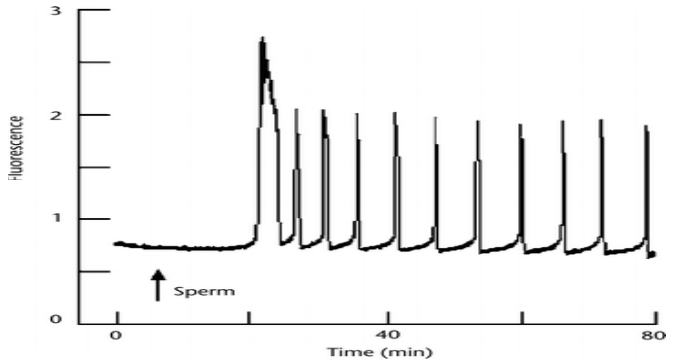
Kepler Universitäts Klinikum

Artificial oocyte activation: Ionophore application

Pro, evidence for clinical readiness

Thomas EBNER

Kepler University, Linz, Austria



OVARIAN CLUB VIII
BUILDING A BRIDGE BETWEEN SCIENCE AND CLINICAL PRACTICE
4-6 NOVEMBER, 2016 · PARIS, FRANCE

Conflict of Interest

Kepler Universitäts Klinikum

This speaker has received payment for expert testimony for MSD, Merck, as well as Glycotope, and he is a consultant for Gynemed.

Outline

Kepler Universitäts Klinikum

1. Principle of OA
2. Artificial oocyte activation (AOA)
3. Indications of ionophore use
4. Possible mechanisms of action
5. Safety issues
6. Future aspects

Principles of oocyte activation

Kepler Universitäts Klinikum

OA is one of the 6 major steps of in vivo and in vitro fertilization

1. Cumulus cell penetration
2. Sperm/oocyte binding and penetration
3. Sperm/oocyte fusion
4. Oocyte activation
5. Sperm processing
6. PN formation

OA is a physiological process entailing

1. the release from MII arrest and completion of second meiotic division and
2. modifications of the zona pellucida to prevent polyspermy

Sperm proteins as potential candidates for OA

1. Citrate synthase
2. Truncated form of c-kit tyrosine kinase receptor
3. Post-acrosomal WW-domain binding protein (PAWP)
4. Phospholipase C ζ

Swain and Pool, 2008; Kashir et al., 2010; Yeste et al., 2016

Kashir et al., HRU 2010, 16 (6), 690-703

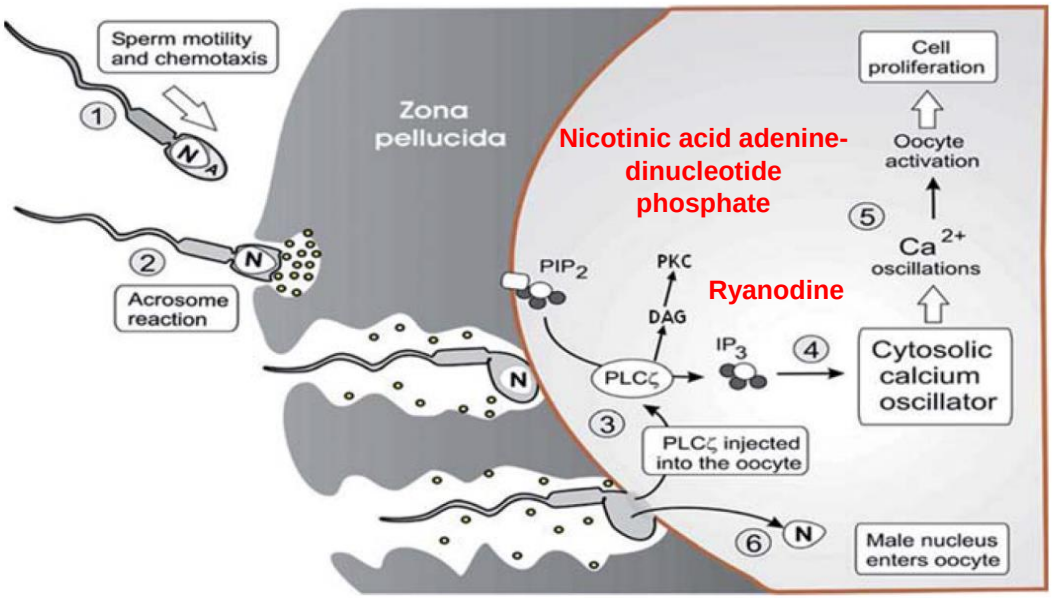


Figure 2 Signalling pathway of PLCζ. Reproduced from Berridge (2009) with permission.

