XXII CONGRESSO BRASILEIRO
DE REPRODUÇÃO ASSISTIDA
Brasília, 01 a 04 agosto de 2018

O QUE FAZER QUANDO A FERTILIZAÇÃO NÃO OCORRE

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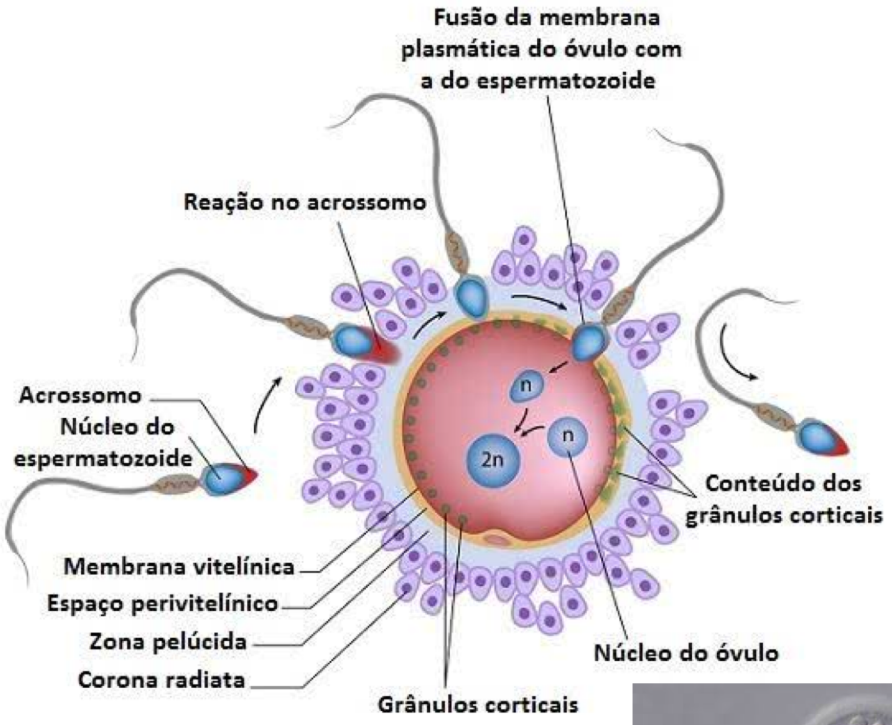
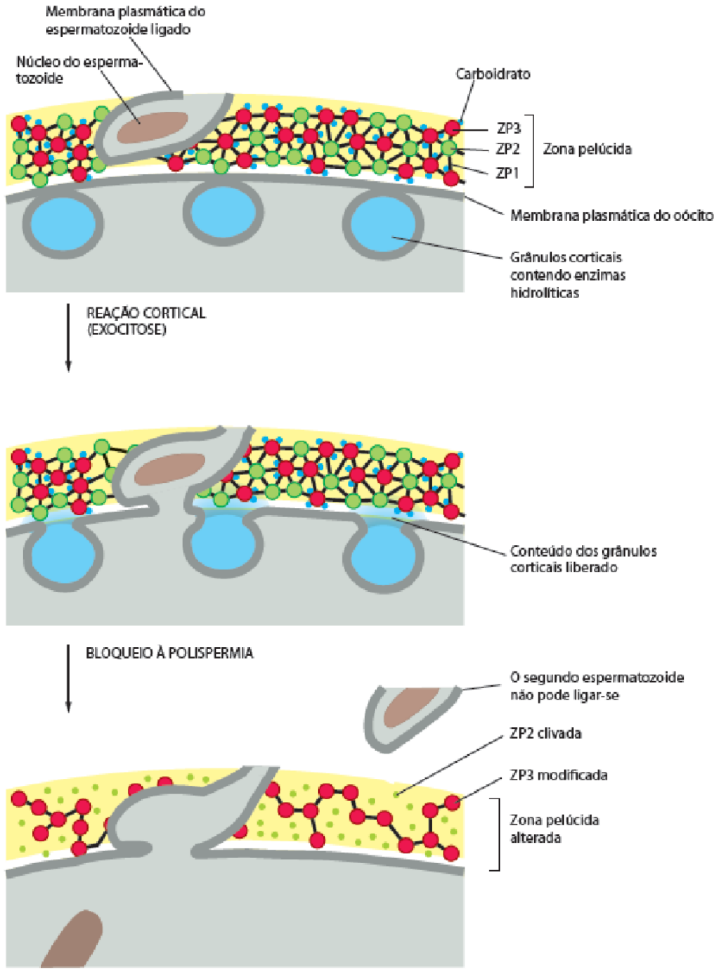
vitæmbrio
REPRODUÇÃO ASSISTIDA

