
In the name
of God...

Royan Institute

# Application of Artificial Oocyte Activation in Assisted reproductive technology

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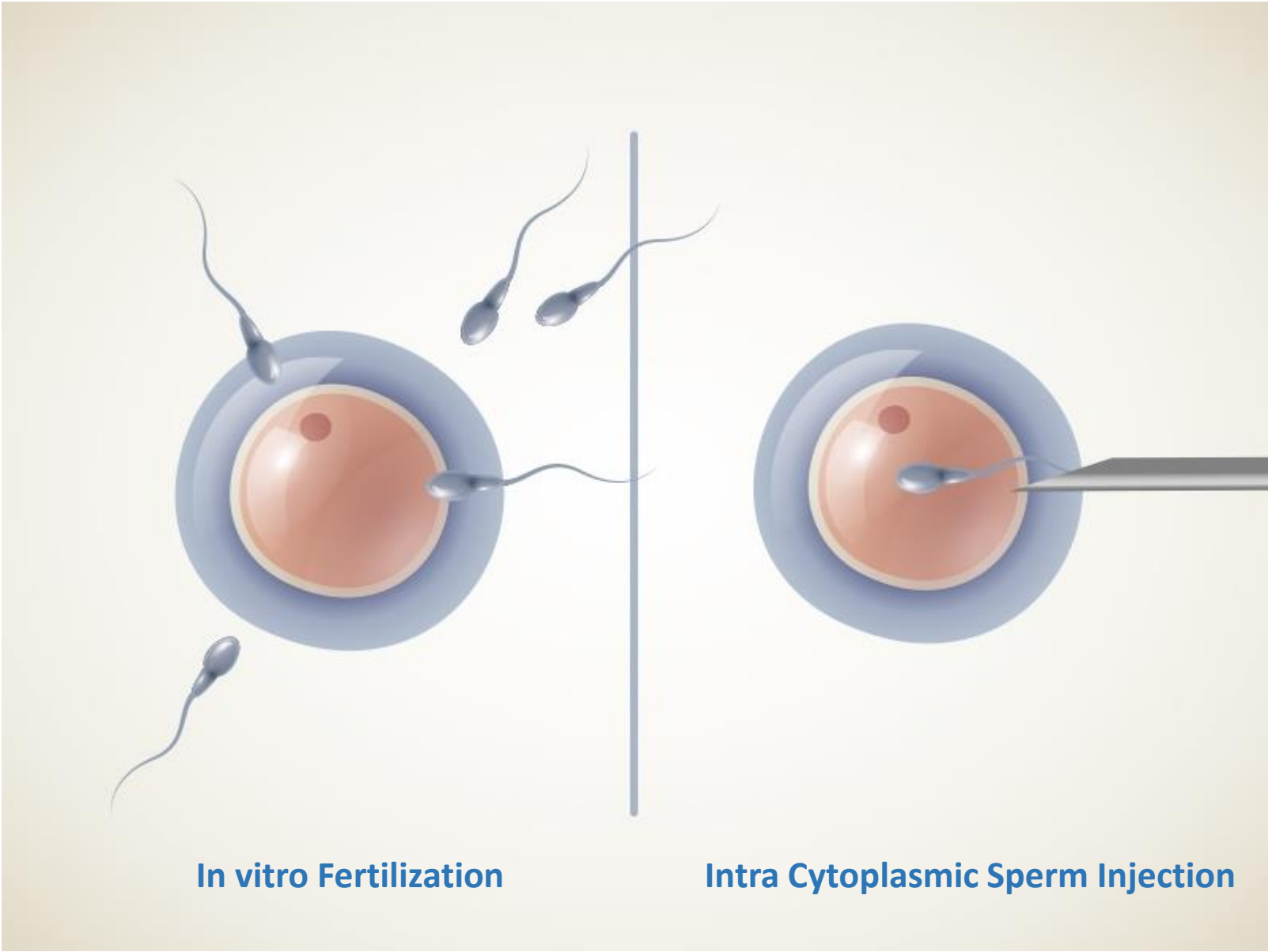
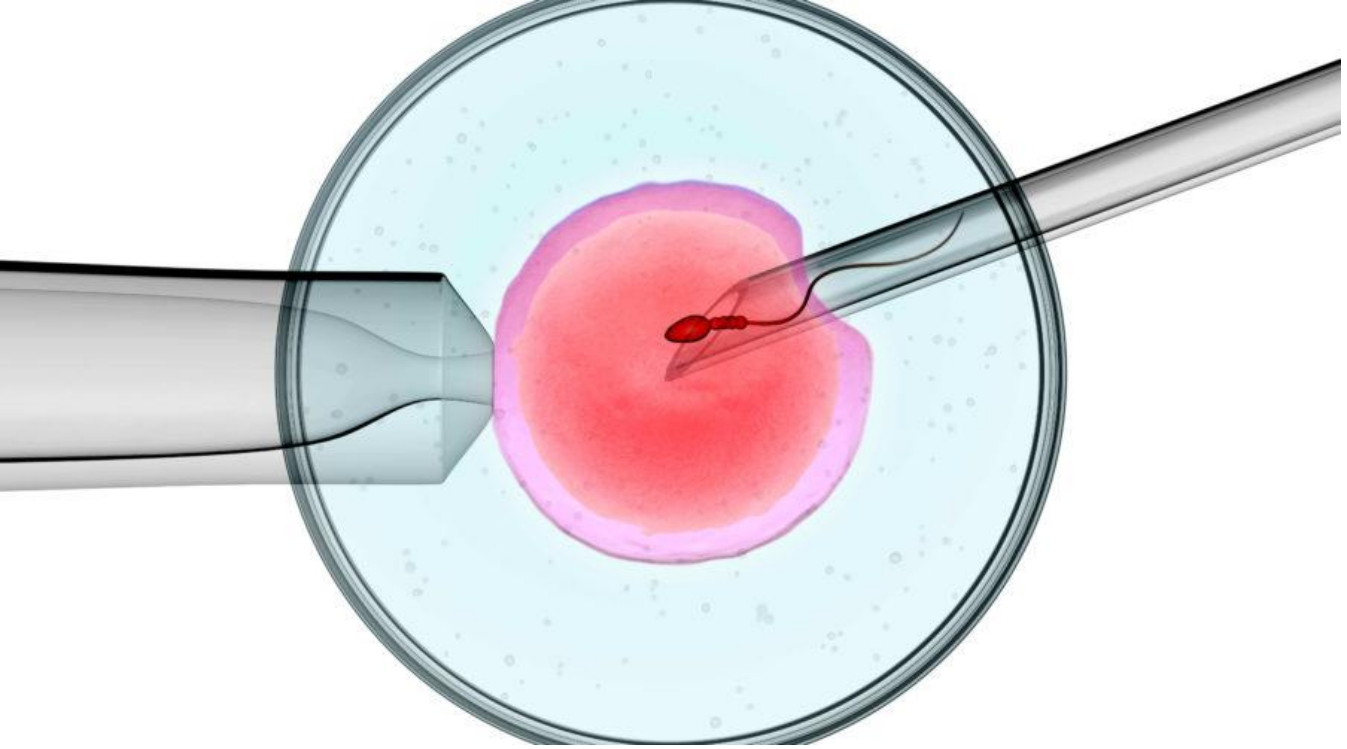
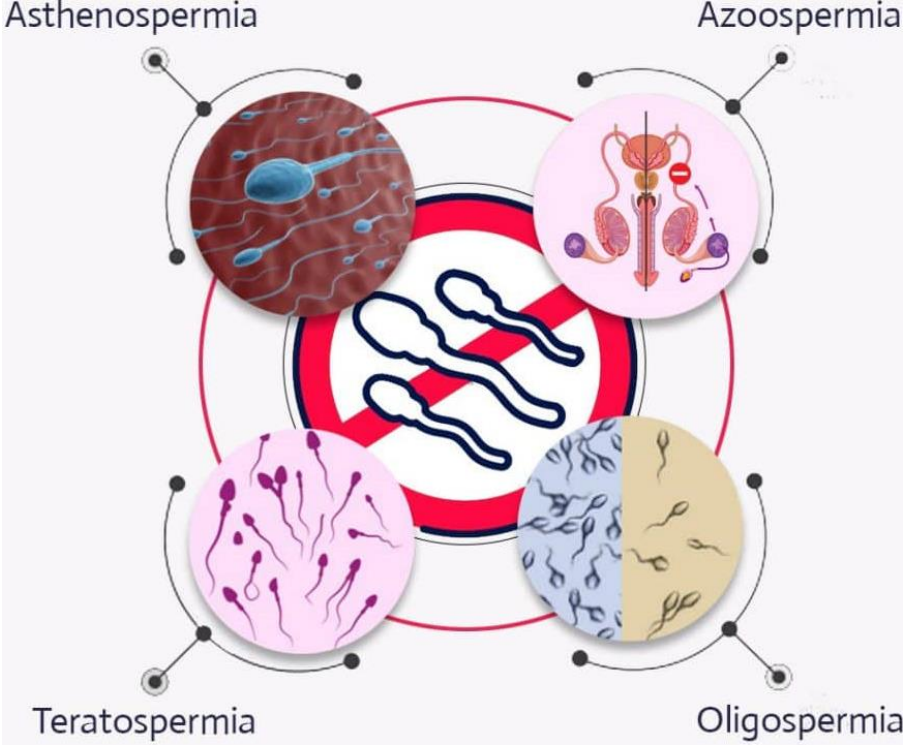