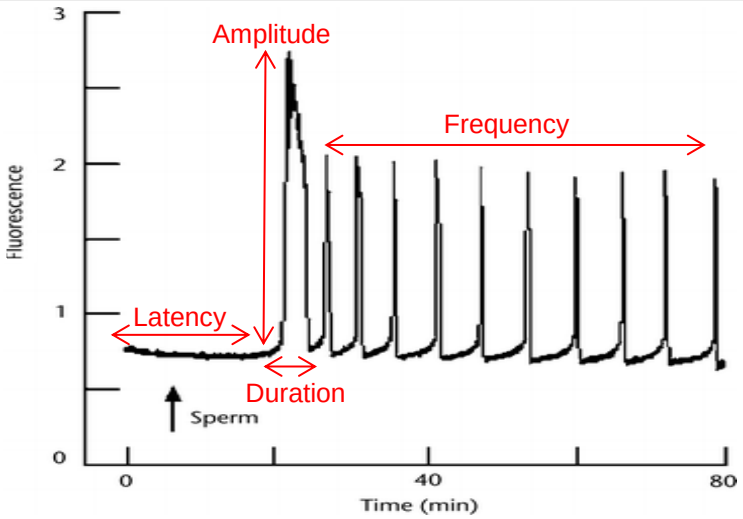
NAADP → NAADPR → Ca^{2+}-release from lysosomes

Sperm-PLCζ → PIP_2 → DAG → Zona reaction

PIP_2 → IP_3 → IP_3R → Ca^{2+} release from ER

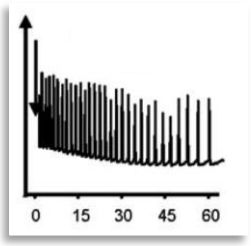
RyR → Ca^{2+} release from ER

- Ca^{2+} signal

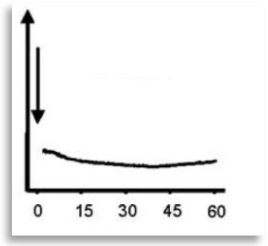


AUC = released Ca^{2+}

- ✓ Sperm induced Ca^{2+} oscillations stimulate mitochondrial respiration
- ✓ The resulting ATP production in turn is required to maintain sperm-triggered Ca^{2+} waves



