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OÖ. GESUNDHEITS- UND SPITALS-AG

Kinderwunsch Zentrum Linz
an der Landes- Frauen- und Kinderklinik

Why do some embryos arrest in vitro?

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Linz, Austria

TAJEV 2014
April 30th – May 4th
Antalya

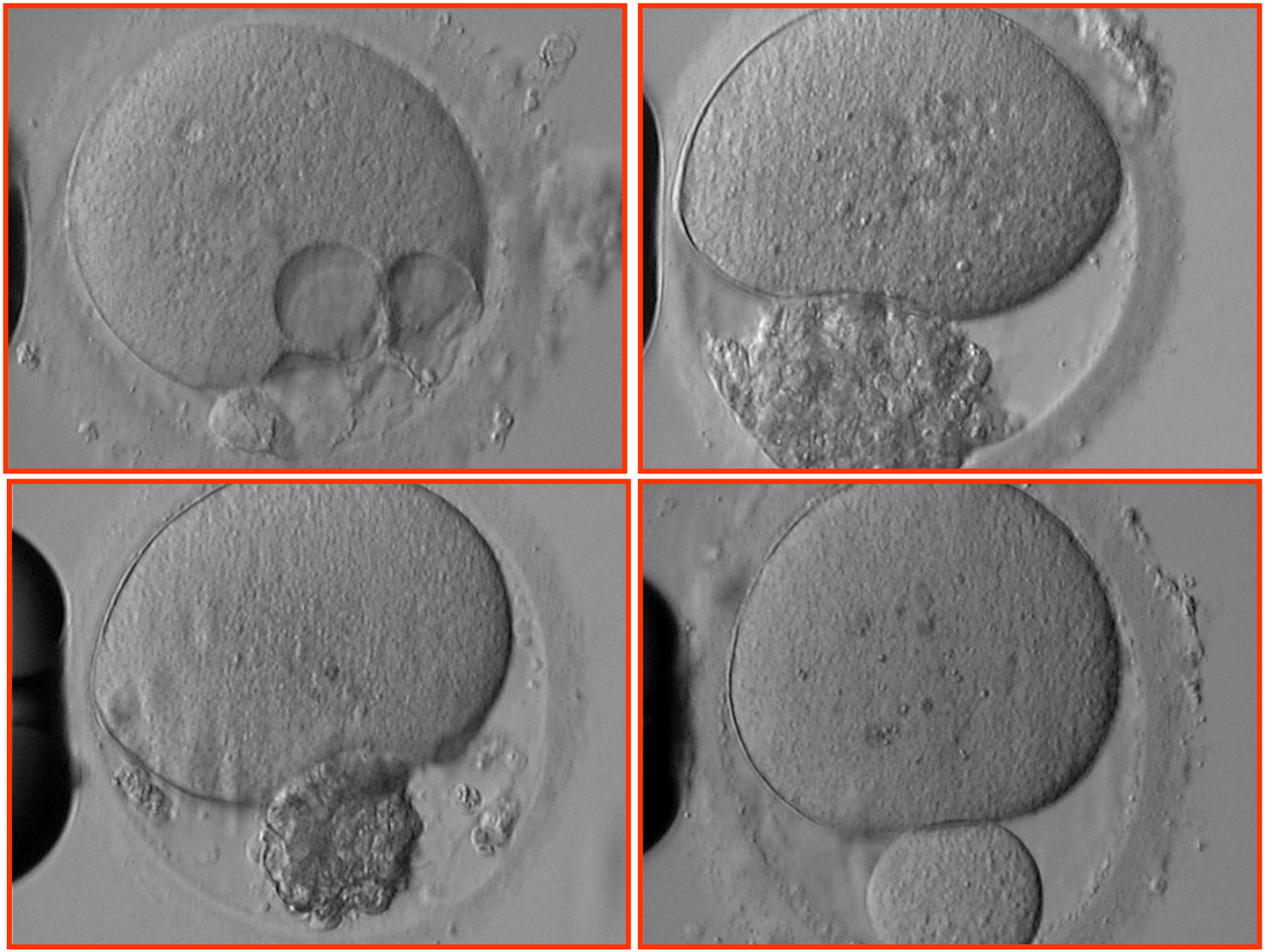
Possible reasons for permanent embryo arrest

- culture conditions
- genetics
- cellular components (e.g. mitotic spindle)