Centro de Fertilidade
VIDA

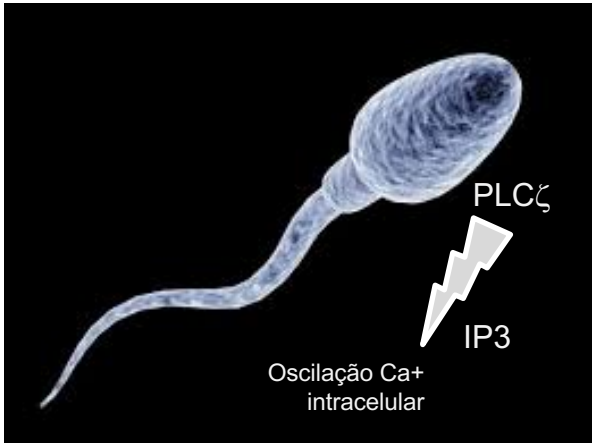
Fases da fertilização

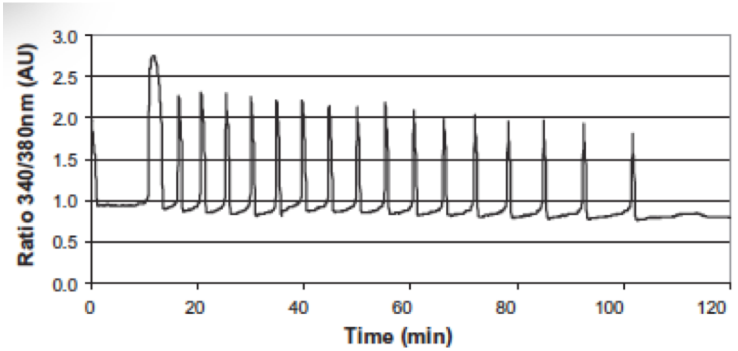




Processos moleculares da fertilização

1. **Liberação de hialuronidase e acrosina → Contato com a corana radiata resulta em perfurações no acrossoma**
2. **Ligação do acrossoma com ZP3 (mediada pela MP, íons de cálcio, prostaglandinas e progesterona)**
3. **Fusão da MP do óvulo com a membrana acrossomal externa**
4. Rearranjos no citoesqueleto para dar suporte à formação do zigoto e desenvolvimento embrionário





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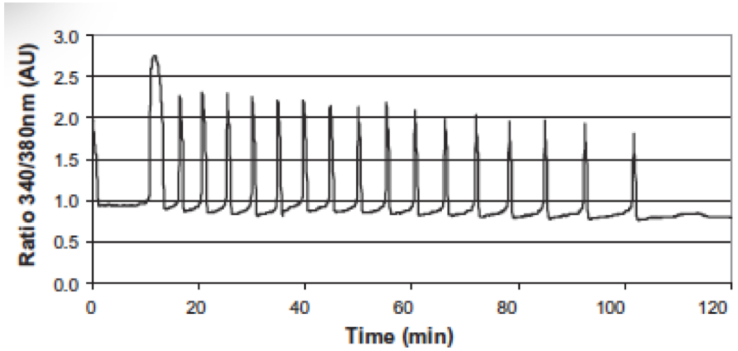

