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Oocyte activation

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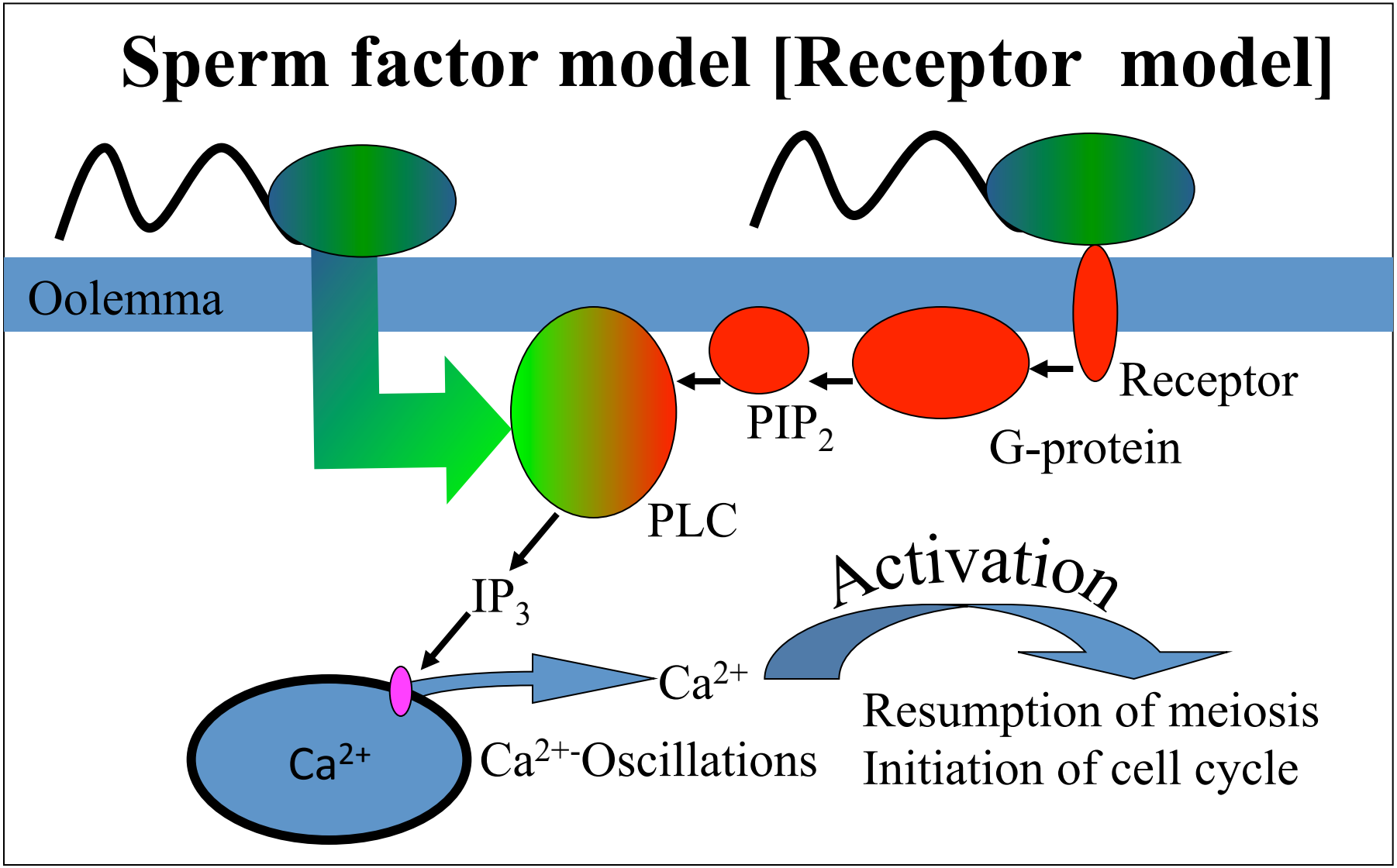
Disclosure

- This speaker was involved in the development and in the implementation of a ready-to-use Ca^{2+}-ionophore
  - As a consequence two travel grants were made available by the developing comapny for two Ph.D. students from this speaker

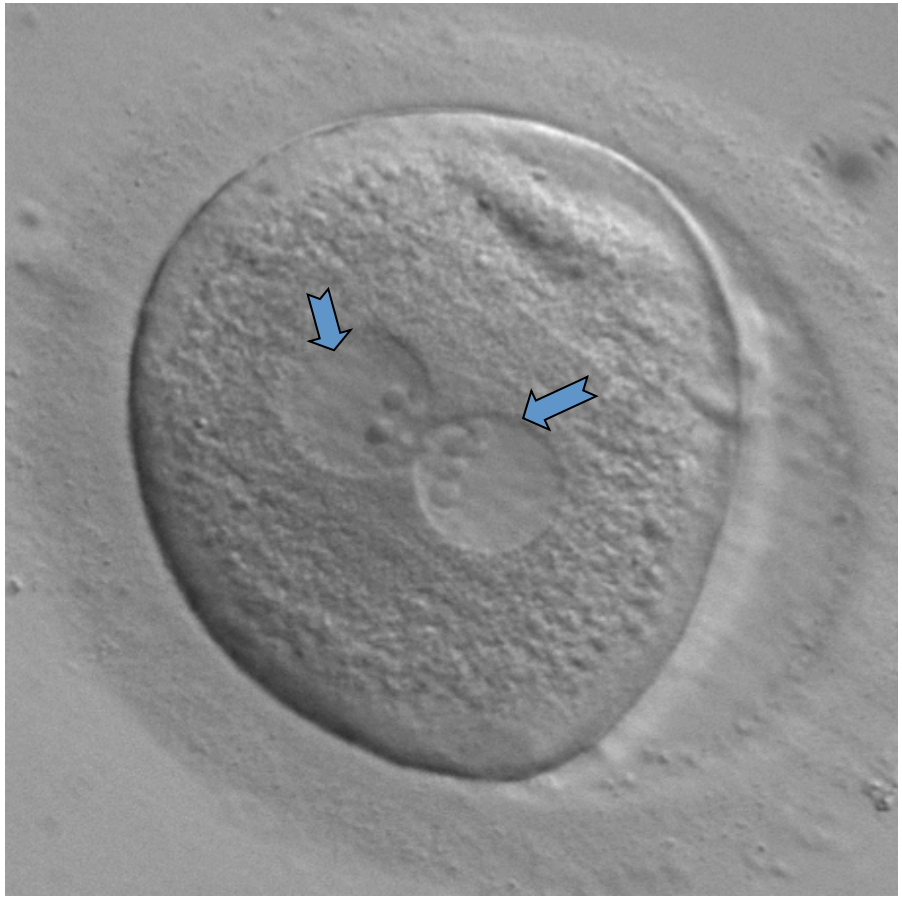
Models of oocyte activation (1995)



Candidates for the oocyte activating factor

- Oscillin (Parrington et al., 1996)
  - contra: Wolosker et al., 1998; Montag et al., 1999
- tr-ckit (Sette et al., 1997)
  - contra: Wu et al., 1998
- PLCγ (Jones et al., 1998)
- Nitric oxide (Kuo et al., 2002)