- ✓ morphological anomalies of oocyte/embryo
- ✓ molecular components

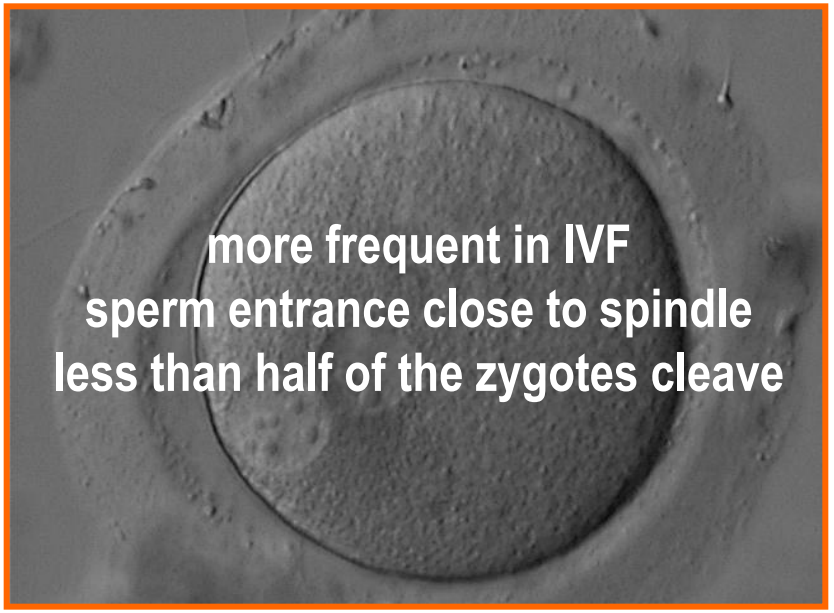
MORPHOLOGICAL ANOMALIES

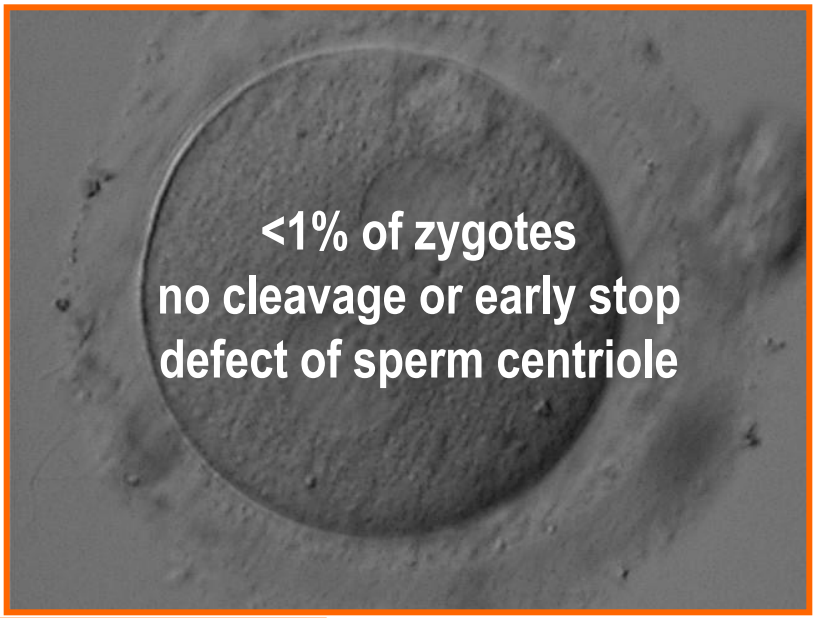
at oocyte stage

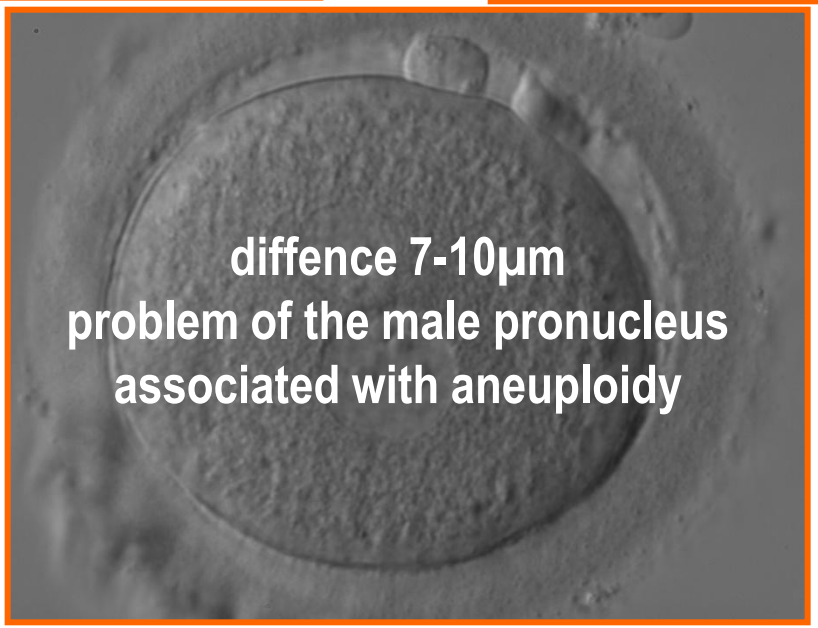


MORPHOLOGICAL ANOMALIES

at zygote stage

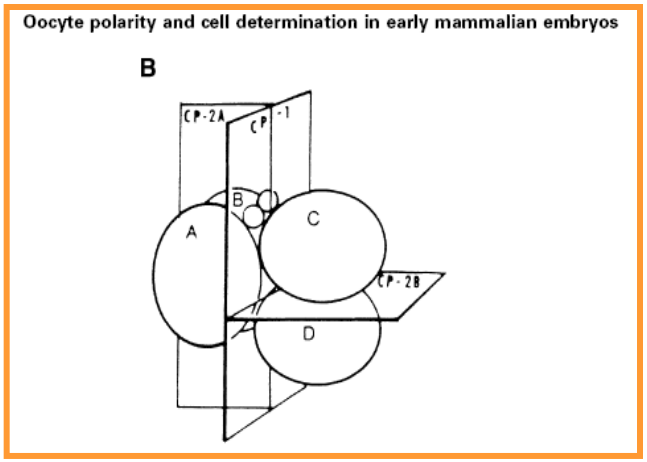




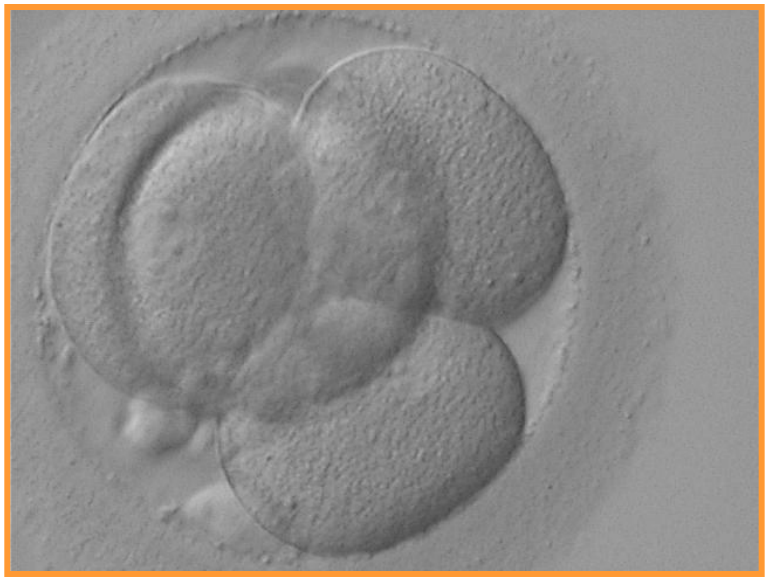


MORPHOLOGICAL ANOMALIES

at cleavage stage

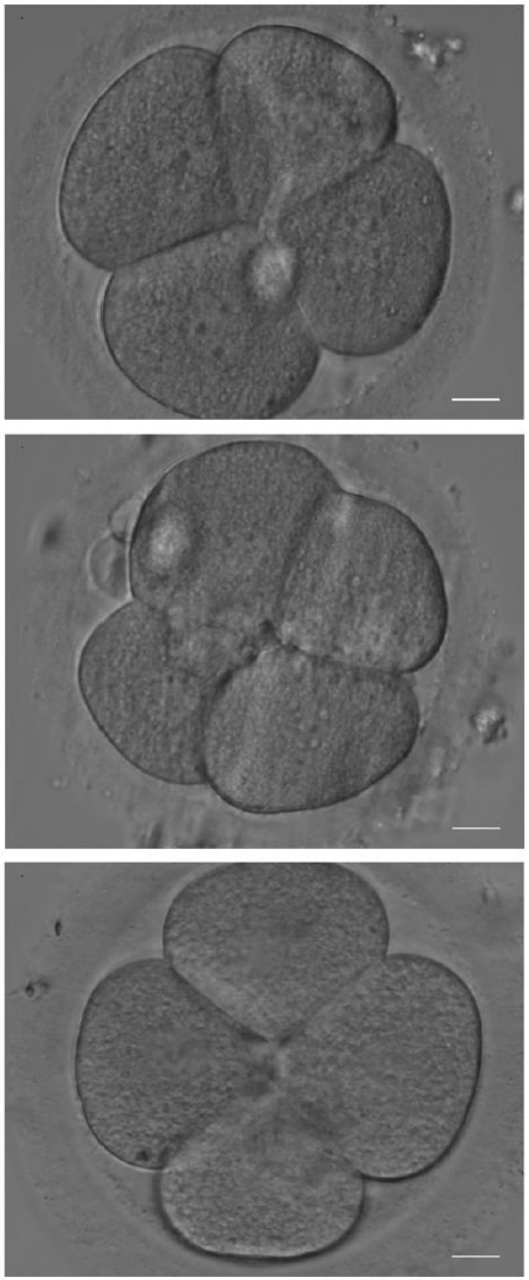


Edwards und Beard, Mol Hum Reprod, 1997



- zona-free embryos
- clover-shaped embryos
- ovoid embryos

1. Clover-shaped embryos



Reproductive BioMedicine Online (2012) **25**, 267–272

Planar embryos have poor prognosis in terms of blastocyst formation and implantation

T Ebner [a,*], M Maurer [b], O Shebl [a], M Moser [a], RB Mayer [a], HC Duba [b], G Tews [a]

[a] *Landes- Frauen- und Kinderklinik, IVF-Unit, Linz, Upper Austria, Austria;* [b] *Landes- Frauen- und Kinderklinik, Department of Human Genetics, Linz, Upper Austria, Austria*

Table 2 Fertilization and preimplantation development in planar embryos and their sibling normal tetrahedral counterparts (data from 50 cycles).

	Planar embryos	*Tetrahedral embryos*	*P-value*
Number of MII oocytes	64	497	
Cleavage	64 (100.0)	494 (99.4)	NS