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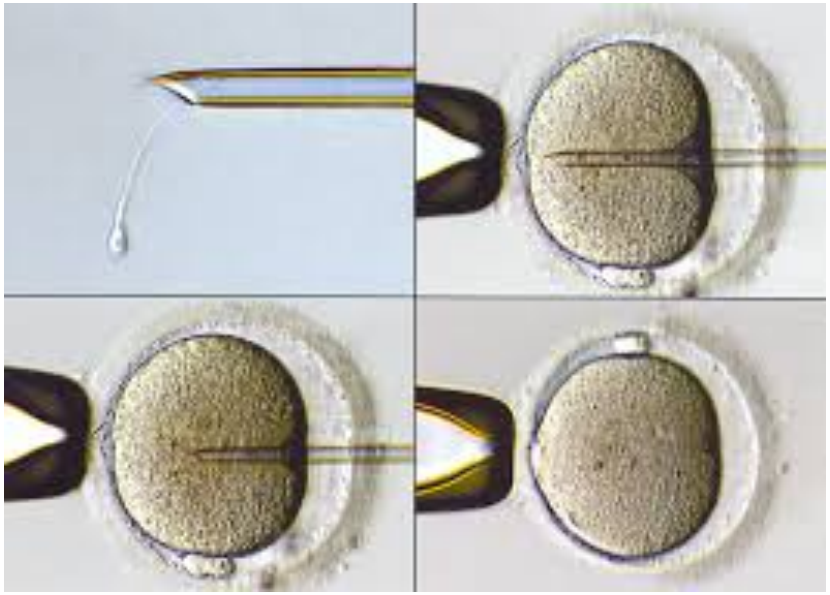
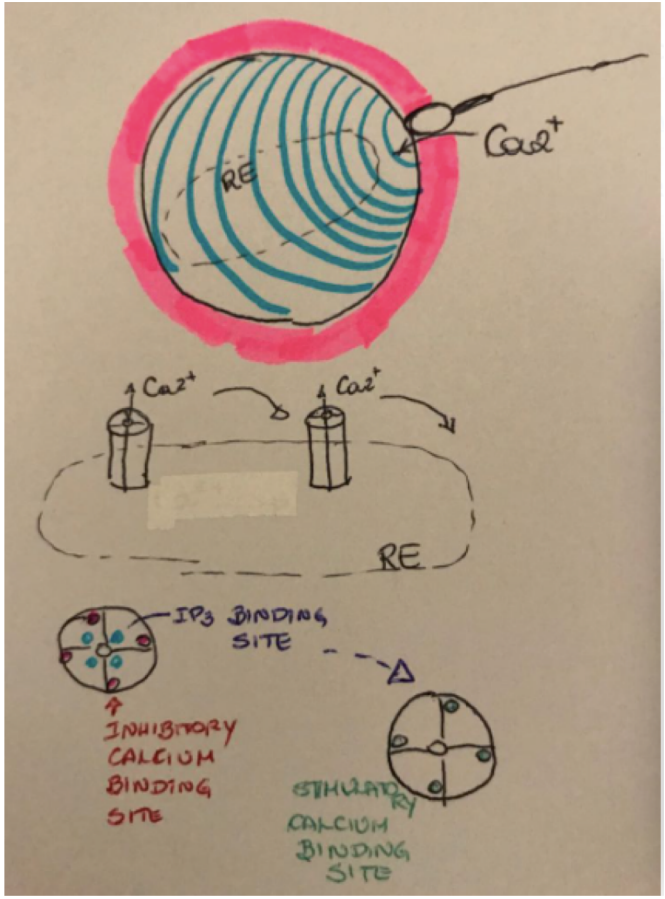
Maturação citoplasmática e nuclear

- Reorganização do RE
- ↑ receptores para o IP3
- ↑ sensibilidade do receptor p/ o IP3
- ↑ estoques [Ca+] no RE
- Redistribuição das proteínas ligadoras ao Ca+ no RE

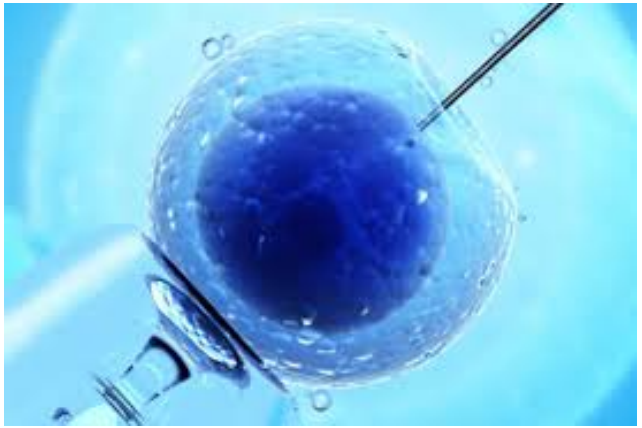
Diferenças para FIV e ICSI





Indicações clássicas para ICSI

- Oligoastenozoospermias clássicas
- Parâmetros seminais sub-óptimos
- **Falha ou baixa fertilização na FIV convencional anterior**



- Azoospermias
- PGTs
- Sorodiscordância

"Quanto" é falha de fertilização?

KPI (Key Performance Indicator)	Competence value	Benchmark value	Vida 2017
ICSI damage rate	<10%	<5%	8,3% (239/2867)
ICSI 2PN rate	>65%	>80%	72% (2064/2867)
IVF 2PN rate	>60%	>75%	43,5% (232/507)
Failed fertilization in IVF	**<5%**		11,3% (6/53)*
Failed fertilization in ICSI (not stressed in the Vienna consensus)	**1 – 3%**		4,9% (23/469)**
Cleavage rate	>95%	>99%	98%
Day 2 embryo development rate	>50%	>80%	NV
Day 3 embryo development rate	>45%	>70%	82%
Blastocyst development rate	>40%	>60%	47,4% à 65,3%
Successful biopsy rate	>90%	>95%	95%
Blastocyst cryosurvival rate	>90%	>99%	90%
Implantation rate (cleavage stage)	>25%	>35%	32%
Implantation rate (blastocyst stage)	>35%	>60%	36%

The Vienna consensus: report of na expert meeting on the development of ART laboratory performance indicators. EHHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine. http://dx.doi.org/10.1016/j.rbmo.2017.06.015