The real activation factor: PLCzeta

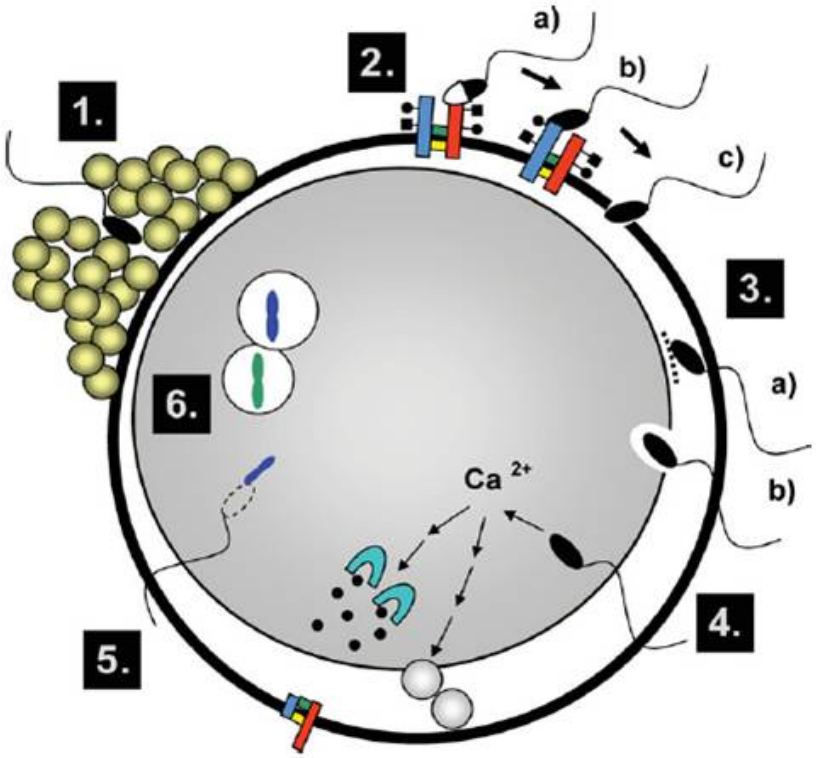


Sperm PLC zeta mediates oocyte activation and initiates the release from the metaphase-II-arrest

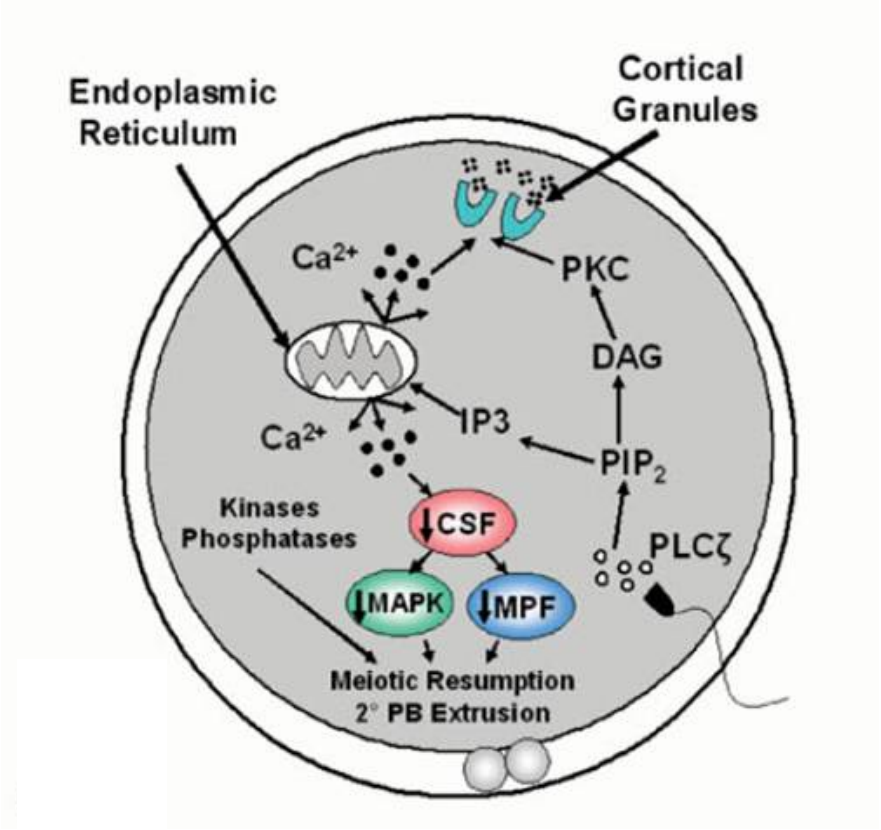
Oocyte activation is a prerequisite for formation of pronuclei, syngamy and initiation of further development

Initiation of activation may differ in IVF vs. ICSI

IVF



ICSI



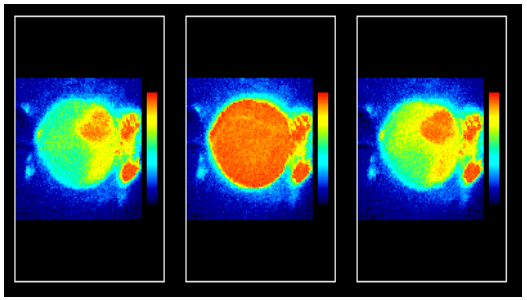
According to Swain and Pool, Human Reproduction Update, 2008

Events related to oocyte activation in IVF and ICSI

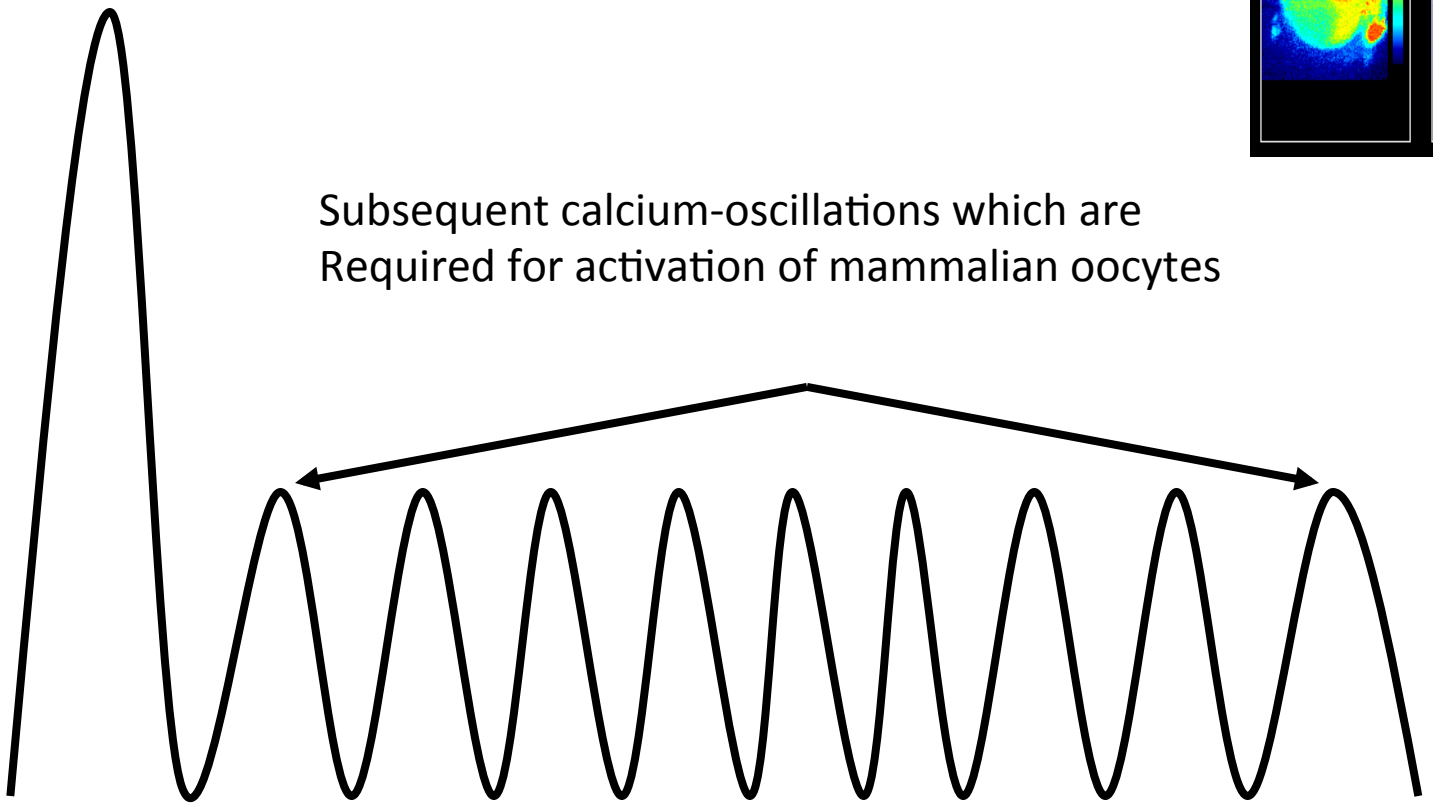
- Rise of intracellular calcium
- Calcium oscillations
- Resumption of meiosis
- Extrusion of second polar body
- Formation of pronuclei
- Initiation of embryonic cell cycles

Oocyte-activation can be measured by the release of intracellular calcium

Initial calcium release, sufficient for activation of oocytes from sea urchin and Xenopus



Subsequent calcium-oscillations which are Required for activation of mammalian oocytes



Mouse Oocyte Activation Test
Is the problem sperm- or oocyte-borne?