Top-quality day-2 embryos	55 (85.9)	340 (68.8)	<0.005
Top-quality day-3 embryos	58 (90.6)	327 (66.2)	<0.001
Multinucleation day 2	1 (1.6)	41 (8.3)	NS
Blastocyst formation	12/53 (22.6)	251/441 (56.9)	<0.001
Good-quality blastocyst	6/12 (50.0)	188/251 (74.9)	NS
Implantation rate	0/11	19/43 (44.2)	<0.01

Values are *n* or *n*/total (%).
For calculation of blastocyst formation, embryos being transferred on day 3 could not be taken into account.
MII = metaphase II; NS = not statistically significant.

➢better quality at cleavage stage
➢less blastocysts
➢no implantation

Figure 1 Planar intracytoplasmic sperm injection (**A**, **B**) o IVF (**C**) embryos on day 2 of preimplantation development Second polar body was either located close to the commoı boundary of all four cells (**A**) or between two of them (**B**) Bars = 14 μm.

✓planar constellation of four blastomeres
✓presumed incomplete cleavage

2. Ovoid embryos

Human Reproduction Vol.23, No.1 pp. 62–66, 2008

Developmental fate of ovoid oocytes

T. Ebner[1], O. Shebl, M. Moser, M. Sommergruber and G. Tews

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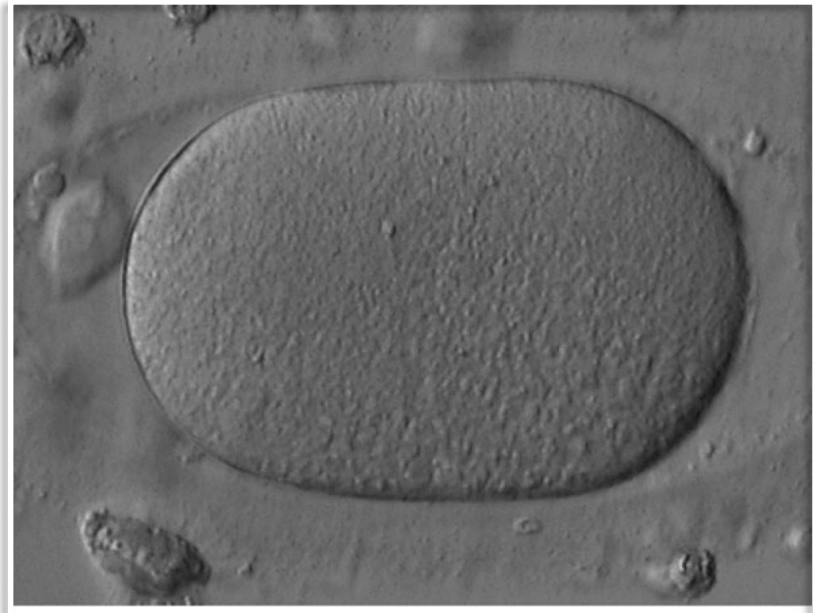


Figure 1: Shape anomalies of oocytes
(**a**) Ovoid oocyte with RI of 1.50 (oocyte) and 1.82 (ZP).