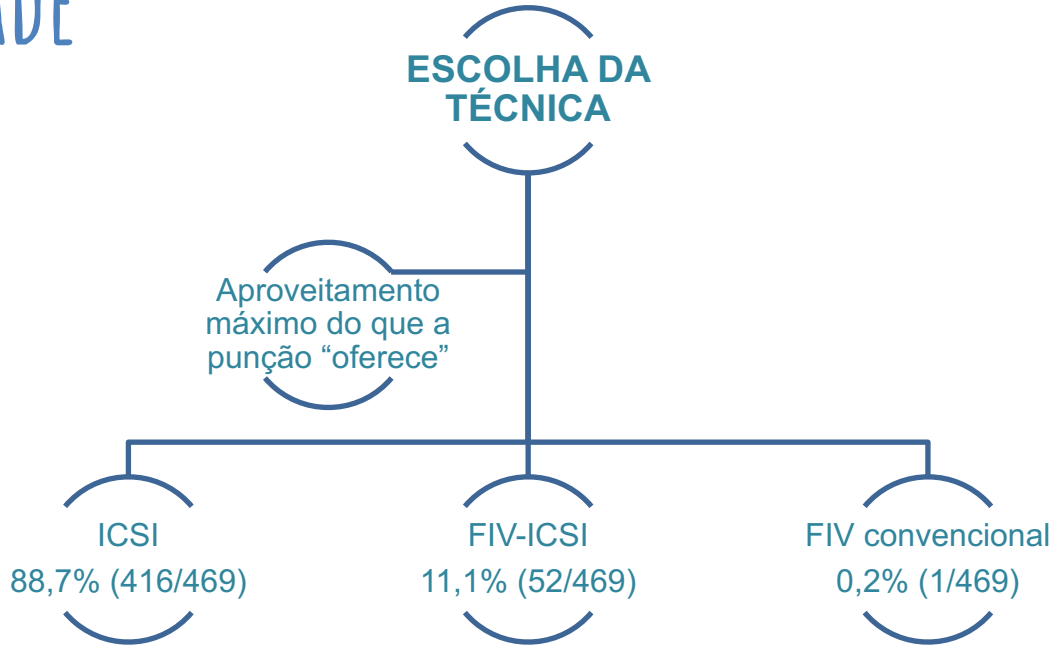
Conduta na escolha da técnica no Vida Centro de Fertilidade

- FIV convencional

  Fertilidade comprovada do casal

- FIV-ICSI

  Avaliado, sempre que o recuperado pós capacitação permite



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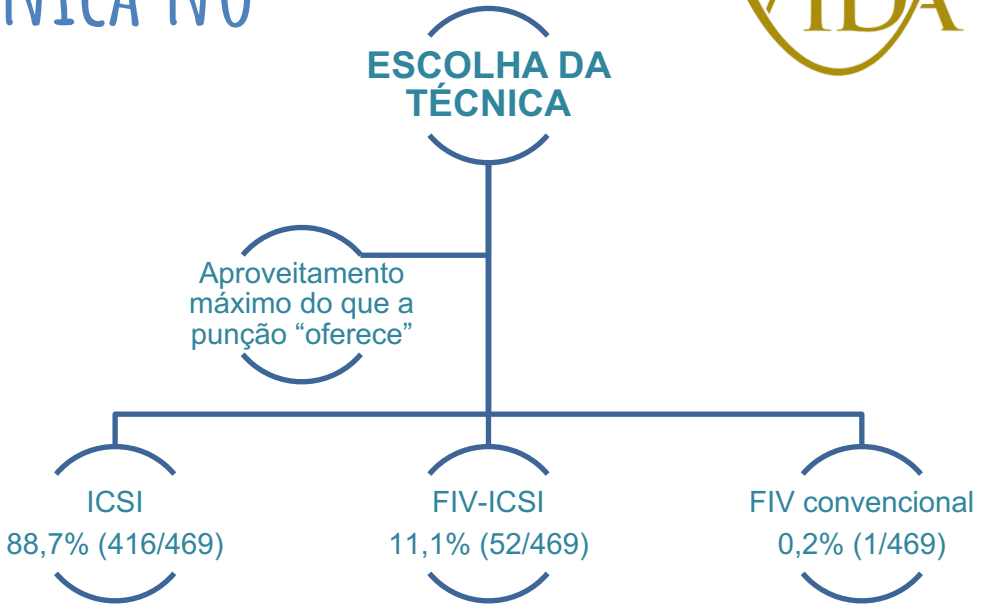


Resultados em 2017 dos casos de FIV-ICSI		
Óvulos inseminados (FIV)	506	
Óvulos maduros FIV	345 (66,6%)	
Óvulos fertilizados FIV	110 (31,9%)	128 (37,1%) – 18 3PN (14,1%)
Óvulos MII injetados	336	
Óvulos fertilizados ICSI	247 (73,5%)	
Falha de fertilização FIV-ICSI	0%	6/53 (11,3%) e 3/52 (5,8%)

"Quanto" é falha de fertilização?