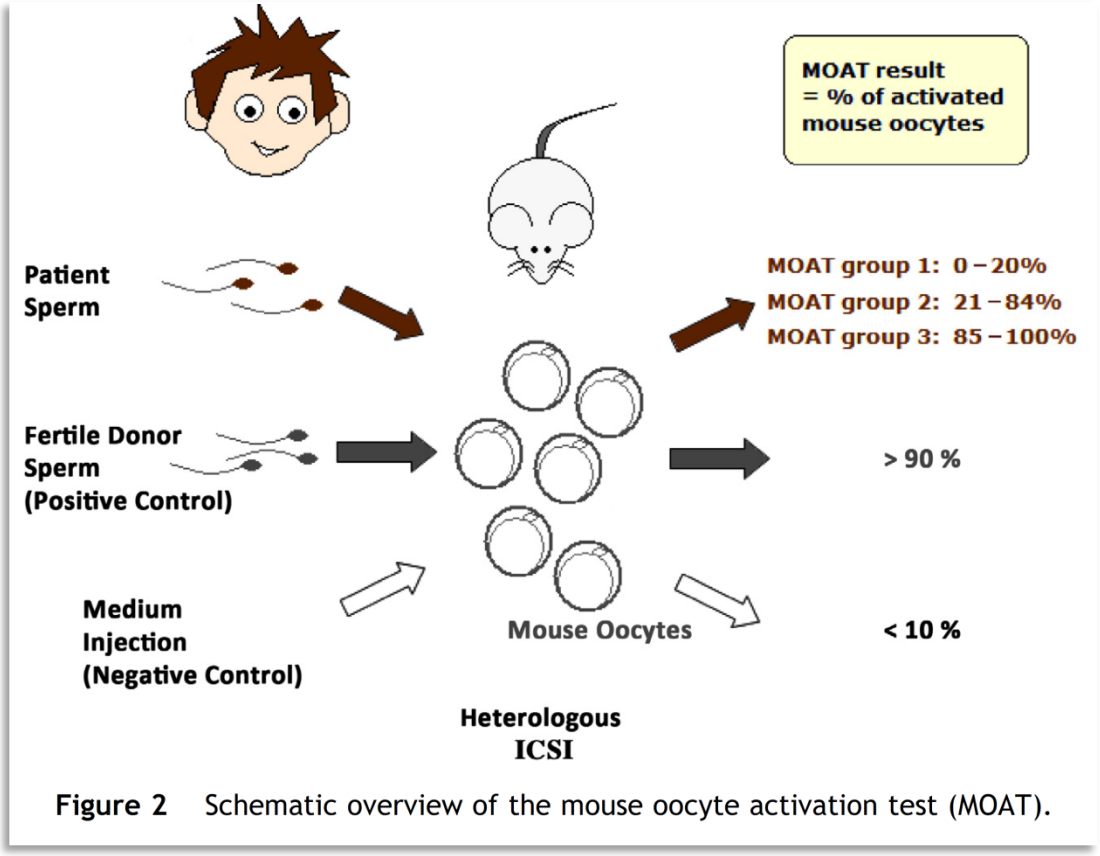


Figure 2 Schematic overview of the mouse oocyte activation test (MOAT).

(Vanden Meerschaut et al., 2014)

- ✓ Offered by the group from Heindryckx / Sutter in belgium

Failed fertilization after ICSI = failed activation?

Possible reasons for fertilization failure	Incidence
Failed activation	15-66%
Failed decondensation of the sperm head	4-45%
Premature condensation of sperm chromatin	2-23%
Spindel- / Centriole defects	6-18%
Suboptimale ICSI	6-23%

According to Swain and Pool, Human Reproduction Update, 2008

What about PLCzeta?

- Some authors have shown that a deficiency of oocyte activation is due to PLC zeta
  - Yoon et al., 2008
  - Heytens et al., 2009
  - Kashir et al., 2012
  - Amdani et al., 2013

Artificial oocyte activation: a means to avoid fertilization failure after ICSI

- Calcium ionophore A23187 Tesarik&Sousa, 1995; Rybouchkin et al., 1997
- Puromycin Murase et al., 2004
- Strontium chloride Murase et al., 2004
- Ionomycin Heindryckx et al., 2005
- 6-DMAP Heindryckx et al., 2009
- Electric pulses Yanagida et al., 1999
- Modified ICSI technique Tesarik et al., 2002; Ebner et al., 2004
- Recombinant PLC zeta Yoon et al., 2008

I. ICSI Tesarik et al. (2002)

Human Reproduction Vol.19, No.8 pp. 1837–1841, 2004
Advance Access publication May 27, 2004
DOI: 10.1093/humrep/deh325

Complete oocyte activation failure after ICSI can be overcome by a modified injection technique

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BACKGROUND: Complete fertilization failure after ICSI is a rare event, and it may happen repeatedly even in cases of normal sperm parameters and good ovarian response. In these cycles, alternative ICSI techniques may prove useful. METHODS: Our modified ICSI (mICSI) is characterized by aspiration close to the opposite membrane (the region of the mitochondria with a high inner mitochondrial membrane potential) which is followed by central deposition of the sperm. The method was applied prospectively to ICSI cycles of patients with a history of complete fertilization failure in previous ICSI cycles. In parallel, mICSI was compared with conventional ICSI in terms of further preimplantation development and treatment outcome. RESULTS: In patients with previous ICSI failures using conventional ICSI (no 2Pn zygotes out of 70 oocytes that had been injected) application of mICSI led to adequate fertilization (53.6%) and pregnancy rates (33.3%) ($P < 0.001$; $P < 0.01$). In patients without previous failed fertilization, no improvement in the rates of fertilization, blastocyst formation, implantation or clinical pregnancy could be seen. CONCLUSIONS: Our data indicate that the present version of ICSI is a reliable alternative to conventional ICSI. However, although it overcomes oocyte-dependent activation failure, routine application does not improve the overall results.

Key words: cytoplasmic maturation/fertilization failure/mitochondria/mitochondrial membrane potential/oocyte activation

II. mICSI Ebner et al. 2004

The effectiveness of intracytoplasmic sperm injection combined with piezoelectric stimulation in infertile couples with total fertilization failure

Volkan Baltaci, M.D.,[a] *Özge Üner Ayvaz, Ph.D.,*[b] *Evrim Ünsal, Ph.D.,*[b] *Yasemin Aktaş, M.Sc.,*[b] *Aysun Baltacı, M.D.,*[b] *Feriba Turhan, M.Sc.,*[b] *Sarp Özcan, M.D.,*[b] *and Murat Sönmezer, M.D.*[c]

[a] Faculty of Medicine, Department of Medical Genetics, Ufuk University, Ankara, Turkey; [b] Gen Art Woman Health and Reproductive Biotechnology Center, Ankara, Turkey; and [c] Ankara University School of Medicine, Department of Obstetrics and Gynecology, Ankara University Center for Research on Human Reproduction, Ankara, Turkey

TABLE 2

Fertilization and embryo grade results after ICSI with piezoelectric activation or conventional ICSI for patients having one previous TFF experience (group I).