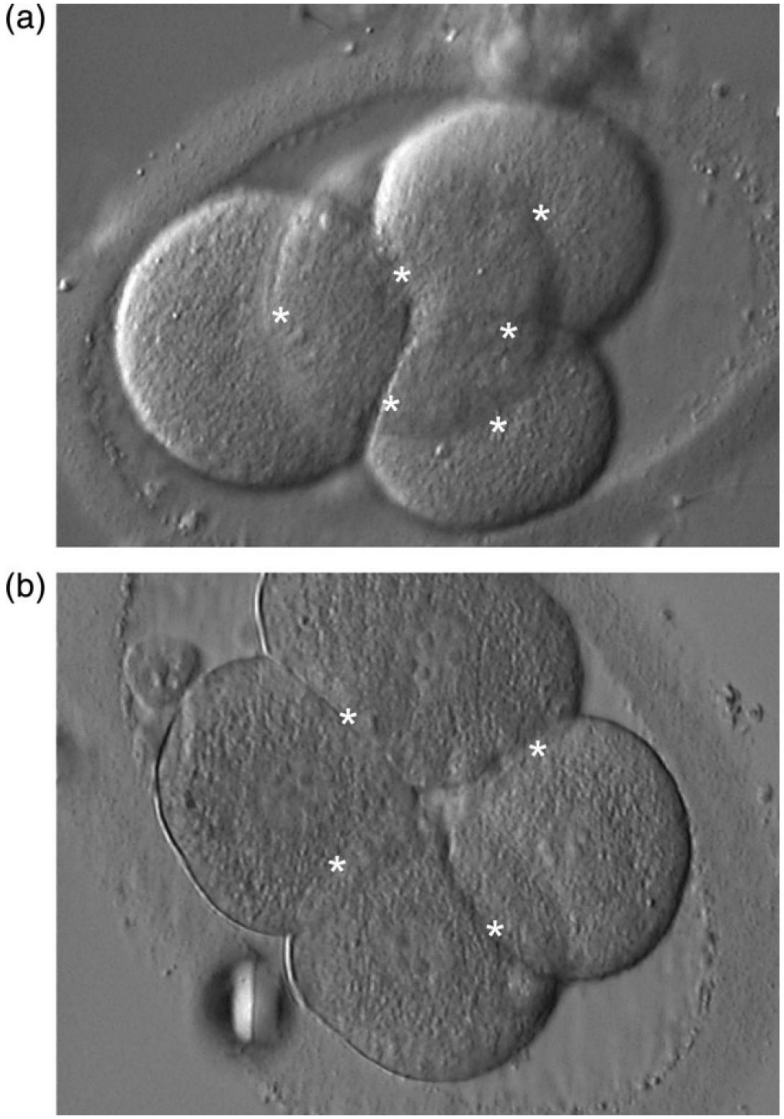
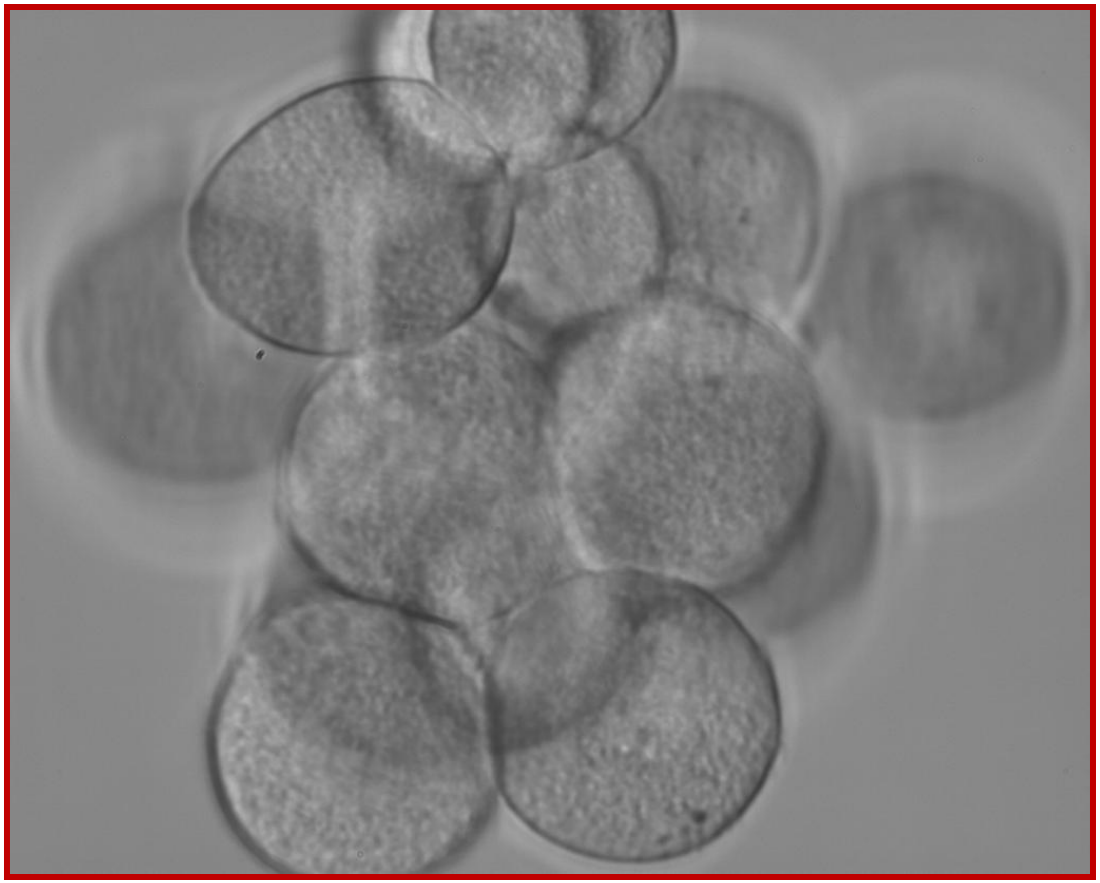


Figure 2: Cleavage patterns of Day 2 embryos
(**a**) Regularly cleaved four-cell embryo with ovoid ZP (RI of 1.40). Asterisks indicate cell–cell contacts between blastomeres ($n = 6$). (**b**) Irregular Day 2 embryo with one cleavage plane (RI of 1.44) and only four cell–cell contacts between blastomeres (asterisks)

Table II. Fertilization and cleavage patterns in ovoid oocytes and their sibling spheric counterparts.