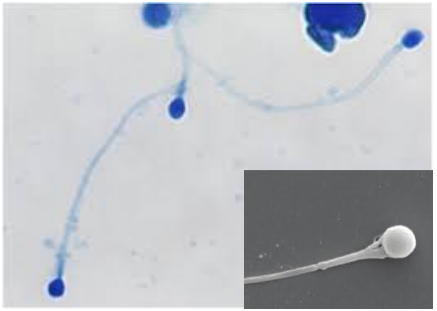
KPI (Key Performance Indicator)	Competence value	Benchmark value	Vida 2017
Failed fertilization in IVF	<5%		**11,5% (6/52)***
Failed fertilization in ICSI (not stressed in the Vienna consensus)	1 – 3%		**4,9% (23/469)****

* Características dos casos de falha na FIV (entre os casos FIV-ICSI)	
Idade (média)	28-38 (33,6 anos)
Óvulos coletados	7 - 26
Óvulos inseminados	3 - 17
Óvulos maduros	0 - 13

** Características dos casos de falha total de fertilização (exclusivamente ICSI)	
Idade (média)	32-45 (39,5 anos)
Óvulos coletados/desvitri	1 – 19 (média 3,9)
Óvulos MII injetados	1 – 14 (total de 59 – média 2,6)
Fatores preponderantes	Baixa reserva ovariana (19) Azo e cripto (2) Endometriose (1) ESCA (1 caso – 4MII/13)

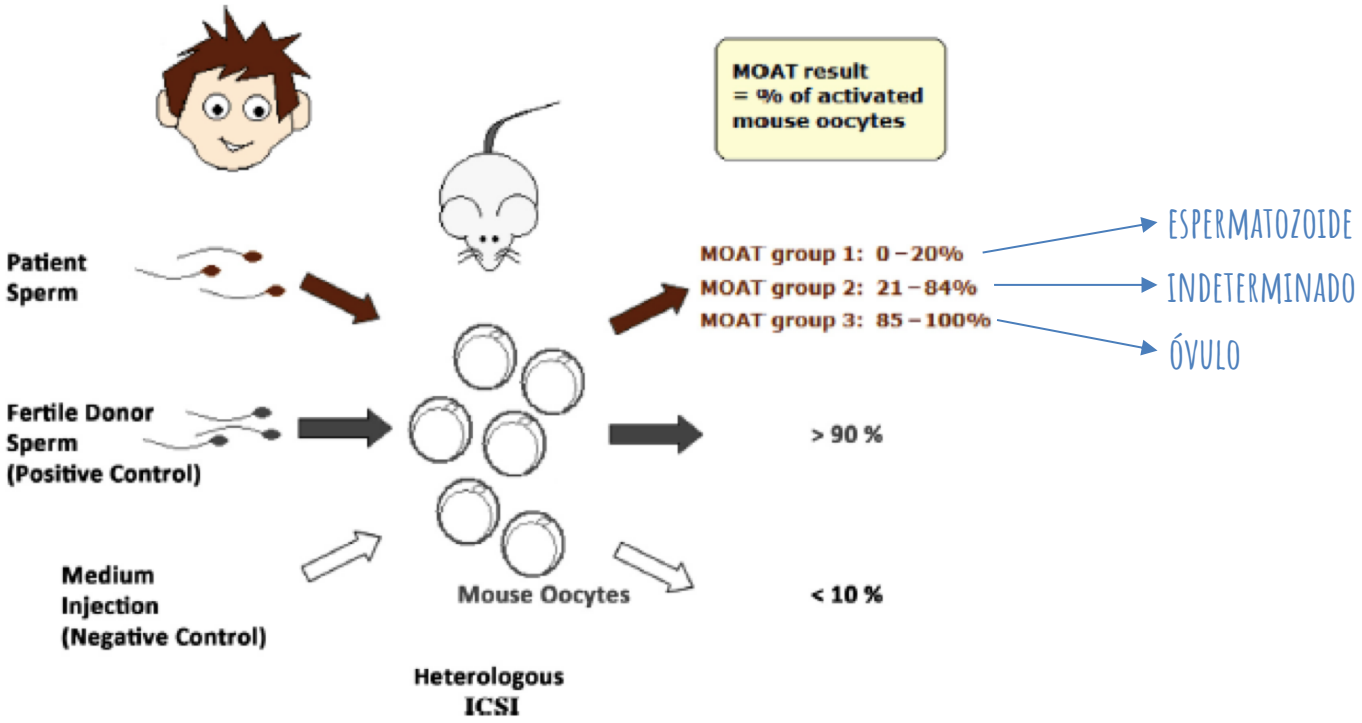
Causas da falha de fertilização em ICSI

- Falha na ativação oocitária (principal)
- Falha na descondensação da cabeça do espermatozoide
- Condensação prematura da cromatina espermática
- Defeitos no fuso (spindle) oocitário
- Defeitos na “sperm aster”



- Problemas técnicos
- Baixa disponibilidade de ovócitos MII
- Ovócitos com alterações morfológicas
- Injeção de espermatozoide sem motilidade
- Teratozoospermia severa (globozoospermia)

Teste para determinar a origem da falha na ICSI



A análise do padrão oscilatório do cálcio confirmam a validade do MOAT.