	Group IA Piezo (+) (n = 123)	Group IB Piezo (–) (n = 88)	*P*
Oocytes fertilized (n)	76	10	
Fertilization rate (%)	62	12	0.001
Grade 1–2 embryos (n, %)	28 (37%)	2 (20%)	0.01
Grade 3–4 embryos (n, %)	48 (63%)	8 (80%)	0.01

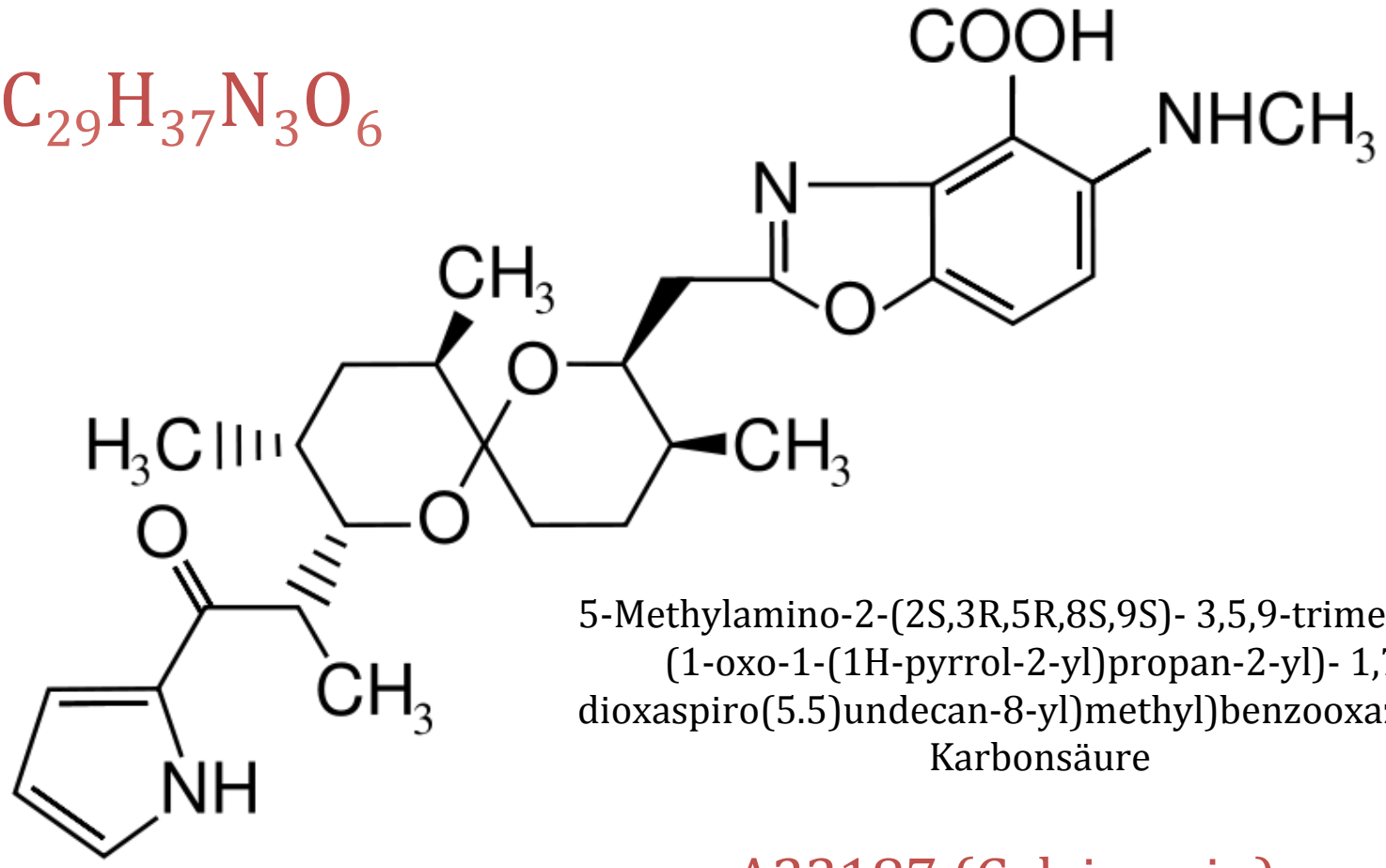
Baltaci. ICSI combined with electrical stimulation. Fertil Steril 2009.

The most commonly used method for oocyte activation: based on a home-made activation solution

1. Perform ICSI
2. Incubate oocytes in 10-20µmol A23187 (Calcimycin in DMSO) for 15-20 min
3. Wash thoroughly in 3-4 wash droplets
4. Culture in-vitro as usual

Ca^{2+}-Ionophore

$C_{29}H_{37}N_3O_6$



5-Methylamino-2-(2S,3R,5R,8S,9S)- 3,5,9-trimethyl-2-(1-oxo-1-(1H-pyrrol-2-yl)propan-2-yl)- 1,7-dioxaspiro(5.5)undecan-8-yl)methyl)benzooxazole-4-Karbonsäure

A23187 (Calcimycin)

Human Reproduction, Vol.27, No.2 pp. 303–313, 2012

When and how should new technology

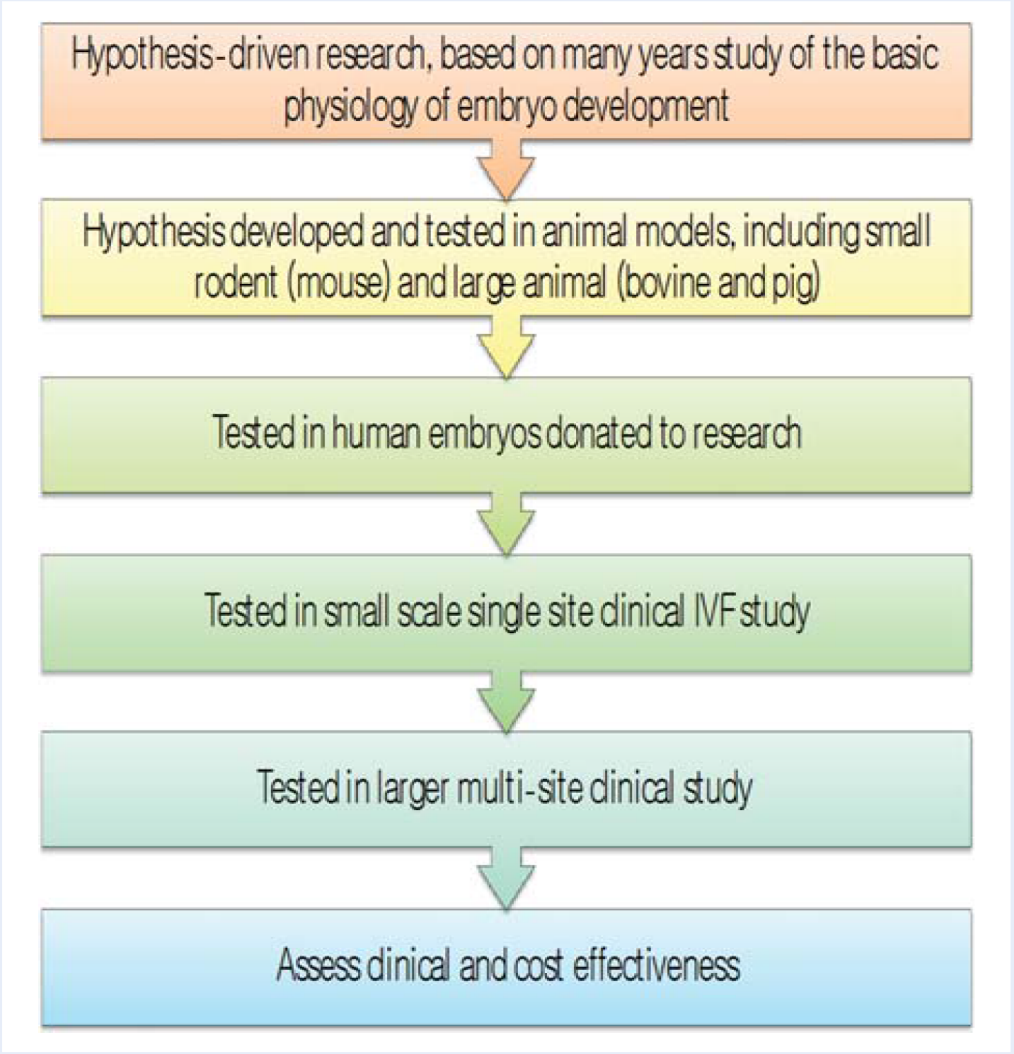
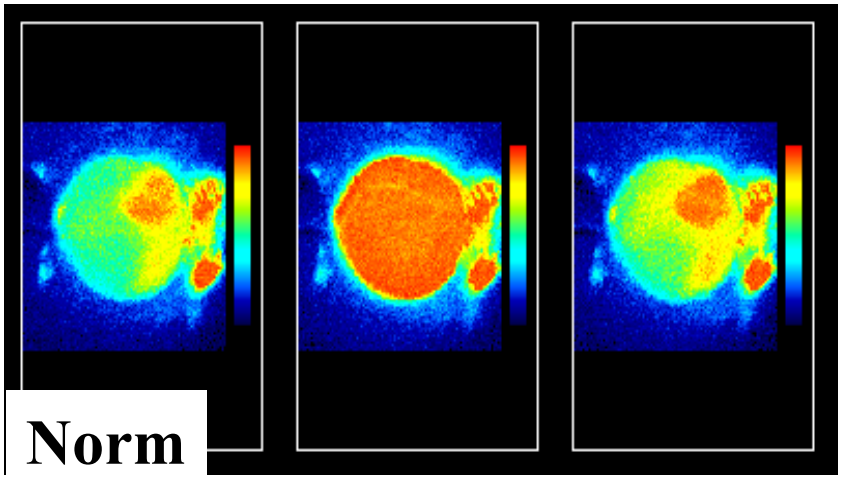