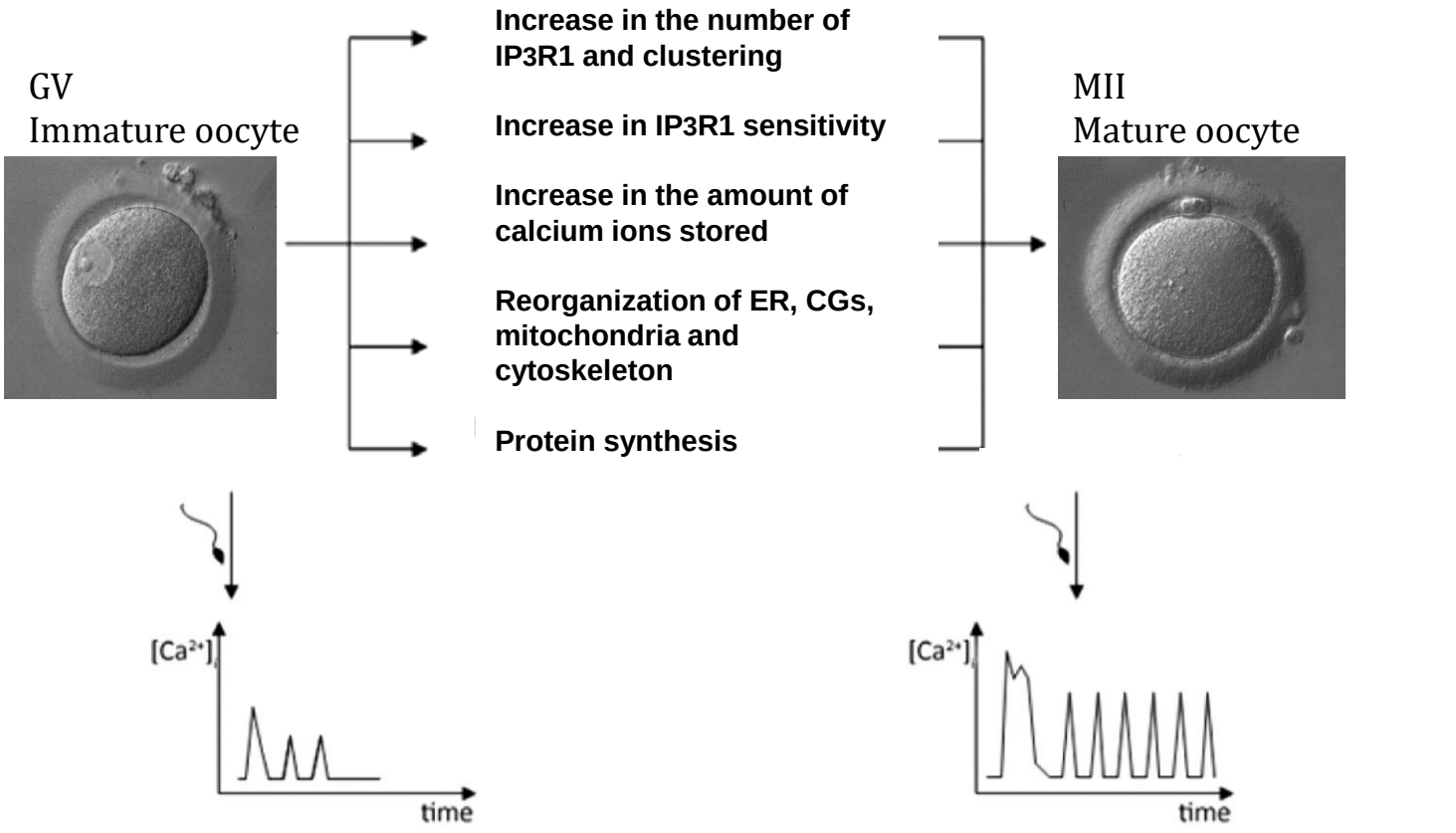
normal FERTILIZATION no



- ✓ The effectiveness of a calcium signal depends on its number, frequency, temporal modulation, and amplitude

Kashir et al., 2010

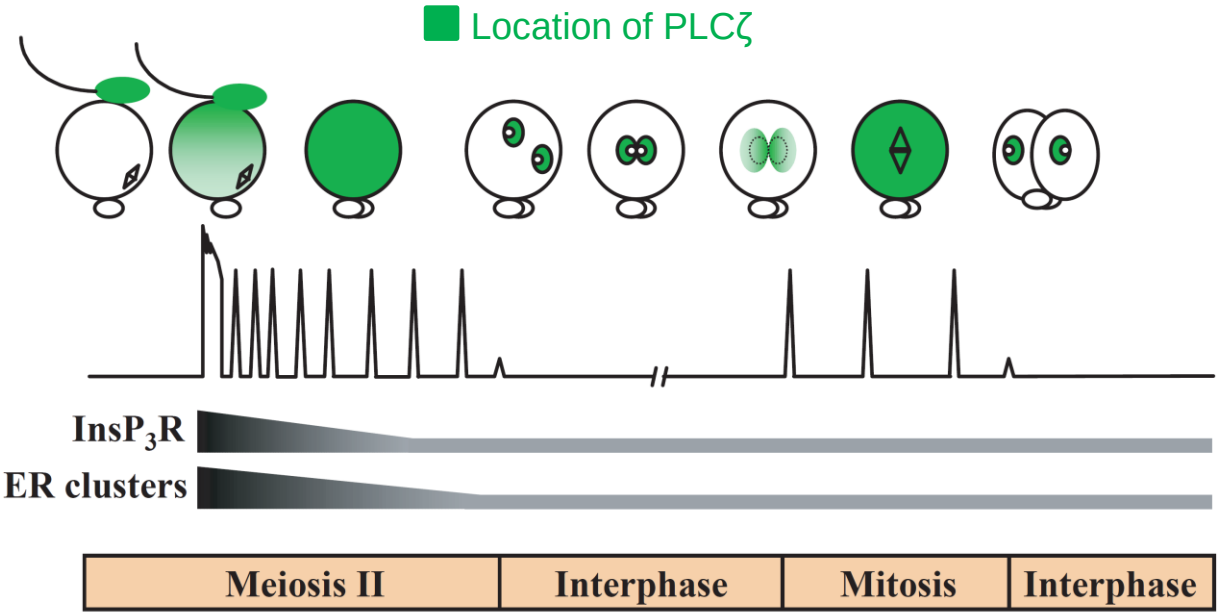
Competency of Ca^{2+} release



Calcium signalling is affected by in vitro maturation, cryopreservation, and oocyte ageing

Courtesy of B. Heindryckx

- Ca^{2+} and mitosis



- ✓ Oscillations cease with formation of both pronuclei
- ✓ Immediately prior to each mitotic division a single Ca^{2+} peak occurs
- ✓ A close correlation between cell division and Ca^{2+} availability has been reported
- ✓ Sinusoidal Ca^{2+} fluctuations observed shortly before every mitotic division disappeared progressively in arrested human embryos
- ✓ Mitosis can be stopped using chelators

