Clinical application of artificial oocyte activation: results from a prospective multi-centre study

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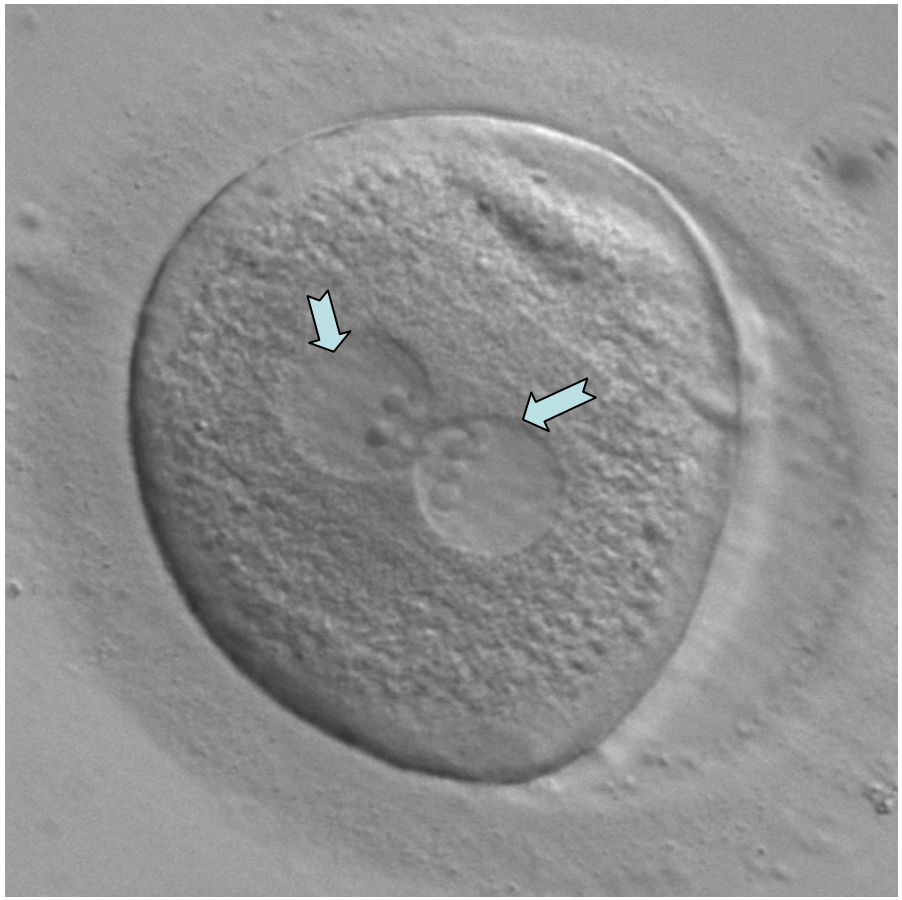
The mechanism of oocyte activation



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

The reason for artificial oocyte activation is fertilization failure

- Fertilization failure can be observed after ICSI
  - Patients with Globozoospermia
  - Patients with normal sperm parameters but consistently low fertilization rates below 50 %
- Some authors have linked this to a deficiency of the oocyte activation factor PLC zeta
  - Yoon et al., 2008; Heytens et al., 2009
  - Review by Kashir et al., Hum Reprod Update 2010
- Failed fertilization is mainly a sperm born problem

Methods of artificial oocyte activation

- Calcium ionophore A23187 Rybouchkin et al., 1997
- Strontium chloride Yanagida et al., 2006
- 6-DMAP Heindryckx et al., 2009
- Electric pulses Yanagida et al., 1999
- Modified ICSI technique Tesarik et al., 2002; Ebner et al., 2004
- Rec PLC zeta Yoon et al., 2008

Results from a retrospective mono-centre study on artificial oocyte activation in the same ICSI patients

	Results from a retrospective mono-centre study (fertilization rate in a previous ICSI cycle < 50%)		
	Pre-cycle without Ca-Act.	**Cycle with Ca-Act.**	**P-value**
Patients	**97**	**97**	
ICSI cycles	**117**	**126**	
Cycles with transfer	**69.2 %**	**87.3 %**	< 0.001
Embryos for transfer (mean)	**1.22**	**1.63**	< 0.001
Fertilization rate	**20.6 %**	**46.7 %**	< 0.001
Pregnancy rate / embryo transfer	**13.9 %**	**27.7 %**	< 0.05

Montag et al., 2009

Design of the multi-centre study

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)

Study design

Method:

– Immediately after ICSI, oocytes were incubated in pre-gased Cult-active medium (Gynemed) for 15 min (calcium ionophore ready-to-use 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 $\geq$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

Overall outcome

– Patients in the study:	101
– Cycles with embryo transfer:	100
– Fertilization rate:	47.7 %
– Number of embryos / transfer:	1.85
– Cycles with positive ß-hCG:	48 (48 %)
– Cycles with positive fetal sac:	37 (37 %)
– Implantation rate:	25.4 %
– Pregnancy loss: 2 EUG, 7 abortions	24.3 %
– Take home baby rate:	28 %

- 35 children born (22 boys, 13 girls)

Results from the prospective multi-centre study - fertilization rate in previous ICSI cycle: 0 %

	Pre-cycle without Ca-Act.	Cycle with Ca-Act.	P-value
Patients	16	16	
ICSI cycles	21	16	
Cycles with transfer	0 %	100 %	
Embryos for transfer (mean)	0.0	1.7	
Fertilization rate	0 %	57 %	
+ßhCG rate	0 %	43.8 %	
Clin. Preg. rate / ET	0 %	31.3 %	
Implantation rate	0 %	26.9 %	
Abortion rate / EUG	0 %	20 %	
Take home baby rate	0 %	25.0 %	

Results from the prospective multi-centre study - fertilization rate in previous ICSI cycle: 1 – 30 %

	Pre-cycle without Ca-Act.	Cycle with Ca-Act.	P-value
Patients	**52**	**52**	
ICSI cycles	**90**	**52**	
Cycles with transfer	**100 %**	**100 %**	
Embryos for transfer (mean)	**1.6**	**1.8**	
Fertilization rate	**20.1 %**	**44.2 %**	< 0.001
+ßhCG rate	**0 %**	**50.0 %**	
Clin. Preg. rate / ET	**0 %**	**40.4 %**	
Implantation rate	**0 %**	**27.1 %**	
Abortion rate / EUG	**0 %**	**19 %**	
Take home baby rate	**0 %**	**32.7 %**	

Results from the prospective multi-centre study - fertilization rate in previous ICSI cycle: 31 – 50 %

	Pre-cycle without Ca-Act.	Cycle with Ca-Act.	P-value
Patients	**34**	**34**	
ICSI cycles	**55**	**34**	
Cycles with transfer	**100 %**	**97 %**	
Embryos for transfer (mean)	**2.0**	**1.9**	
Fertilization rate	**39.4 %**	**50.9 %**	< 0.005
+ßhCG rate	**3.6 %**	**45.5 %**	< 0.001
Clin. Preg. rate / ET	**3.6 %**	**33.3 %**	< 0.001
Implantation rate	**3.0 %**	**22.2 %**	< 0.001
Abortion rate / EUG	**100 %**	**36.4 %**	< 0.1
Take home baby rate	**0 %**	**21.2 %**	< 0.001

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 %**

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 deficiency, thus enabling fertilization rates close to 50%

- Artificial oocyte activation may not help as an universal tool to enhance fertilization rates in every patient

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