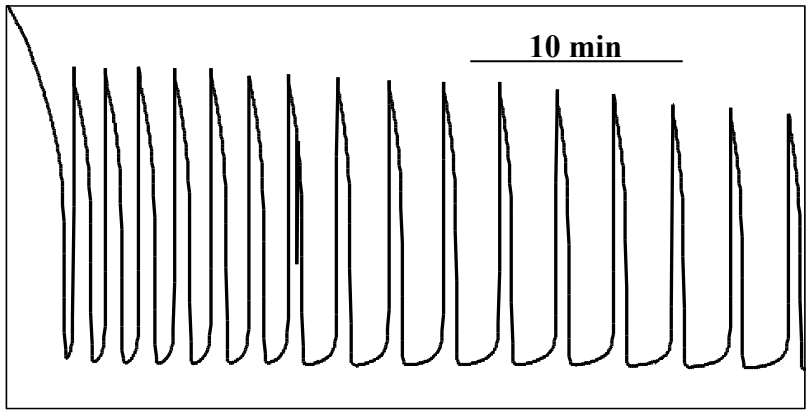
Figure 1 Ideal paradigm of hypothesis-driven basic research.

Mouse oocyte activation test

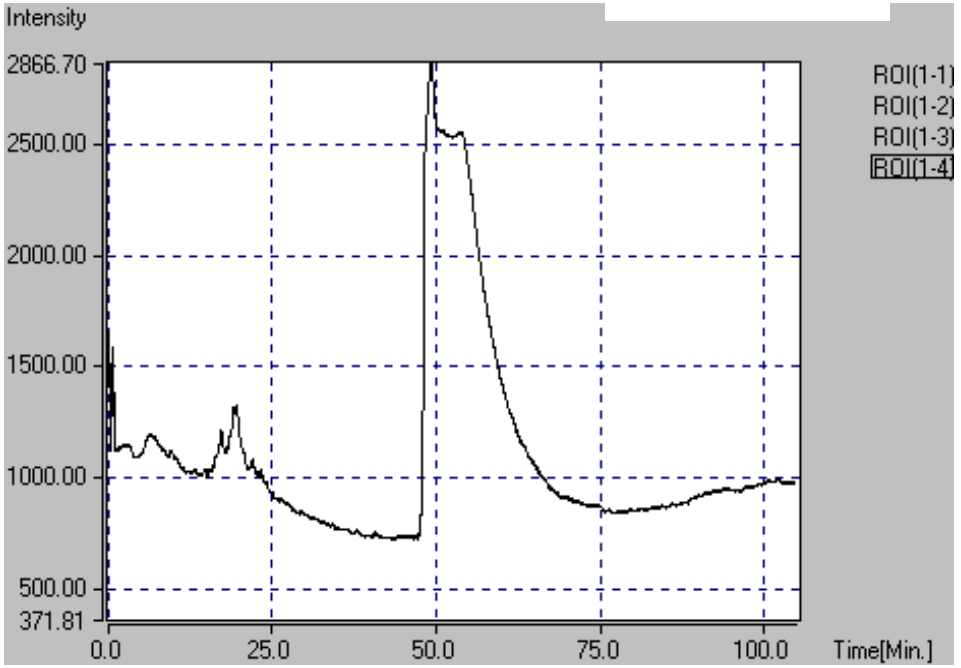
Injection of human sperm or human sperm extract into mouse oocytes combined with Ca^{2+}-measurements



Norm



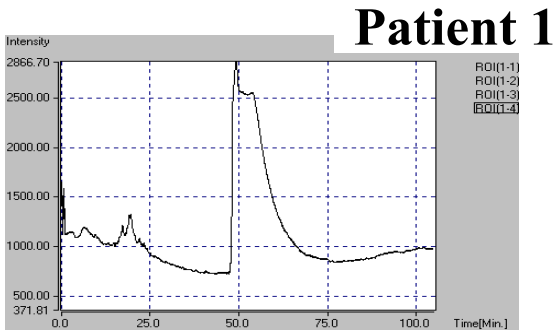
Patient 1



Case Patient 1 – based on a grant project

Young couple: woman: fertile / male: OAT

- 1. ICSI 02/2002:
  - 9 oocytes: 1 x 2PN
- 2. ICSI 04/2002:
  - 8 oocytes: 1 x 2PN
- MOAT-result
  - Impaired activation capacity
  - Couple agreed to artficial oocyte activation (Ca^{2+}-ionophore A23187)
- 3. ICSI 06/2002
  - 8 oocytes:
  - 4 conventional ICSI: 0 x 2PN
  - 4 ICSI + artificial activation: 3 x 2PN
  - Intact pregnancy, male, 3540 g, 49 cm, healthy



Artificial activation does not work for all!

- 2nd patient: MOAT showed impaired activation potential
  => artificial oocyte activation was succesfull

- 3rd patient: normal MOAT
  the couple wanted also artificial activation
  but again failed fertilization

Patients 4 to 100

- No previous work-up with MOAT
- Strict indication list
  - Fertilization in previous ICSI cycle < 30 (50)%
  - TESE cases with immotile sperm
- Treatment based on home-made activation solution
- Patients signed informed consent
  - Experimental procedure
- Patients were sensitized to provide birth data

Reproductive BioMedicine Online (2012) **24**, 521–526

The benefit of artificial oocyte activation is dependent on the fertilization rate in a previous treatment cycle

Markus Montag [a,b,*], Maria Köster [b], Katrin van der Ven [b], Ulrike Bohlen [b], Hans van der Ven [b]

[a] *Department of Gynecological Endocrinology and Fertility Disorders, University of Heidelberg, Voßstr. 9, 69115 Heidelberg, Germany;* [b] *Department of Gynecological Endocrinology and Reproductive Medicine, University of Bonn, Bonn, Germany*

Table 1 Results of artificial oocyte activation in patients with 0% fertilization rates in an initial cycle (group 1).