In vitro Fertilization
Intra Cytoplasmic Sperm Injection






## When is ICSI recommended, and for who?

- There is low sperm count and/or poor sperm motility and/or poor abnormal morphology;
- Previous IVF treatments resulted in none or very few fertilized eggs;
- Surgical sperm retrieval is required (due to a medical condition, vasectomy or an extremely low sperm count);
- Embryo testing is being performed, such as Pre-Implantation Genetic Testing for Aneuploidy (PGT-A) ;
- Frozen sperm is being used that may be of low quality.

Reported ICSI fertilization rates are between **70 and 80%,** usually about **10% higher** than that of conventional IVF methods. Unfortunately, TFF still occurs in ICSI cycles.

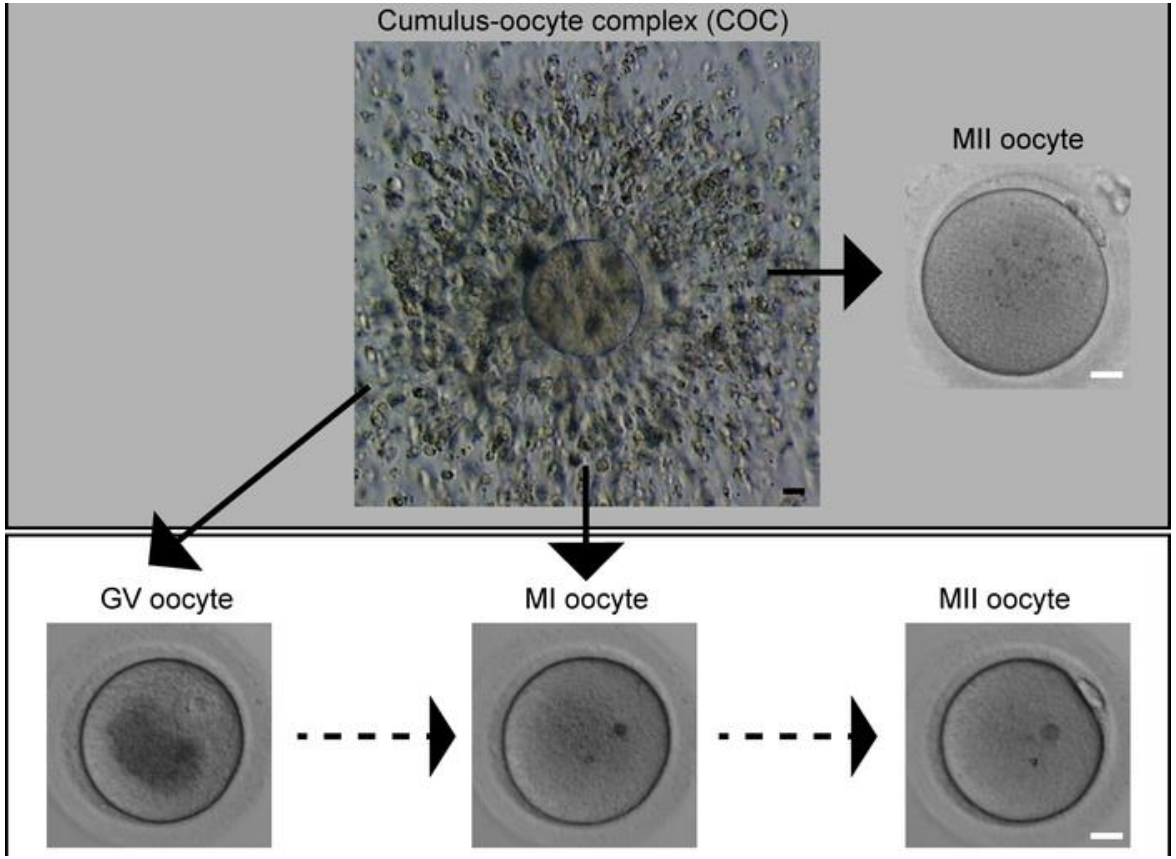
Total fertilization failure (TFF),which is the failure of fertilization in all oocytes, occurs in **5–10% of all cycles in Europe and the USA.**