Courtesy of B. Heindryckx

Larman et al., J Cell Sci 2004, 117, 2513-2521

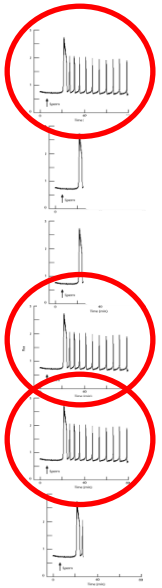
- Artificial oocyte activation (AOA)

I. Modified ICSI-technique

II. Electrical activation

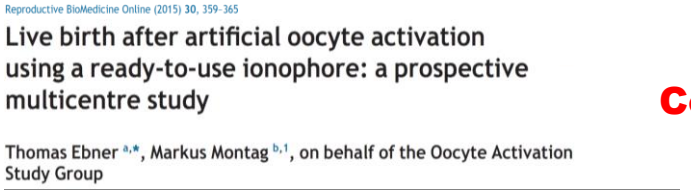
III. Chemical activation

Less effective
Limited to some species
Meiotic spindle disruption



- ✓ Strontium chloride (Kishikawa et al., 1999)
- ✓ Puromycin (Murase et al., 2004)
- ✓ 6-DMAP (Heindryckx et al., 2009)
- ✓ Phorbol esters (Cuthbertson and Cobbold, 1995)
- ✓ Thimerosal (Fissore et al., 1995)
- ✓ Calcium ionophore (Rybouchkin et al., 1997)
  - ionomycin
  - calcimycin (A23187)

- Indications



Reproductive BioMedicine Online (2015) 30, 359–365

Live birth after artificial oocyte activation using a ready-to-use ionophore: a prospective multicentre study

Thomas Ebner [a,*], Markus Montag [b,1], on behalf of the Oocyte Activation Study Group

Complete fertilization failure in previous ICSI cycle(s)

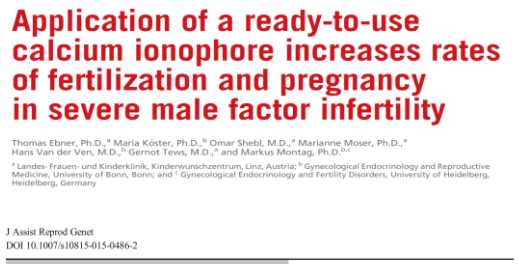
Reproductive BioMedicine Online (2012) 24, 521–526

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

Low fertilization rate (<30%) in previous ICSI cycle(s)



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

Severe male factor (crypto- and azoospermia)

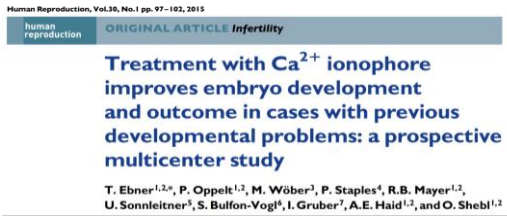
J Assist Reprod Genet
DOI 10.1007/s10815-015-0486-2

CASE REPORT

Healthy twin live-birth after ionophore treatment in a case of theophylline-resistant Kartagener syndrome

T. Ebner [1,3] · M. Maurer [2,3] · P. Oppelt [1,3] · R. B. Mayer [1,3] · H. C. Duba [2,3] · W. Costamoling [4] · O. Shebl [1,3]

Severe male factor (Kartagener syndrome)



Human Reproduction, Vol.30, No.1 pp. 97–102, 2015

human reproduction ORIGINAL ARTICLE *Infertility*

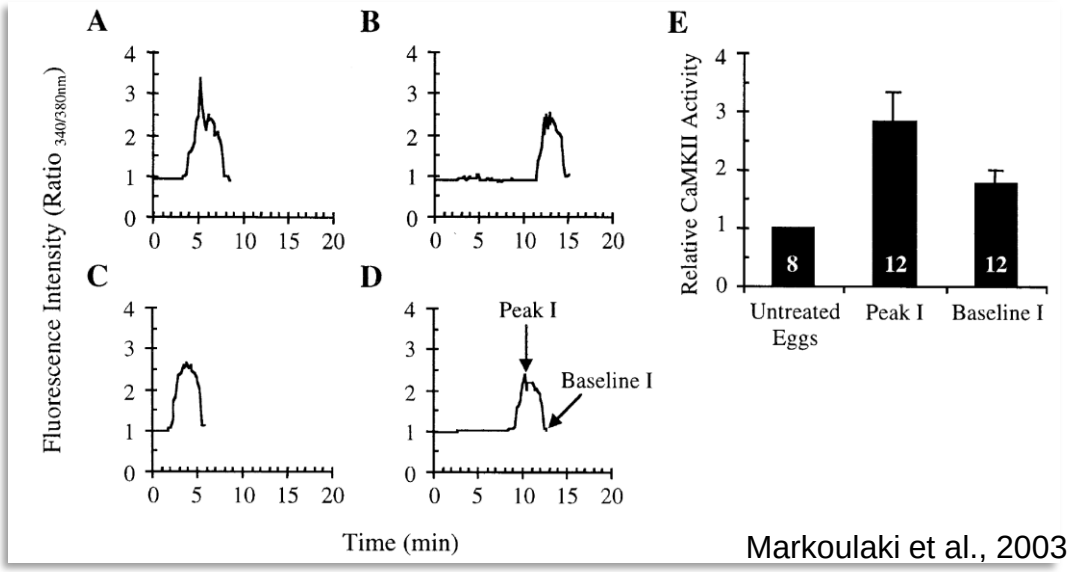
Treatment with Ca^{2+} ionophore improves embryo development and outcome in cases with previous developmental problems: a prospective multicenter study

