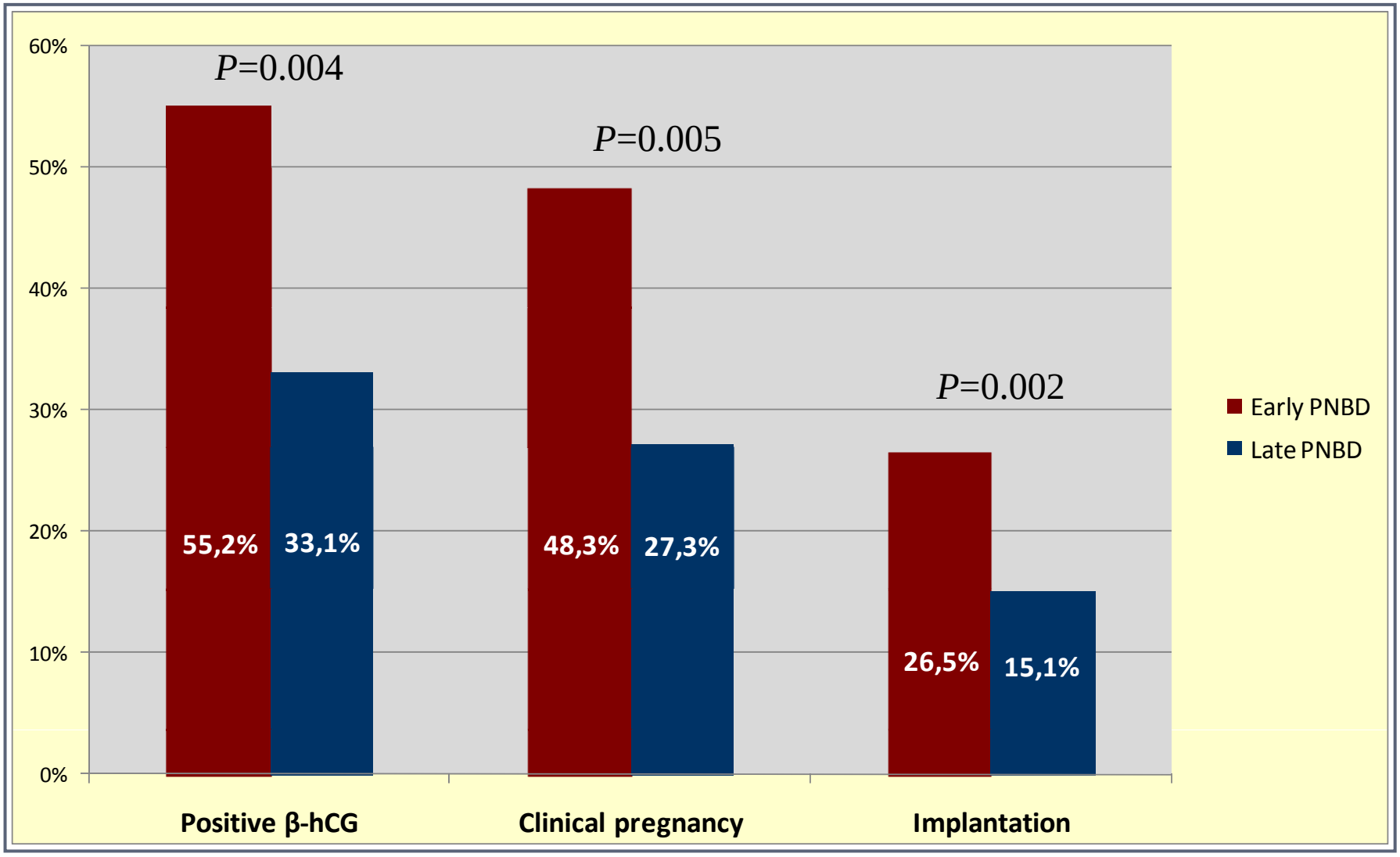


Confirmed by Hesters et al. 2008

Pregnancy and implantation rates in early and late PNBD cycles

	Early PNBD	Late PNBD	*P*-value
Number of IVF-ET cycles	58	139	–
Number of oocytes	6.7 ±4.0	7.2 ±3.6	NS
Number of ET embryos	2.7 ± 1.0	2.9 ± 0.9	NS
+βhCG	55.2%	33.1%	0.004
Clinical pregnancy	48.3%	27.3%	0.005
Implantation rate	26.5%	15.1%	0.002