Vanden Meerschaut, F et al. Diagnostic and prognostic value of calcium oscillatory pattern analysis for patients with ICSI fertilization failure. Hum. Reprod. 2013, 28:87–98.

Ativação oocitária assistida (AOA – assisted oocyte activation)

Métodos de ativação artificial

- Físico: campo elétrico gerado por corrente de voltagem contínua, fazendo com que as proteínas carregadas na bicamada lipídica da membrana celular se movam, formando poros na membrana e permitindo o influxo de cálcio extracelular.
- Mecânico: rompimento da membrana plasmática e aspiração vigorosa do citoplasma (ICSI modificada)

Ativação oocitária assistida (AOA – assisted oocyte activation)

Métodos de ativação artificial

- Químico: Um ionóforo (inomicina ou calcimicina) é uma molécula lipossolúvel, geralmente sintetizada por microorganismos, que transportam ions através da bicamada lipídica da membrana celular. Os ionóforos de cálcio ajudam a ativar o oócito aumentando a permeabilidade de cálcio da membrana celular, permitindo assim que o cálcio extracelular flua para a célula. Este tratamento causa um único pico na concentração de íons cálcio, mas não há oscilações de cálcio subsequentes.

Ativação oocitária assistida (AOA – assisted oocyte activation)

Table 1 Types of artificial oocyte activation methods and reported cases in clinical research

Types of artificial oocyte activation methods	Mechanism of oocyte activation	Cases of pregnancy and delivery in clinical research
Calcium ionophore†	Increase in Ca^{2+} permeability of cell membrane	+ (Hoshi *et al*. 1995 A23187[27])
Electrostimulation	Pore formation in cell membrane, entry of extracellular Ca^{2+}	+ (Yanagida *et al*. 1999[28])
Strontium	Release of endogenous Ca^{2+}	+ (Yanagida *et al*. 2006[29])
Ethanol	Increase in Ca^{2+} permeability of cell membrane	
Puromycin‡	Inhibition of protein synthesis	+ (Murase *et al*. 2004[30])
Cycloheximide	Inhibition of protein synthesis	

†Calcium ionophores include A23187 and ionomycin. ‡Puromycin is used in combination with A23187.

Yanagida K et al. The present status of artificial oocyte activation in assisted reproductive technology. Reproductive Medicine and Biology 2008; *7*: 133–142.

Estudos

- AOA viabiliza a fertilização em casos de falha e proporciona a gravidez
- Estudos heterogêneos e com casuística pequena → resultados variáveis
- A maioria não utilizou o teste diagnóstico anterior (ex: MOAT ou ICSI heteróloga)

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) Tapered heads (n = 23) Bent necks (n = 15)	 36.7 39.3 49.4	 82.7 81.7 48.2	 0.0008 0.005 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 14.3 (1–33) 47 (34–65) 85.8 (66–100)	57.8 58.3 63.4 77.9	S S S 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) TESE OA (n = 24) PESE OA (n = 49)	 44.0 65.2 65.8	 44.7 55.0 67.0	 NS NS NS
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Montag et al. (2012)	Single exposure to 10 μmol/l calcimycin for 15 min immediately following ICSI	ICSI with previous: Failed fertilization (n = 27) Low fertilization (n = 38) Very low fertilization (n = 24)	 0 19.3 (0–29) 36.8 (30–50)	 41.6 44.4 56.1	 <0.05 <0.001 <0.001
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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

Values are in parentheses are 95% CI. Case reports are not included in this overview.
AOA = assisted oocyte activation; ICSI = intracytoplasmic sperm injection; NOA = nonobstructive azoospermia; OA = obstructive azoospermia; NS = no significant; PESE = percutaneous epididymal sperm extraction; S = significant difference but no P-values provided in the paper; split AOA cycle = conventional ICSI and ICSI combined with AOA on sibling oocytes from the same cycle; TESE = testicular sperm extraction.

Vanden Meerschaut, F et al. Assisted oocyte activation following ICSI fertilization failure. Reproductive Bio-Medicine Online (2014), http://dx.doi.org/10.1016/j.rbmo.2014.01.008

Estudos

30 casos de FF anterior com ICSI + AOT	PR
MOAT group 1	34%
MOAT group 2	43%
MOAT group 3	17%

Características dos casos de falha total de fertilização de ICSI no Vida Centro de Fertilidade	
Fatores preponderantes	Baixa reserva ovariana (19) Azo e cripto (2) Endometriose (1) ESCA (1 caso – 4MII/13)