T. Ebner[1,2,*], P. Oppelt[1,2], M. Wöber[3], P. Staples[4], R.B. Mayer[1,2], U. Sonnleitner[5], S. Bulfon-Vogl[6], I. Gruber[7], A.E. Haid[1,2], and O. Shebl[1,2]

Developmental arrest, delay, and problems

- Theoretical mechanism of action?

✓ The downstream protein CaMKII oscillates in temporal synchrony with the repititive Ca^{2+}-peaks



Markoulaki et al., 2003

This indicates that artificial Ca^{2+} recruitment can result in a proper downstream response

- Theoretical mechanism of action?

✓ CaMKII activity rises and falls with the initial Ca^{2+} transient in a directly proportional manner (Markoulaki et al., 2003)

This reflects the situation commonly found in artificial oocyte activation (which is the presence of a single Ca^{2+} peak)

✓ Once activated autophosporylation of CaMKII keeps the enzyme active even without the presence of calcium (Johnson et al., 1998)

This scenario would explain how a single calcium stimulus can be transmitted without a physiological repetitive pattern

✓ In human, a reduction in frequency can be compensated by both a higher amplitude as well as a longer total duration of the Ca^{2+} signal (Nikiforaki et al., 2014)

Oocyte activation is tolerant to pertubations of the Ca^{2+} oscillation pattern as long as the total calzium amount is uncompromised (and passes a critical threshold)

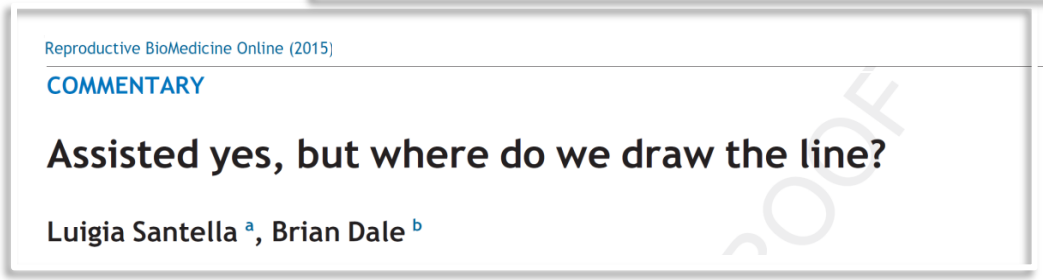
- Safety issues

Reproductive BioMedicine Online (2015) 30, 323–324

EDITORIAL

A plea for caution and more research in the 'experimental' use of ionophores in ICSI

Jonathan van Blerkom, Jacques Cohen, Martin Johnson



Reproductive BioMedicine Online (2015)

COMMENTARY

Assisted yes, but where do we draw the line?

Luigia Santella [a], Brian Dale [b]

Current Trends in Clinical Embryology 2015; 2 (4): 149-152

Commentary

Oldie but Goldie or opening Pandora's box?

Thomas Ebner[1,2]
Omar Shebl[1,2]
Lodovico Parmegiani[3]

COMMENTARY

Artificial oocyte activation: evidence for clinical readiness

T Ebner [a,*], M Montag [b]

✓If it is not obvious that artificial oocyte activation is needed a sibling oocyte approach should be applied

✓Assisted oocyte activation is not beneficial for all patients (Montag et al., 2012; Vanden Meerschaut et al.,2012)

✓Artificial oocyte activation still needs to be considered as experimental (but seems to be safe)

Oldie but Goldie or opening Pandora´s Box

- 5 years of ready-to-use ionophore treatment in Linz, Austria (2011-2015) -

8.9% (305/3416)
Ionophore applied

40.1% (112/279)
positive hCG
18 no ET
8 OHSS

32.3% (90/279)
Live birth rate

98
+28 vit
Children

i. Is the miscarriage rate higher than would normally be expected?