Pregnancy and implantation rates in early and late PNBD cycles

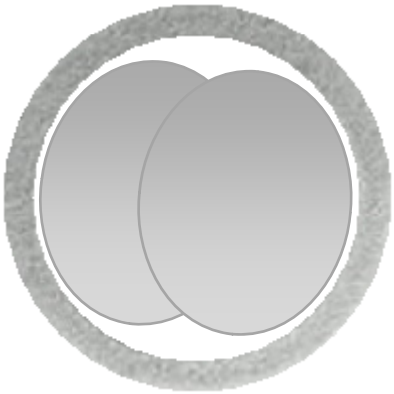


Early pronuclear breakdown

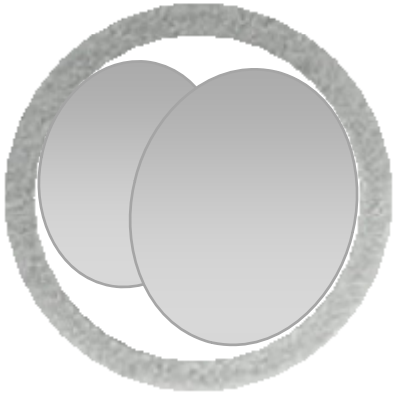
- Should be assessed between 22-25 hours post insemination
- Strongly correlates with the dynamics of subsequent cleavage cycles
- Has limited effect on further embryo morphology
- Strongly correlates with the implantation potenital of the embryos

Morphology of the early cleaved embryo I.

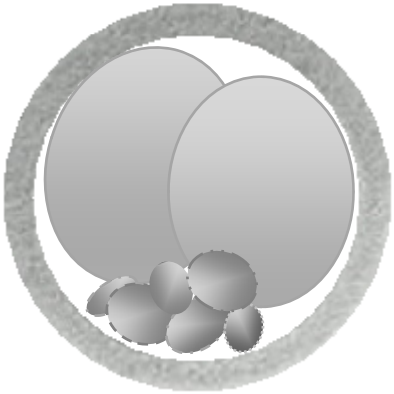
Cleavage pattern



Even



Uneven



Fragmented

Ciray et al. 2006

Even early cleaved embryos:

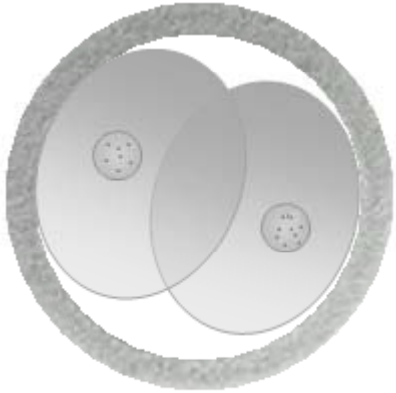
- Better embryo morphology
- Higher implantation rate

Confirmed by:

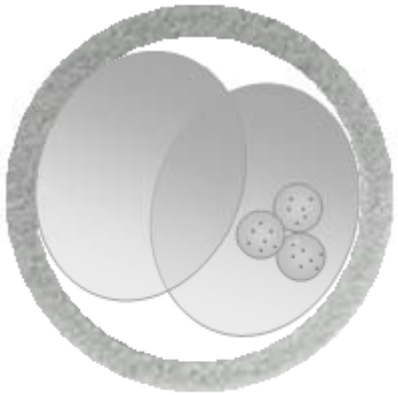
- Terriou et al. 2007
- Hesters et al. 2008

Morphology of the early cleaved embryo II.