	Standard ICSI	*Artificial oocyte activation*	*P-value*
Patients (*n*)	27	27	–
Female age (years)	36.1 ± 4.2	37.8 ± 3.4	NS
ICSI cycles (*n*)	31	39	–
Oocytes retrieved	5.4 ± 2.8	7.4 ± 3.8	<0.05
Oocytes injected	3.7 ± 2.4	5.9 ± 3.5	<0.001
Fertilization/injected oocyte	0 (0/114)	41.6 (96/231)[a]	<0.05
ET cycles	0 (0/31)	82.1 (32/39)	<0.05
Embryos transferred (mean)	0	1.46	<0.05
Positive βHCG/ET	0	25.0 (8/32)	<0.05
Clinical pregnancy/ET	0	18.8 (6/32)	<0.05
Implantation/embryos transferred	0	12.3 (7/57)	<0.05
Abortion/clinical pregnancy	0	16.7 (1/6)	<0.05
Take-home baby/ET	0	15.6 (5/32)	<0.05
Take-home baby/cycle	0	12.8 (5/39)	<0.05

Values are mean ± SD or % (*n*/total), unless otherwise indicated.
ET = embryo transfer; NS = not significant.
[a]No fertilization occurred in seven patients in seven activation cycles.

Table 2 Results of artificial oocyte activation in patients with 1–29% fertilization rates in an initial cycle (group 2).

	Standard ICSI	*Artificial oocyte activation*	*P-value*
Patients (*n*)	38	38	–
Female age (years)	33.3 ± 4.4	35.3 ± 3.7	<0.05
ICSI cycles (*n*)	47	58	–
Oocytes retrieved	8.0 ± 4.0	7.8 ± 4.1	NS
Oocytes injected	6.2 ± 3.6	6.1 ± 3.5	NS
Fertilization/injected oocyte	19.3 (72/373)	44.4 (161/363)[a]	<0.001
ET cycles	100.0 (47/47)	87.9 (51/58)	<0.05
Embryos transferred (mean)	1.48	1.57	NS
Positive βHCG/ET	14.9 (7/47)	37.3 (19/51)	<0.05
Clinical pregnancy/ET	12.8 (6/47)	31.4 (16/51)	<0.05
Implantation/embryos transferred	8.6 (6/70)	17.6 (16/91)	NS
Abortion/clinical pregnancy	0 (0/6)	12.5 (2/16)	NS
Take-home baby/ET	12.8 (6/47)	27.5 (14/51)	NS
Take-home baby/cycle	12.8 (6/47)	24.1 (14/58)	NS

Values are mean ± SD or % (*n*/total), unless otherwise indicated.
ET = embryo transfer; NS = not significant.
[a]No fertilization occurred in six patients in seven activation cycles. These patients had only one oocyte fertilized in the previous cycles without activation.

0% fertilization rate in previous cycle

Table 1 Results of artificial oocyte activation in patients with 0% fertilization rates in an initial cycle (group 1).

	Standard ICSI	*Artificial oocyte activation*	*P-value*
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[a]No fertilization occurred in seven patients in seven activation cycles.

1-29% fertilization rate in previous cycle

Table 2 Results of artificial oocyte activation in patients with 1–29% fertilization rates in an initial cycle (group 2).

	Standard ICSI	*Artificial oocyte activation*	*P-value*
Patients (*n*)	38	38	–
Female age (years)	33.3 ± 4.4	35.3 ± 3.7	<0.05
ICSI cycles (*n*)	47	58	–
Oocytes retrieved	8.0 ± 4.0	7.8 ± 4.1	NS
Oocytes injected	6.2 ± 3.6	6.1 ± 3.5	NS
Fertilization/injected oocyte	19.3 (72/373)	44.4 (161/363)[a]	<0.001
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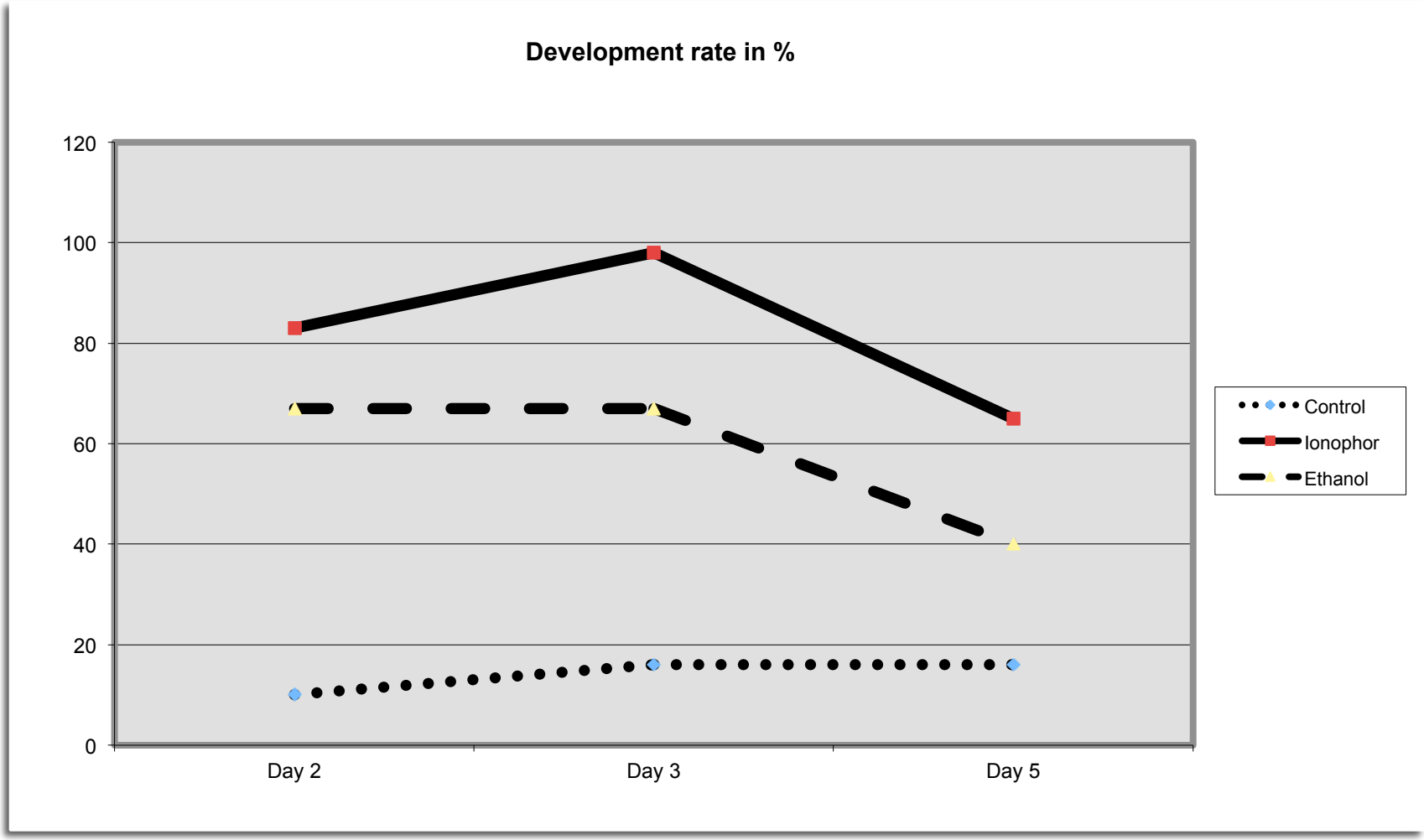
Implantation/embryos transferred	8.6 (6/70)	17.6 (16/91)	NS
Abortion/clinical pregnancy	0 (0/6)	12.5 (2/16)	NS
Take-home baby/ET	12.8 (6/47)	27.5 (14/51)	NS
Take-home baby/cycle	12.8 (6/47)	24.1 (14/58)	NS

Values are mean ± SD or % (*n*/total), unless otherwise indicated.
ET = embryo transfer; NS = not significant.
[a]No fertilization occurred in six patients in seven activation cycles. These patients had only one oocyte fertilized in the previous cycles without activation.