No

19.6% (22/112)
- 6x biochemical
- 16x missed abortions

ii. If available for genetic analysis are the products of conception euploid or not?

YES

iii. Do miscarriage rates occur at the same stage of gestation common after unassisted ICSI?

YES

15 HA-
- 12x week 5
- 3x week 7

7 HA+
- 2x week 8
- 4x week 10
- 1x week 18

iv. Is the incidence of minor and major congenital malformations within acceptable limits?

YES

0.8% 1/126
- Hypoplastic left heart syndrome (major, vitrified)

Artificial oocyte activation with calcium ionophore does not cause a widespread increase in chromosome segregation errors in the second meiotic division of the oocyte

Antonio Capalbo, Ph.D.,[a,b] Christian S. Ottolini, B.Sc.,[c,d] Darren K. Griffin, Ph.D.,[d] Filippo Maria Ubaldi, M.D., M.Sc.,[a,b] Alan H. Handyside, Ph.D.,[c,d,e] and Laura Rienzi, B.Sc., M.Sc.[a,b]

100µM

59.3% euploid

TABLE 2

Aneuploidies identified in normal and abnormal activated oocytes.

Patient	Oocyte ID	aCGH analysis	SNP genotyping analysis	No. of polar PB, PN	Amplification type
Normally activated (2PB1PN and 2PB0PN)					
1	1.1	−5, −10, +20	−5, −10, +20	2PB, 1PN	SurePlex
	1.2	+11, −21	+11, −21	2PB, 1PN	SurePlex
2	2.1	+22	+22	2PB, 1PN	SurePlex
	2.3	+1	+1[a]	2PB, 1PN	SurePlex
	2.5	Euploid	Euploid	2PB, 1PN	SurePlex
4	4.1	−4	−4	2PB, 1PN	SurePlex
	4.2	Euploid	Euploid	2PB, 1PN	SurePlex
	4.3	Euploid	Euploid	2PB, 1PN	SurePlex
	4.4	Euploid	Euploid	2PB, 1PN	SurePlex
5	5.1	Euploid	N/A	2PB, 1PN	SurePlex
	5.2	−13	N/A	2PB, 1PN	SurePlex
	5.3	Euploid	N/A	2PB, 1PN	SurePlex
6	6.2	Euploid	Euploid	2PB, 1PN	MDA
	6.3	Euploid	Euploid	2PB, 1PN	MDA
7	7.3	Euploid	Euploid	2PB, 1PN	MDA
	7.4	−4	−4	2PB, 1PN	MDA
8	8.1	−6, −18, +20	−6, −18, +20	2PB, 1PN	MDA
	8.2	Euploid	Euploid	2PB, 1PN	MDA
	8.3	Euploid	Euploid	2PB, 1PN	MDA
9	9.1	N/A	Euploid	2PB, 1PN	MDA
10	10.1	N/A	−17	2PB, 1PN	MDA
	10.2	N/A	Euploid	2PB, 1PN	MDA
	10.3	N/A	Euploid	2PB, 1PN	MDA
	10.4	N/A	Euploid	2PB, 1PN	MDA
Normally activated 2PB0PN					
2	2.2	++15	+15	2PB, 0PN	SurePlex
3	3.1	Euploid	Euploid	2PB, 0PN	SurePlex
7	7.2	−13, +20, −22	−13, +20,[a] −22	2PB, 0PN	MDA
Abnormally activated					
2	2.4	−13	−13	2PB, 2PN	SurePlex
6	6.1	Euploid	N/A	1PB, >2PN	MDA
7	7.1	Euploid	N/A	2PB, 2PN	MDA
11	11.1	+1, +4, +15, +16, +17, −18, −22	N/A	2PB, 2PN	SurePlex

Note: aCGH = array comparative genomic hybridization; N/A = not available; PB = polar body; PN = pronucleus; SNP = single-nucleotide polymorphism.
[a] Aneuploidies occured following a meiosis two segregation error.

Capalbo. Aneuploidies after artificial activation. Fertil Steril 2015.

„There is no evidence that AOA causes a widespread increase in chromosome segregation errors in meiosis II“

Reproductive BioMedicine Online (2011) 23, 234–244

Methodology matters: IVF versus ICSI and embryonic gene expression

Phillip J Bridges [a], Myoungkun Jeoung [a], Heyoung Kim [a], Jung Ho Kim [b], Dong Ryul Lee [b], CheMyong Ko [a,*], Doris J Baker [a,*]

[a] Division of Clinical and Reproductive Sciences, University of Kentucky, Lexington, KY 40536, USA; [b] Fertility Center of CHA Gangnam Medical Center, CHA University, 135-080 Seoul, South Korea

$SrCl_2$

132 genes in total
(197 in ICSI vs IVF)

Table 4 Functional classification of genes that differed in blastocysts derived by ICSI with chemical activation (ICSI-A) versus IVF ($P < 0.05$).