Nuclear morphology



Mononucleated



Nonmononucleated
multinucleated or
no visible nucleI

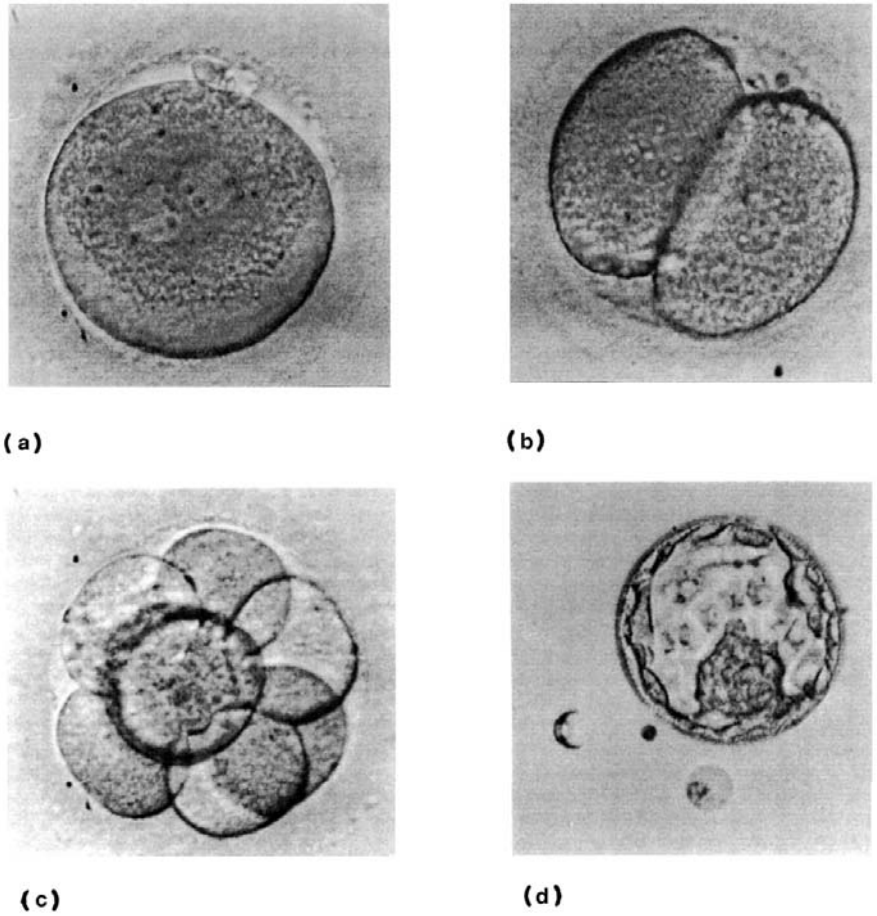
Ciray et al. 2006

Mononucleated early cleaved embryos:

- Better embryo morphology
- Higher implantation rate

Early cleavage in scoring systems

Fisch et al. 2001: The Graduated Embryo Score (GES)



(a) Embryo evaluated at 16–18 h post insemination demonstrating nucleolar alignment along the pronuclear axis;

(b) embryo evaluated at 25–27 h post insemination demonstrating symmetrical blastomere cleavage and no fragmentation;

(c) embryo evaluated at 64–67 h post insemination demonstrating symmetrical cleavage, eight cells and no fragmentation;

(d) fully expanded blastocyst at ~120 h post insemination.

Early cleavage in scoring systems

Table I. Graduated Embryo Scoring of cleavage-stage embryos

Evaluation	Hours after insemination	Developmental milestone	Score
1	16–18	Nucleoli aligned along pronuclear axis	20
2	25–27	Cleavage regular and symmetrical	30
		Fragmentation[a]: Absent	30
		<20%	25
		>20%	0
3	64–67	Cell number and grade[b]:	
		7, I; 8, I; 8, II; 9, I	20
		7, II; 9, II; 10, I; 11, I; compacting I	10
Total score			100

[a]If the embryo was not cleaved at 25–27 h, grading of fragmentation should occur at the 64–67 h evaluation.
[b]Grade I = symmetrical blastomeres and absent fragmentation; grade II = slightly uneven blastomeres and <20% fragmentation; grade III = uneven blastomeres and >20% fragmentation.

GES: predicting blastocyst formation, pregnancy and implantation

Fisch et al. 2001

The cause of early cleavage

Shoukir et al. 1997
„it could be an intrinsic factor within oocyte and embryo”

Lundin et al. 2001
Higher „metabolic fitness” of the embryo

The cause of early cleavage

Paternal factor

- Centrioles introduced by the spermatozoon
- Differences of individual sperm to stimulate calcium transients
- DNA status of the spermatozoa

Maternal factor

- Oocyte maturity (cytoplasmic and nuclear)
- Correct spatial arrangement of the oocyte
- Oocyte's ability to respond to the calcium transients stimuli
- DNA replication (shorter S-phase)
- Chromosomal abnormalities

Conclusions I.

Optimal time of assessment:

- 26 (±1) hours for early cleavage
- 23 (±1) hours for singamy

Early cleavage (or singamy) correlates with

- faster development
- better embryo quality
- higher blastocyst formation rate
- higher implantation and pregnancy rates

Conclusions II.

Morphology of early cleaved embryos correlates to further embryo development and implantation

Assessment of early cleavage or early pronuclear breakdown **should be included in the scoring system** to select the most viable embryos for transfer