European Tissue Directive

- Use of a home-made activation solution no longer acceptable
- Alternatives:
  - Non-chemical activation
    - -> no experience / no large data in literature
  - Modified ICSI techniques
    - -> few papers / applicability for different indications?
  - Recombinant PLCzeta
    - -> mouse data on importance of proper frequence/amplitude
  - Chemical activation using a commercial product
    - -> extensive experience / company contact

Development of mouse oocytes after artificial activation with a ready-to-use ionophore (A23187)



The next step: A prospective multi-centre study

M. Montag, Bonn, Germany / T. Ebner, Linz, Austria

Non-randomized prospective study

- Study period from September 2009 to October 2010
- Patient cycles were reported on the day of ICSI

6 study centres aiming to 100 cycles in total

- 5 centres in Germany, 1 in Austria

Patient inclusion criteria:

- Fertilization rate of < 50% in a previous ICSI cycle
- Maternal age < 40 years of age
- No endometriosis or PCO
- At least 3 M-II-oocytes for ICSI in the trial cycle
- Ejaculated spermatozoa only (no Cryo or TESE sperm)

Ebner & Montag, RBMOnline, in press

Methodology and evaluation

Method:

– Immediately after ICSI, oocytes were incubated for 15 min in a commercial ready-to-use calcium ionophore medium
– Oocytes were thoroughly washed in culture medium and incubated as usual

Evaluation criteria:

– Fertilization rate
– Transfer rate
– Implantation- / Pregnancy-rate
– Pregnancy outcome
– Take home baby rate

Recruited patients

– Patients recruited/received activation: n = 111

– 10 patients with activation had to be excluded
  - Failed IVF in pre-cycle: n = 1
  - TESE sperm: n = 2
  - Maternal age ≥40: n = 3
  - Fertilization rate in pre cycle 50%: n = 4
  - 4 of these patients got pregnant with activation

– Patients remaining in the study: n = 101

Ebner & Montag, RBMOnline, in press

Treatment outcome in the study group

Started cycle	101
Cycles with at least one fertilization	100
Pregnancy	37
Ectopic pregnancy	2
Clinical pregnancy	35
Multiple pregnancy	10/35 (28.6)
Vanishing twins	3
Miscarriage	7
Implantation rate	47/185 (25.4)
Live birth	28
Children born from singleton pregnancy	18
Children born from twin pregnancy	17
Malformation	1 (2.9)

Ebner & Montag, RBMOnline, in press

The results of the prospective study confirm those of the retrospective study

	Comparison of the overall study results (fertilization in pre-cycle < 50%)	
	Retrospective study	**Prospective study**
Patients	**97**	**101**
Mean age ♀	**36.3**	**37.3**
ICSI cycles	**126**	**101**
Cycles with transfer	**87.3 %**	**99.0 %**
Embryos for transfer (mean)	**1.63**	**1.85**
Fertilization rate	**46.7 %**	**47.7 %**
Pregnancy rate / embryo transfer	**27.7 %**	**37.0 %**
Take home baby rate / transfer	**24.0 %**	**28.0 %**

Neonatal outcome of children born

	Singleton pregancy	twin pregnancy
Children	18	17
Delivery		
Median (week)	39	37
Range (week)	32-42	30-41
Length (cm)		
Median (cm)	50	48
Range (cm)	45-54	39-54
Weight (g)		
Median (g)	3180	2440
Range (g)	2510-4040	1475-3890
Malformation	1	0

Ebner & Montag, RBMOnline, in press

Application of a ready-to-use calcium ionophore increases rates of fertilization and pregnancy in severe male factor infertility

Thomas Ebner, Ph.D.,[a] Maria Köster, Ph.D.,[b] Omar Shebl, M.D.,[a] Marianne Moser, Ph.D.,[a] Hans Van der Ven, M.D.,[b] Gernot Tews, M.D.,[a] and Markus Montag, Ph.D.[b,c]

[a] Landes- Frauen- und Kinderklinik, Kinderwunschzentrum, Linz, Austria; [b] Gynecological Endocrinology and Reproductive Medicine, University of Bonn, Bonn; and [c] Gynecological Endocrinology and Fertility Disorders, University of Heidelberg, Heidelberg, Germany

TABLE 1

Results of the prospective application of A23187 as compared with the previous cycles without the use of ionophore.

	A23187 cycle	Previous cycles	*P* value
No. of cycles	75	88	
COC collected (mean ± SD)	9.6 ± 5.8	11.2 ± 8.3	NS
Fertilization rate	379/666 (56.9)	244/704 (34.7)	<.001
Azoospermia	228/354 (64.4)[b]	91/220 (41.4)[c]	<.001
Cryptozoospermia	151/312 (48.4)[b]	153/484 (31.6)[c]	<.001
Blastocyst formation[a]	67/119 (56.3)	20/79 (25.3)	<.001
No. of ETs	73 (97.3)	79 (89.8)	NS
Implantation rate	42/126 (33.3)	6/150 (4.0)	<.001
Positive β-hCG	34 (46.6)	6 (7.6)	<.001
Clinical pregnancy rate	29 (29.7)	5 (6.3)	<.001
Live-birth rate	25 (34.2)	1 (1.3)	<.001
Children born	32	1	

Note: Values in parentheses are percentages. β-hCG = β human chorionic gonadotropin; COC = cumulus-oocyte complex; ET = embryo transfer; NS = not statistically significant; SD = standard deviation.

[a] Exclusive data from Kinderwunsch Zentrum Linz.

[b] $P<.001$.

[c] $P<.05$.

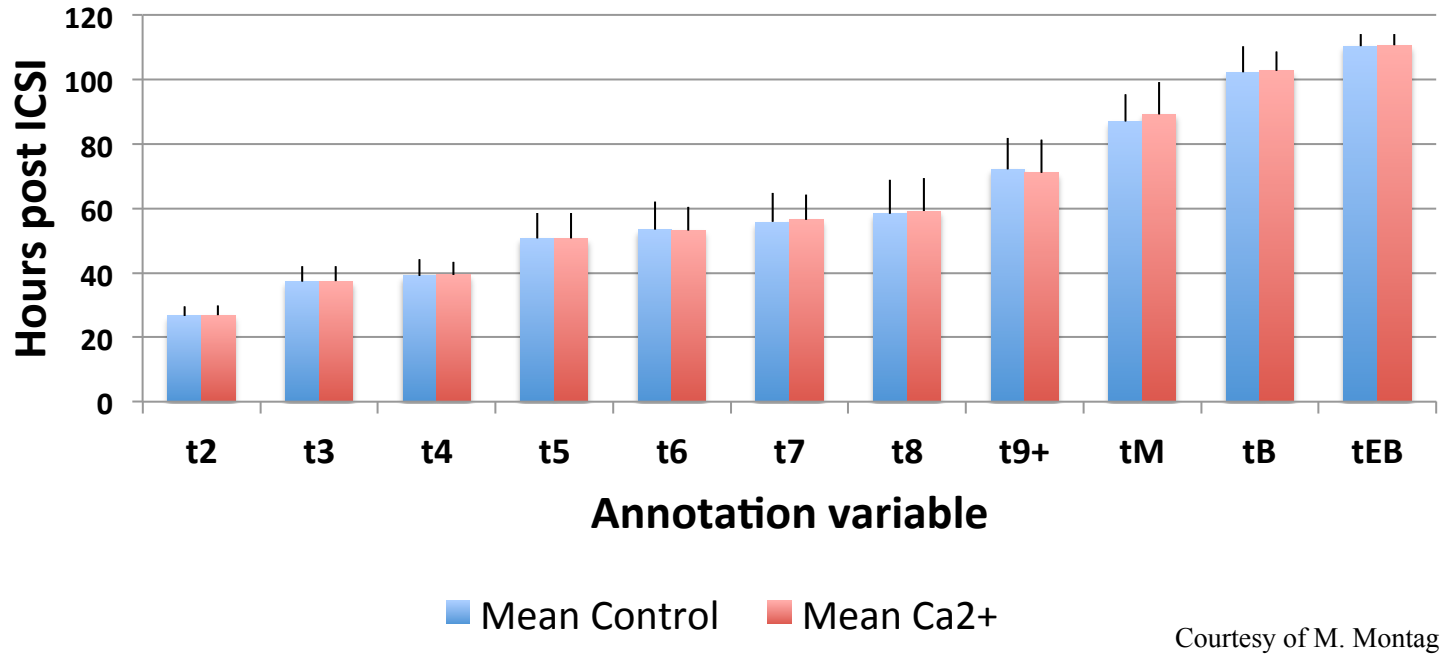
Ebner. Ionophore and severe male infertility. Fertil Steril 2012.