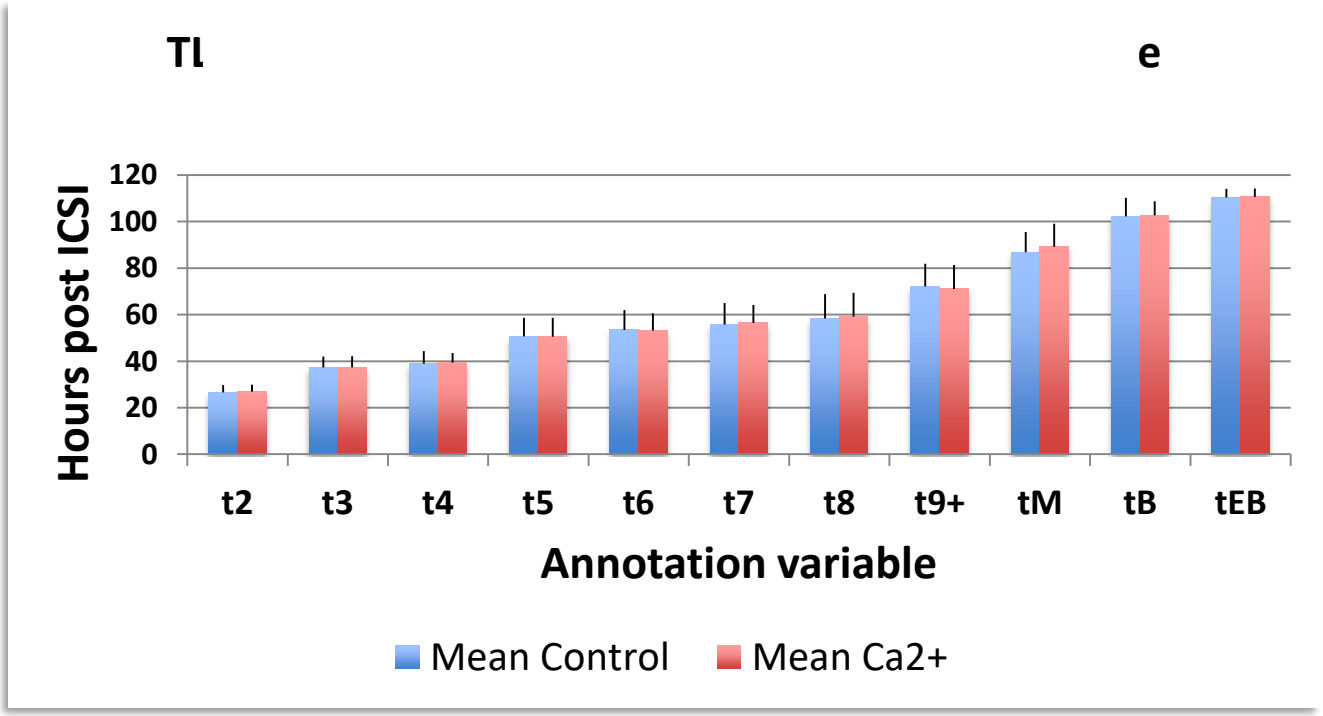
Type	Function	Name	Entities	P-value
Development	Cellular	Ureteric bud branching	16	0.00469
	Neural	Peripheral nervous system development	14	0.00398
		Neural crest cell development	5	0.00798
	Organ	Organ morphogenesis	98	0.00007
		Thyroid gland development	5	0.00147
		Inner ear morphogenesis	29	0.00197
		Middle ear morphogenesis	6	0.00324
		Embryonic gut development	5	0.00792
		Pattern specification process	28	0.00890
	Structural	Cartilage development	28	0.00659
		Embryonic skeletal system morphogenesis	26	0.00720
Metabolism		Lipid glycosylation	7	0.00147
		Retinol metabolic process	10	0.00297
Response	Cellular	Glial cell differentiation	10	0.00072
		[illegible]cytosolic calcium ion concentration	40	0.00005
			265	0.00036
			[illegible]	
		Synaptic transmission		
	Other	Detection of chemical stimulus involved in [illegible]		[illegible]
		Visual perception	93	0.00001
		Response to stimulus	84	0.00006
		Digestion	17	0.00629
		Acute-phase response	15	0.00840
Signalling		G-protein coupled receptor protein signalling pathway	216	0.00000
		Signal transduction	805	0.00008
		Cell surface receptor linked signal transduction	115	0.00014
		G-protein signalling, coupled to cyclic nucleotide second messenger	14	0.00026
		Cell–cell signalling	103	0.00115
		Inositol phosphate-mediated signalling	5	0.00651
		G-protein signalling, coupled to cAMP nucleotide second messenger	10	0.00783