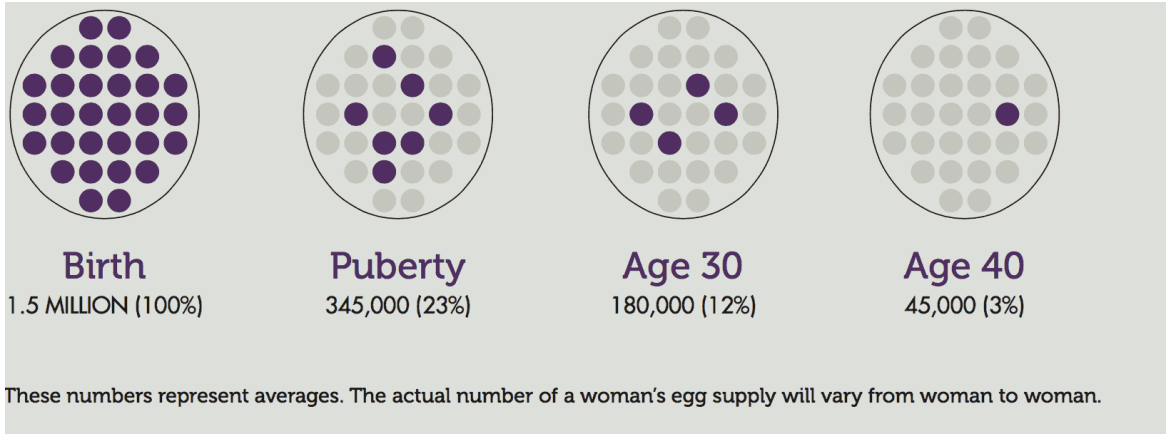
TFF after ICSI is **defined** as **the lack of male and female pronuclei formation**, a sign of fertilization, at the standard checking time of 17 ± 1 h post ICSI, in all oocytes retrieved during a cycle.

Sun B et al., 2021

**TFF rates depend** heavily on the number of mature (MII) oocytes available.

- One retrospective study found a TFF rate of **2.14%** in cycles with 3 or more MII oocytes and a TFF rate of **17.4%** **in** cycles with 1 or 2MII oocytes available.



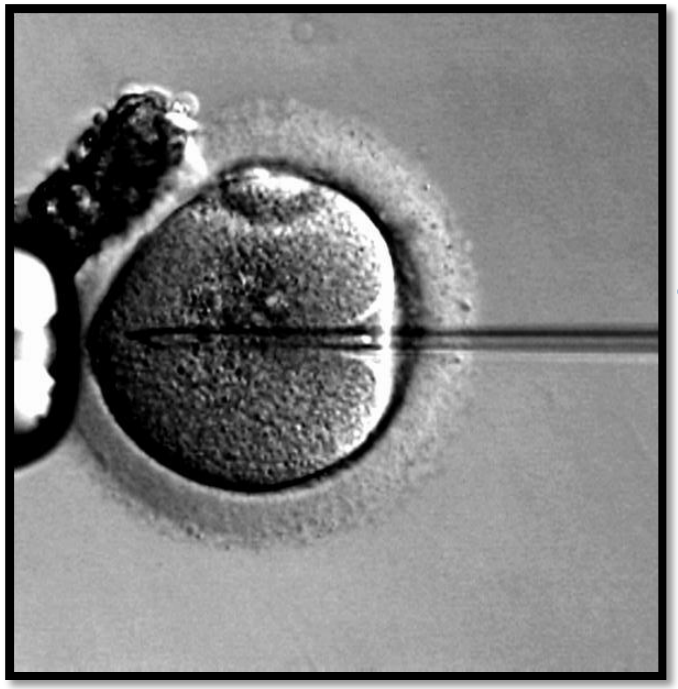




Excluding cycles with diminished oocyte reserve, TFF rates were found to be 1–5%.