Does artificial oocyte activation affect embryo development

ESHRE, London, 2013

O-215 Does oocyte activation influence morphokinetic parameters of embryos: a comparative analysis using time-lapse imaging

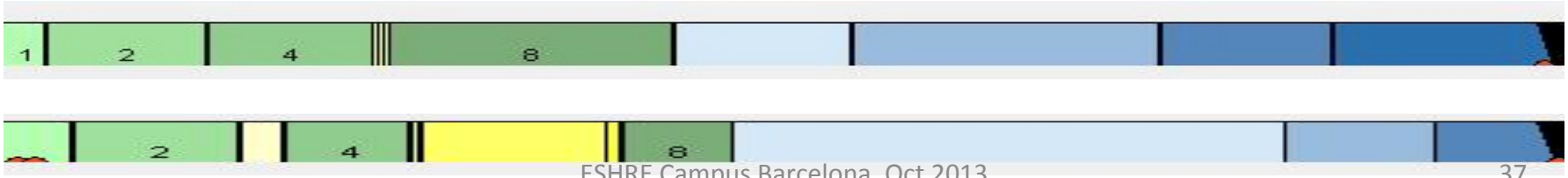
M. Montag, B. Toth, J. Weigert, and T. Strowitzki
Universitäts-Frauenklinik, Abt. Gynäkol. Endokrinologie & Fertilitätsstörungen, Heidelberg, Germany



Courtesy of M. Montag

Does artificial oocyte activation affect embryo development

	Study group	Control group
Duration 2-cell stage (t3 – t2)	10.5 ± 3.2	10.6 ± 3.0
Duration 4-cell stage (t5 – t3)	13.5 ± 4.5	13.3 ± 5.3
Time in 3-cell stage (t4 – t3)	1.6 ± 3.1	2.0 ± 3.4



P-133 Embryo morphokinetic after artificial oocyte activation by using calcium ionophore

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	Control	iCa	Sig
Age	39.35 ± 2.7	38 ± 2.9	>0.05
Fertilization rate	66.28 ± 19.63	58.88 ± 21.77	>0.05
% TQE	41.01 ± 29.84	47.18 ± 33.66	>0.05
Number ET	1.74 ± 0.53	1.80 ± 0.52	>0.05
Pn Fading	26.15 ± 3.40	23.50 ± 3.65	0.03
T4	41.70 ± 5.57	38.24 ± 3.47	<0.01
T5	54.92 ± 5.60	50.17 ± 7.38	<0.01

	Montag et al.	Taboas Lima et al.
Cases	13	14
Product	ready-to-use	homemade
Type	A23187	ionomycin
Exposure	15 min	20 min
Concentration	10-20 µM	> 50 µM

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Assisted oocyte activati
fertilization failure

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Table 1 Overview of the calcium ionophore AOA protocols used in human assisted reproduction treatment.

Reference	*AOA protocol*	*Cases*	*Fertilization rate (%)*		
			Conventional ICSI	*AOA*	*P-value*
Moaz et al. (2006)	Twofold exposure to 10 μmol/l ionomycin for 10 min at 1 h and 1.5 h following ICSI	Abnormal sperm morphology			
		Amorphous heads (n = 18)	36.7	82.7	0.0008
		Tapered heads (n = 23)	39.3	81.7	0.005
		Bent necks (n = 15)	49.4	48.2	NS
Heindryckx et al. (2008)	Injection of 0.1 mol/l $CaCl_2$ together with spermatozoa during ICSI, followed 30 min later by a 2-fold exposure to 10 μmol/l ionomycin for 10 min, 30 min apart	Previously failed or low fertilization after conventional ICSI (n = 30)	14 (0–22)	75	<0.001
Nasr-Esfahani et al. (2008)	Single exposure to 10 μM ionomycin for 10 min	Severe teratozoospermia with a split AOA cycle (n = 78)	0	57.8	S
			14.3 (1–33)	58.3	S
			47 (34–65)	63.4	S
			85.8 (66–100)	77.9	NS
Borges et al. (2009a)	Single exposure to 5 μM calcimycin for 30 min, immediately following ICSI	ICSI with spermatozoa from:			
		TESE NOA (n = 29)	44.0	44.7	NS
		TESE OA (n = 24)	65.2	55.0	NS
		PESE OA (n = 49)	65.8	67.0	NS
Borges et al. (2009b)	Single exposure to 5 μM calcimycin for 30 min immediately following ICSI	ICSI with spermatozoa from:			
		Ejaculated (n = 46)	76.2	69.4	NS
		Epididymal (n = 41)	66.6	48.9	NS
		Testicular (n = 70)	56.1	50.6	NS
Montag et al. (2012)	Single exposure to 10 μmol/l calcimycin for 15 min immediately following ICSI	ICSI with previous:			
		Failed fertilization (n = 27)	0	41.6	<0.05
		Low fertilization (n = 38)	19.3 (0–29)	44.4	<0.001
		Very low fertilization (n = 24)	36.8 (30–50)	56.1	<0.001
Vanden Meerschaut et al. (2012)	Injection of 0.1 mol/l $CaCl_2$ together with spermatozoa during ICSI, followed 30 min later by a 2-fold exposure to 10 μmol/l ionomycin for 10 min, 30 min apart	Suspected oocyte-related activation failure with a split AOA cycle and ICSI with previous:			
		Failed fertilization (n = 5)	25.0	72.7	<0.001
		Low fertilization (n = 7)	60.4	75.0	NS
Ebner et al. (2012)	Single exposure to a ready-to-use calcimycin solution for 15 min immediately following ICSI	Azoo- or cryptozoospermia (n = 66)	34.7	56.9	<0.001

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Deemeh MR1, Tavalaee M1, Nasr-Esfahani MH2.

Conclusions

- In the majority of patients with either low or failed fertilization after ICSI the underlying incidence is a deficiency in sperm-mediated oocyte activation
- Artificial oocyte activation can overcome a sperm-born activation problem, but it may not help as an universal tool to enhance fertilization rates in every patient
- Artificial oocyte activation has been introduced in the lab
  - Case study with experimental back ground investigations
  - Experimental study for restricted indications (LBR / Children born)
  - Animal study with a ready-to-use product
  - Verification in a multi-center observational study (LBR / Children born)
  - Investigation of morphokinetic development
- Not all activation methods are equally well assessed
- Some may change morphokinetic patterns of embryos

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Thank you for your attention!
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