	Ovoid oocytes	Round oocytes	*P*-value
Number of MII oocytes	137	690	
Fertilization (2PN)	102 (74.5)	524 (75.9)	0.71
Cleavage	99 (97.1)	514 (98.1)	0.50
Cleavage pattern (Day 2)			
Six contact points	60 (60.6)	480 (93.4)	<0.001
Five contact points	12 (12.1)	30 (5.8)	<0.05
Four contact points	27 (27.3)	4 (0.8)	<0.001
Prolonged culture to day 5	84	408	
Compaction (Day 4)			
Six contact points	26/60 (43.3)	175/387 (45.2)	0.79
Five contact points	4/12 (33.3)	7/17 (41.2)	0.67
Four contact points	0/12	0/4	
Blastocyst formation (Day 5)			
Six contact points	13/60 (21.7)	120/387 (31.0)	0.14
Five contact points	2/12 (16.7)	7/17 (41.2)	0.16
Four contact points	0/12	0/4	
Overall blastulation:	**18%**	**32%**	

3. Zona-free embryos

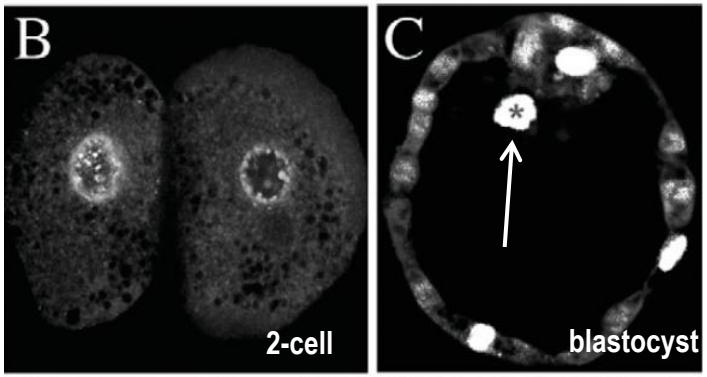


HUMAN MODEL

- Approximately 10% of all human embryos stop cleavage until 4-cell stage.
  - 1% (early cleavage 26-28 hpi)
  - 15% (32 hpi)
- 40% of all patients have at least one embryo with permanent arrest

- Before 8-cell-stage (day 3) no morphological, biochemical or molecular markers of apoptosis can be found
- Thus, permanent developmental arrest is a NON-apoptotic process
- On the other hand, in 2-4-cell embryos certain agents can induce mitochondrial depolarisation and inhibition of protein kinases which can lead to apoptotic behavior

- Consequently, the molecular machinery of apoptosis is inherent, but it cannot be used properly, e.g., due to immature mitochondria.

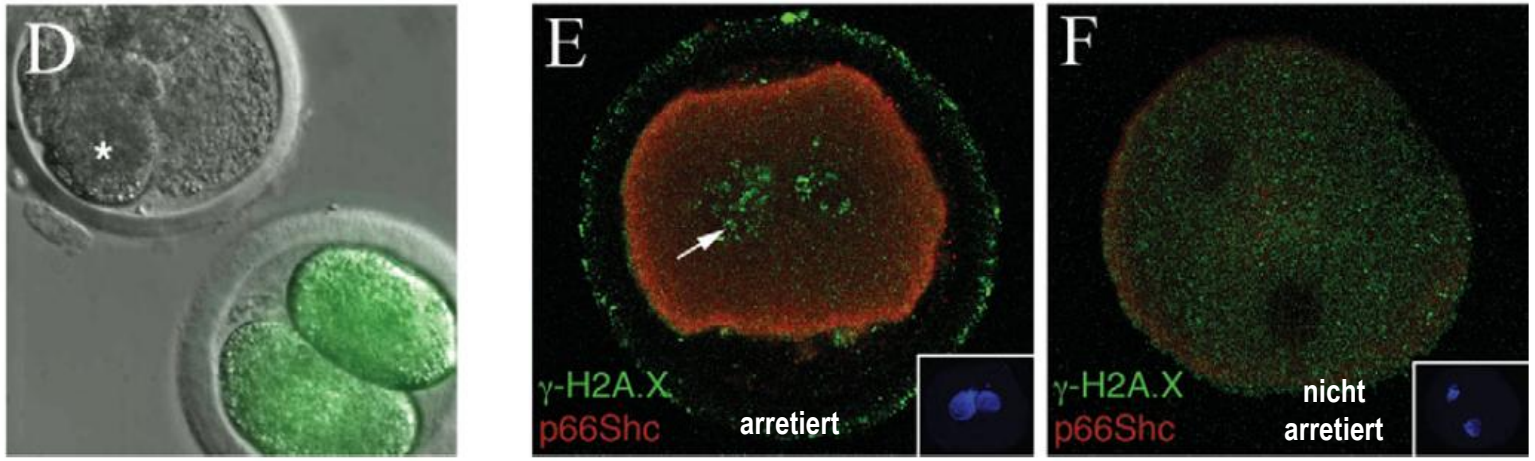


Betts und Madan; Mol Hum Reprod, 2008

Cellular SENESCENCE

2- to 4-cell embryos characterized by permanent arrest are metabolic active and show high intracellular levels of free radicals (ROS), both of which are indicators of beginning embryonic senescence.