(Table 4 continued on next page)

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

Reproductive BioMedicine Online (2014) 28, 54–63

Neonatal and neurodevelopmental outcome of children aged 3–10 years born following assisted oocyte activation

Frauke Vanden Meerschaut [a,*], Evelien D'Haeseleer [b], Hannelore Gysels [c], Ylenia Thienpont [c], Griet Dewitte [d], Björn Heindryckx [a], An Oostra [d], Herbert Roeyers [c], Kristiane Van Lierde [b], Petra De Sutter [a]

- ✓ Language skills
- ✓ Motor skills
- ✓ Cognition

were found to be within expected ranges

Table 3 Scores of the follow up by assessment.

Test and age group	*No. of children*	*Norm*	*Score*			
Cognition	21		Verbal IQ	Performal IQ	Full scale IQ	
WPPSI-III-NL (≤7 years)	20	100	102.8 (96.4–109.2) (77–129)	105.0 (97.8–112.2) (74–142)	104.2 (97.5–110.8) (85–144)	
WISC-III-NL (>7 years)	1	100	123	130	131	
Language	20		Receptive scale	Expressive scale	Total percentile score	
RTOS (<5 years)	8	50	82.0 (63.4–100.7)[a] (35.5–99)	73.3 (49.4–97.2)[b] (30–99)	78.2 (57.2–99.2)[c] (33.3–99.0)	
CELF-IV-NL (≥5 years)	12	50	32.5 (7.3–57.7)[a] (3.6–94.5)	40.9 (16.6–65.2)[b] (7.1–88.5)	40.1 (16.8–63.4)[c] (9.1–89.7)	
Motor skills	19		Manual dexterity	Aiming and catching	Balance	Total percentile score
Movement ABC-II-NL (3–6 years)	17	50	42.0 (24.4–58.7) (1–95)	43.8 (29.5–58.0) (2–95)	41.5 (25.1–57.9) (5–91)	37.1 (22.3–52.0) (2–84)
Movement ABC-II-NL (7–10 years)	2	50	56.5 (NA) (50–63)	21.0 (NA) (5–37)	77.0 (NA) (63–91)	50.0 (NA) (37–63)
Autism screening	16		Score			
SCQ (≥2 years)	16	<15	6.4 (4.5–8.3) (0–13)			
Maladaptive behaviour screening	19		Internalizing broadband	Externalizing broadband	Total score	
CBCL (1.5–5 years)	11	<70	50.5 (42.7–58.2) (33–74)	47.6 (42.1–53.0) (35–60)	48.6 (42.5–54.8) (30–63)	
CBCL (6–18 years)	8	<70	38.4 (33.6–43.2) (33–50)	39.1 (35.0–43.2) (34–48)	37.3 (32.0–42.5) (29–45)	
TRF (1.5–5 years)	4	<70	47.5 (41.3–53.7) (44–53)	44.8 (36.8–52.7) (38–49)	45.5 (41.7–49.3) (44–49)	
TRF (6–18 years)	4	<70	43.5 (31.7–55.7) (37–52)	42.7 (40.9–44.1) (41–43)	42.5 (32.1–52.9) (33–48)	

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

Future aspects

What to do if there is no success with ionophore treatment?

- ✓increase exposure time
- ✓switch to 2-fold exposure
- ✓switch to combination of 2 ionophores (e.g., calci- and ionomycin)
- ✓more invasive approach (e.g., direct injection of $CaCl_2$ & x2 ionomycin)
- ✓use recombinant PLCζ

- Summary

Kepler Universitäts Klinikum

Taken together, it seems that human oocytes can respond to a wide range of intracellular Ca^{2+} signalling parameters and have a surprisingly high rate of tolerance for prolonged changes in cytosolic Ca^{2+}

So far neonatal outcome is promising

AOA is in the transition process from experimental to clinical
(provided that it is applied with a proper indication)

Thank you very much for your kind attention

Prof. Dr. Peter OPPELT
Assoc.Prof. Dr. Omar SHEBL

Dr. Alwin HABELSBERGER
Dr. Elisabeth RADLER
Dr. Richard B. MAYER

Manuela PUCHNER
Doris BRANDSTETTER
Esther MITTERMAIR
Laudia HADJARI