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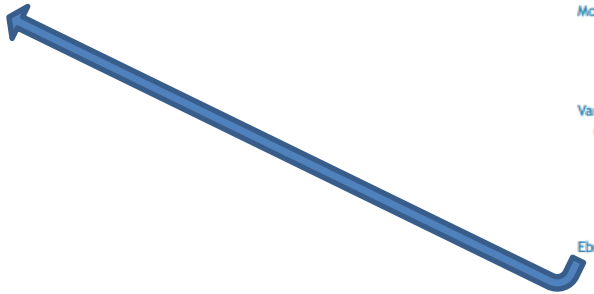
Estudos

	ICSI	ICSI + AOA
Tx fertilização	34,7%	56,9%
No. transferências	79	73
No. gestações clínicas	5	29
Abortos	4 (80%)	4 (11,8%)



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Follow-up das crianças nascidas de ICSI+AOA

Estudo	No. de crianças	Tratamento
Vanden Meerschaut et al. 2014	21 (3-10 anos)	Injeção de 0.1 mol/L CaCl2 na ICSI seguindo, 30 min depois, de 2 exposições, com intervalo de 30 min, de ionicina 10µmol/L
Takisawa et al. 2011	10	A23187 (calcimicina)
Takisawa et al. 2011	12	Cloreto de estrôncio
Sato et al. 2011	Peso e altura de 21 crianças até os 6 anos	????

Ainda não existe confirmação da segurança genética e de *imprinting* anormal para o uso combinado da ICSI com os métodos de ativação de oócitos.

Vanden Meerschaut, F et al. Assisted oocyte activation following ICSI fertilization failure. Reproductive Bio-Medicine Online (2014), http://dx.doi.org/10.1016/j.rbmo.2014.01.008

Metanálise

Table III Summary of findings of RCTs on the effect of ICSI-AOA compared with ICSI-only in oocytes of women with previous fertilization failure, and as a rescue method in oocytes that failed to fertilize.

Outcome	Absolute risk		Relative effect RR (95% CI)	Oocytes (studies)	Observed effect	Quality of the evidence
	ICSI-only	ICSI-AOA				
Previous fertilization failure						
Embryos at cleavage stage (Day 2–3)	11.4%	61.8%	5.4 (3.0–9.9)	211 oocytes (1)	Benefit	Very low[a]
Embryos at blastocyst stage (Day 5–6)			Not reported by the included studies.			
Rescue method						
Embryos at cleavage stage (Day 2–3)	8.8%	75.7%	8.7 (2.3–32.8)	204 oocytes (2)	Benefit	Very low[b]
Embryos at blastocyst stage (Day 5–6)	0%	4.7%	2.0 (0.1–35.0)	104 oocytes (1)	*	Very low[a]

RR, risk ratio; CI, confidence interval.

*The estimate was very imprecise, not being possible to ascertain whether ICSI-AOA is related to harm, no effect or benefit.

[a]Quality of evidence was downgraded because of very serious imprecision, serious inconsistency of the results and high risk of bias of the included studies.

[b]Downgraded because of very serious imprecision, and because evidence comes from only one small study deemed at high risk of bias.