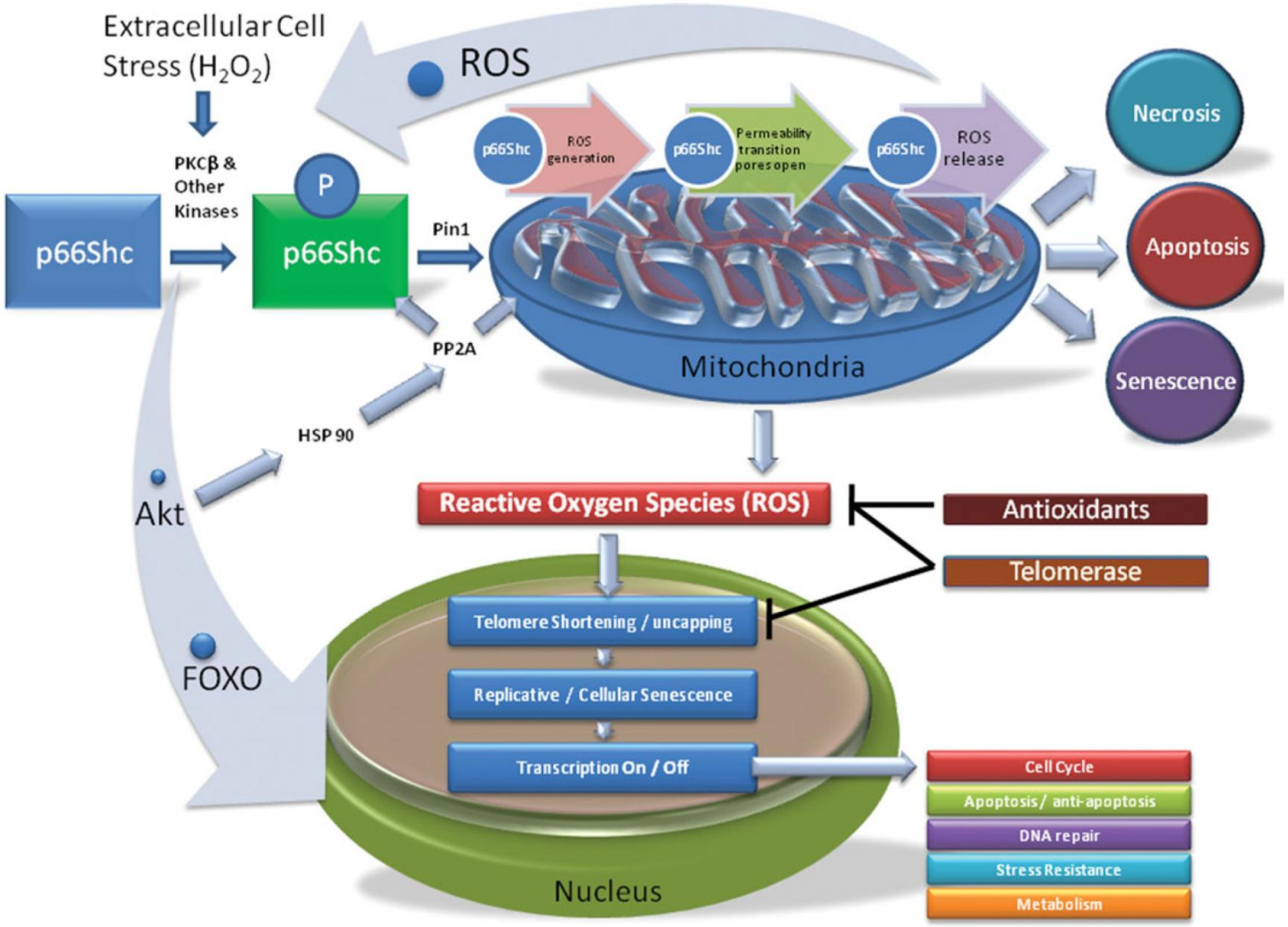
This is further supported by the finding that arrested embryos display high levels of the adapter protein p66Shc which is known for ist apoptotic response to oxidative stress.



Betts und Madan; Mol Hum Reprod, 2008

Increased levels of ROS damage the embryo by causing lipid peroxidation, protein oxidation, and inducing DNA-strand breaks which in turn may result in telomere shortening.

If the lenght of the telomere falls below a critical threshold apoptosis/senescence is the logical consequence.


Extracellular Cell Stress (H_2O_2)
ROS
PKCβ & Other Kinases
p66Shc
P
p66Shc
Pin1
p66Shc
ROS generation
p66Shc
Permeability transition pores open
p66Shc
ROS release
Necrosis
Apoptosis
Senescence
Mitochondria
PP2A
HSP 90
Akt
FOXO
Reactive Oxygen Species (ROS)
Antioxidants
Telomerase
Telomere Shortening / uncapping
Replicative / Cellular Senescence
Transcription On / Off
Nucleus
Cell Cycle
Apoptosis / anti-apoptosis
DNA repair
Stress Resistance
Metabolism

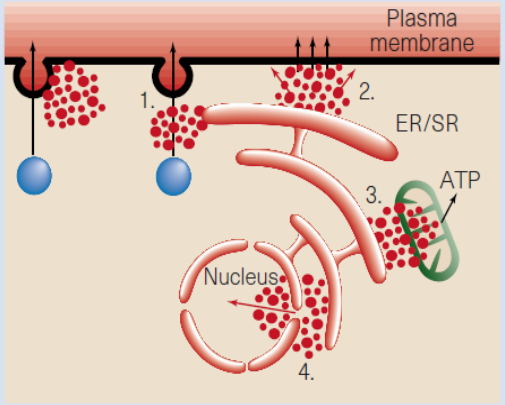
Calcium — a life and death signal

Michael J. Berridge, Martin D. Bootman & Peter Lipp

One of the most versatile and universal signalling agents in the human body is the calcium ion, Ca^{2+}. How does this simple ion act during cell birth, life and death, and how does it regulate so many different cellular processes?

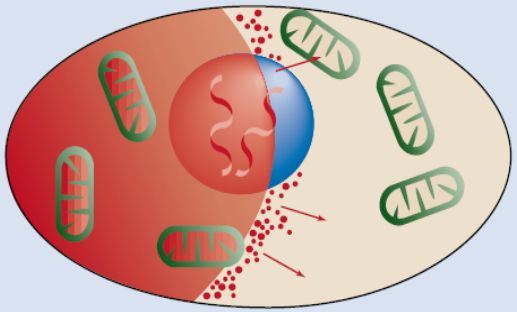
NATURE | VOL 395 | 15 OCTOBER 1998 | www.nature.com

a Elementary events



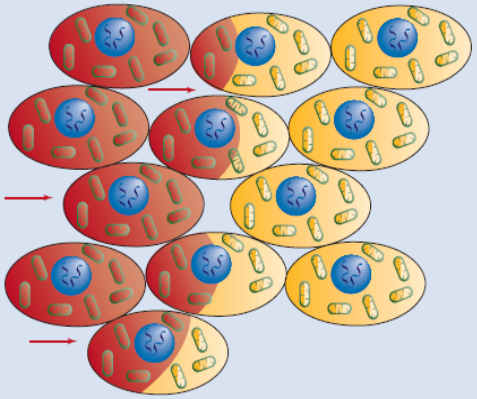
Growth-cone migration
Membrane excitability
Mitochondrial metabolism
Vesicle secretion
Smooth muscle relaxation
Mitosis
Synaptic plasticity

b Global Ca^{2+} wave (intracellular)



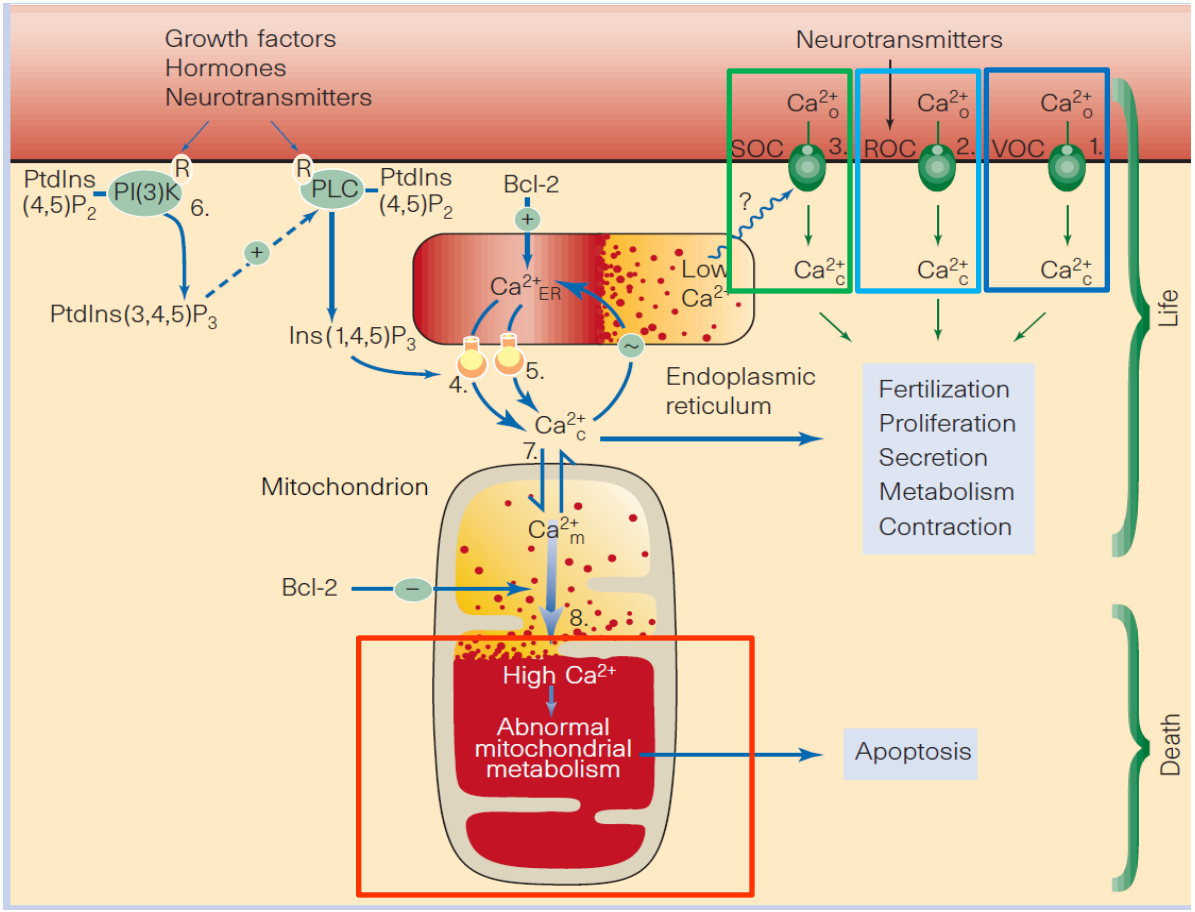
Fertilization
Smooth muscle contraction
Skeletal muscle contraction
Cardiac muscle contraction
Liver metabolism
Gene transcription
Cell proliferation

c Global Ca^{2+} wave (intercellular)



Wound healing
Ciliary beating
Glial cell function
Bile flow
Insulin secretion
Smooth muscle-induced nitric oxide synthesis in endothelium

How does calcium enter the cell?



1. voltage-dependent Ca^{2+}-channels in excitable cells

2. receptor-bound Ca^{2+}-channels associated with neurotransmitter signalling

3. from intracellular Ca^{2+}-storages

4. Overloading with Ca^{2+} can lead to changes in mitochondrial metabolism and apoptosis

CALCIUM and MITOSIS

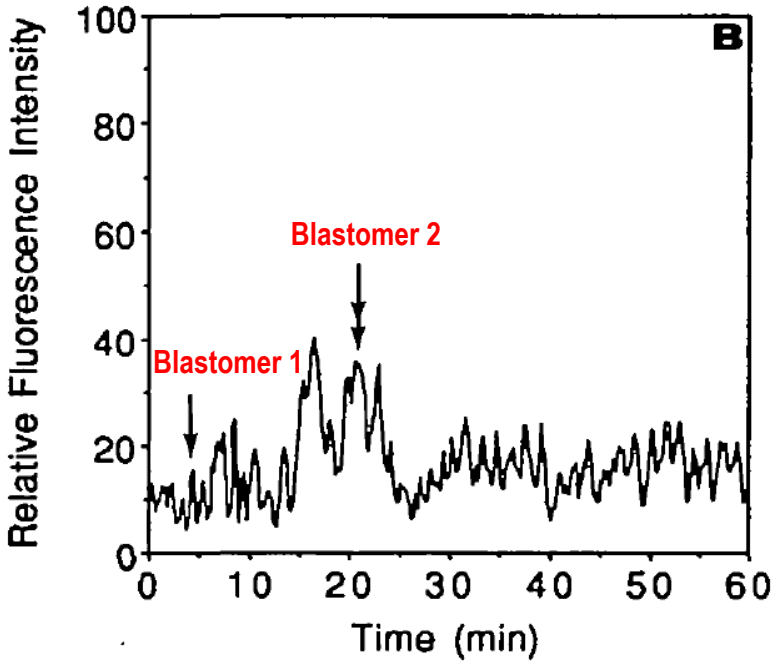
- The role of Ca^{2+} in mitosis is discussed controversially
- Studies on C. elegans and X. laevis show, that changes in intracellular calcium balance initiate and regulate cell division
- In human, Ca^{2+} -peaks were observed preceeding cell division. More interestingly, these Ca^{2+} -oscillationen were undetectable in arrested embryos *(Sousa et al.,1996).*
- In animal models it could be shown that Ca^{2+} -chelators block mitosis whereas calcium ionophore restart division *(Wong et al. 2005).*