Sfontouris, IA et al. 2015. Hum Reprod Advance Access.
doi:10.1093/humrep/dev136

Metanálise

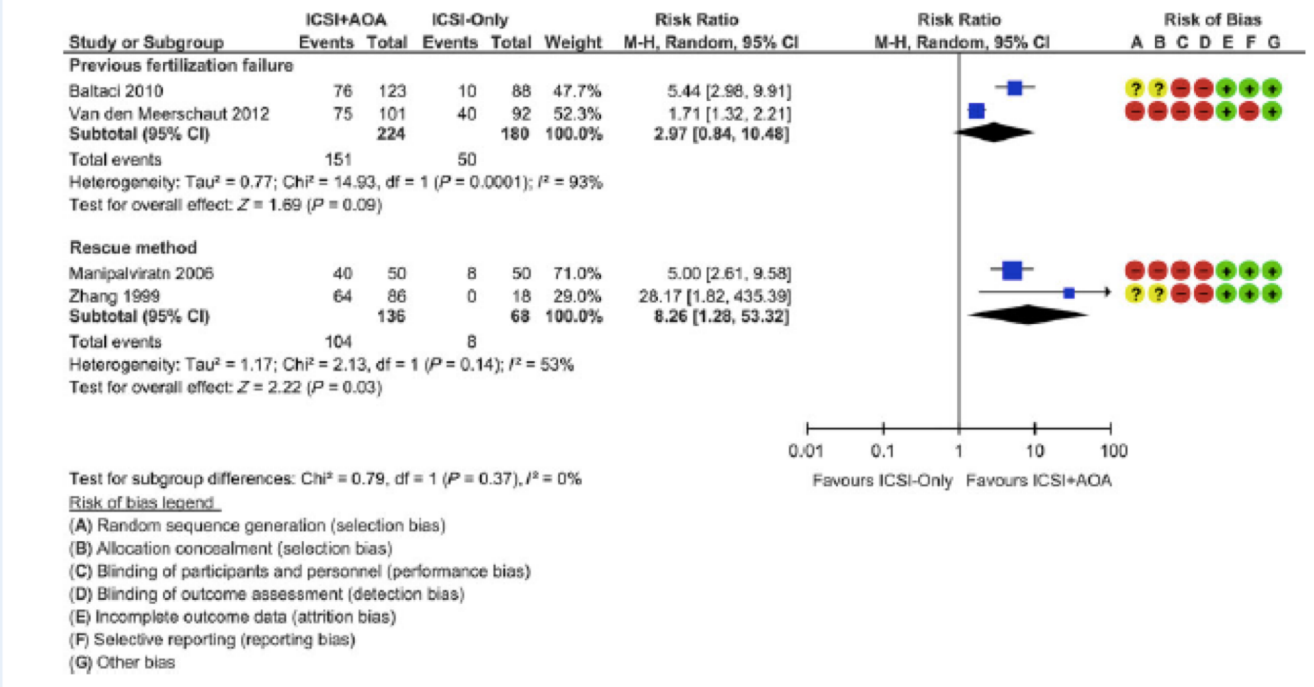

Figure 3 Forest plot for fertilization per oocyte randomized reported by the studies included in the oocyte-based review.

Sfontouris, IA et al. 2015. Hum Reprod Advance Access. doi:10.1093/humrep/dev136

Metanálise

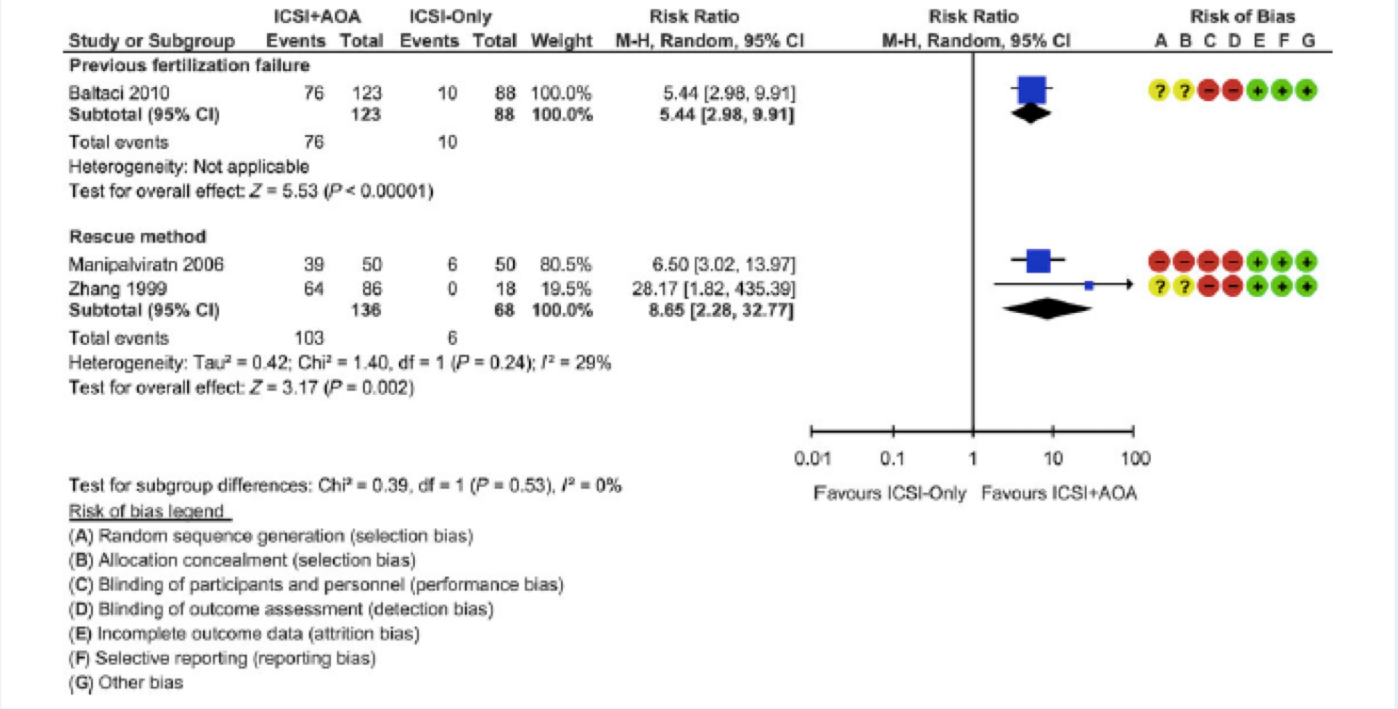

Figure 2 Forest plot for embryos at cleavage stage per oocyte randomized reported by the studies included in the oocyte-based review.

Sfontouris, IA et al. 2015. Hum Reprod Advance Access. doi:10.1093/humrep/dev136

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