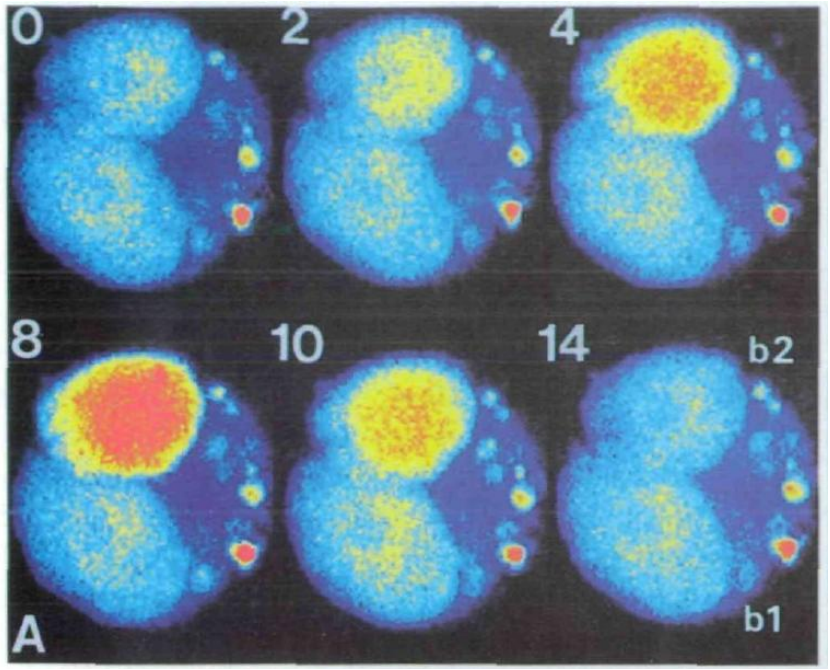
Molecular Human Reproduction vol.2 no.12 pp. 967–977, 1996

Developmental changes in calcium dynamics, protein kinase C distribution and endoplasmic reticulum organization in human preimplantation embryos

Mario Sousa[1], Alberto Barros[2] and Jan Tesarik[3,4,5]

[1]Laboratory of Cell Biology, Institute of Biomedical Sciences, [2]Laboratory of Medical Genetics, Faculty of Medicine, University of Porto, Porto, Portugal, [3]Department of Biochemistry and Molecular Biology, University of Granada Faculty of Sciences, Granada, Spain, and [4]Laboratoire d'Eylau, 55 Rue Saint-Didier, 75116 Paris, France





Ca^{2+}-signal is different from the typical oscillations during oocyte activation

Multicentre trial in Austria

(Ethical votum D-17-13, Upper Austria)

INDICATION

I. Complete developmental arrest in previous cycle (no transfer)

II. Less than 15% blastocyst formation in previous cycle (e.g., 1/7)

III. Developmental delay of all embryos (at least 24h)

Main outcome measures

I. Blastulation

I. Fertilization

II. Embryo quality

III. Rates of implantation/ pregnancy

EXCLUSION

I. Complete fertilization failure after ICSI

II. Exclusively bad quality embryos

III. Refusal of participation

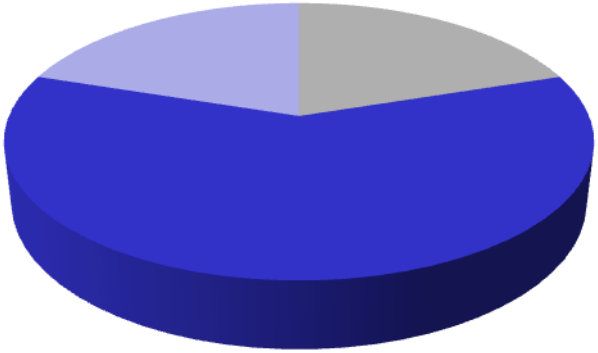
PATIENTS

- 36 patients
  - complete arrest: n=10
  - ≤ 15% blastocysts: n=14
  - developmental delay: n=12
- 40 previous cycles (x24 same protocol)
  - 7,5% hCG positiv; 2,5% Lebendgeburt
- Age: 32±5 a
- AMH 3.3±1.9; FSH 8.4±2.5; LH 5.8±2.6
- E_2 at ovulation induction: 1826±1102
- COC: 10±6
- x13 long protocol, x23 Antagonist protocol

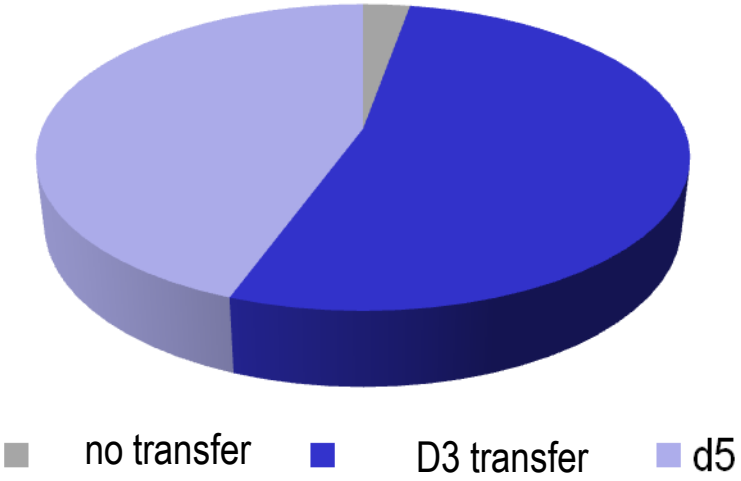
… normal collective

Results I

Previous cycle



Ionophore A23187



Results II

	Previous cycle	Ionophore	P-value
2Pn	191/302 (63.3)	205/285 (71.9)	0.025
Cleavage d2	149/191 (78.0)	203/205 (99.0)	<0.0001
Survival transfer day	52/191 (27.2)	145/205 (70.7)	<0.0001
blastulation	13/90 (14.4)	90/174 (51.7)	<0.0001
positive hCG	3/40 (7.5)	12/36 (33.3)	0.005
clinical pregnancy/live birth	1/40 (2.5)	11/36 (30.6)	0.0008

	Change of protocol	Identical stimulation	
2Pn	83/111 (74.8)	174/216 (80.6)	
Cleavage d2	81/83 (97.6)	122/122 (100)	
Survival transfer day	54/83 (65.1)	91/122 (74.6)	
blastulation	39/69 (56.6)	50/102 (49,0)	n.s.

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Thank you very much for your kind attention

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Dr. Richard B. MAYER
Dr. Marianne MOSER
Fr. Manuela PUCHNER
Fr. Renate WIESINGER