Sun B et al., 2021

- Among the TFF cases, roughly **10% were due to technical** errors where sperm DNA lie outside of the oocyte.

- A majority of the cases point to **failed oocyte activation**.



**Technical errors**

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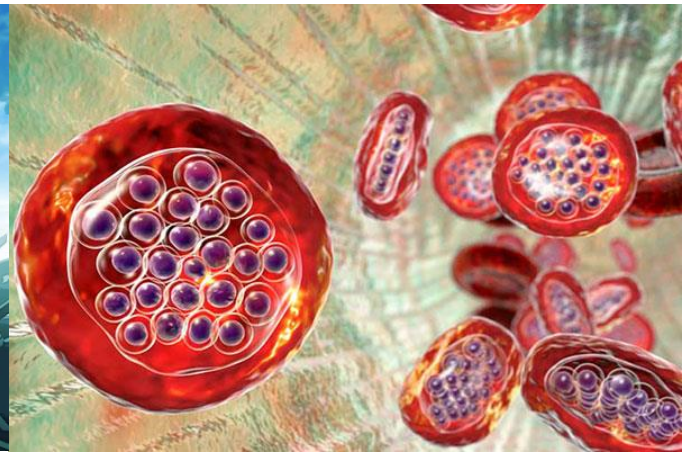
**While the main etiology of TFF has been extensively reported as oocyte activation failure, mechanisms underlying oocyte activation failure have yet to be elucidated.**



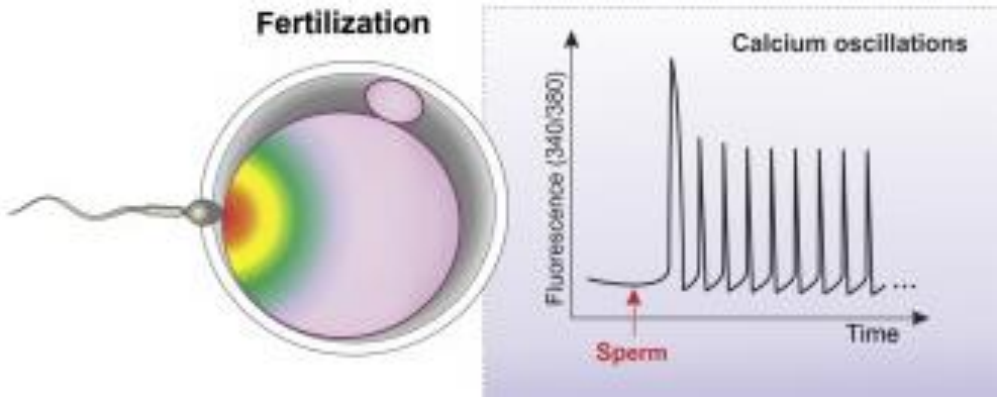
Sun B et al., 2021

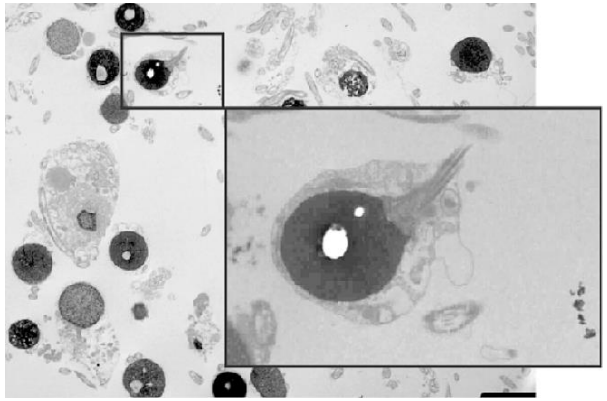
**First:** It is often unclear whether the defect comes from the sperm, the oocyte, or both.

**Second**: Proteins, organelles, and metabolic pathways of both gametes need to function properly in cascades of cellular events in a successful oocyte activation.

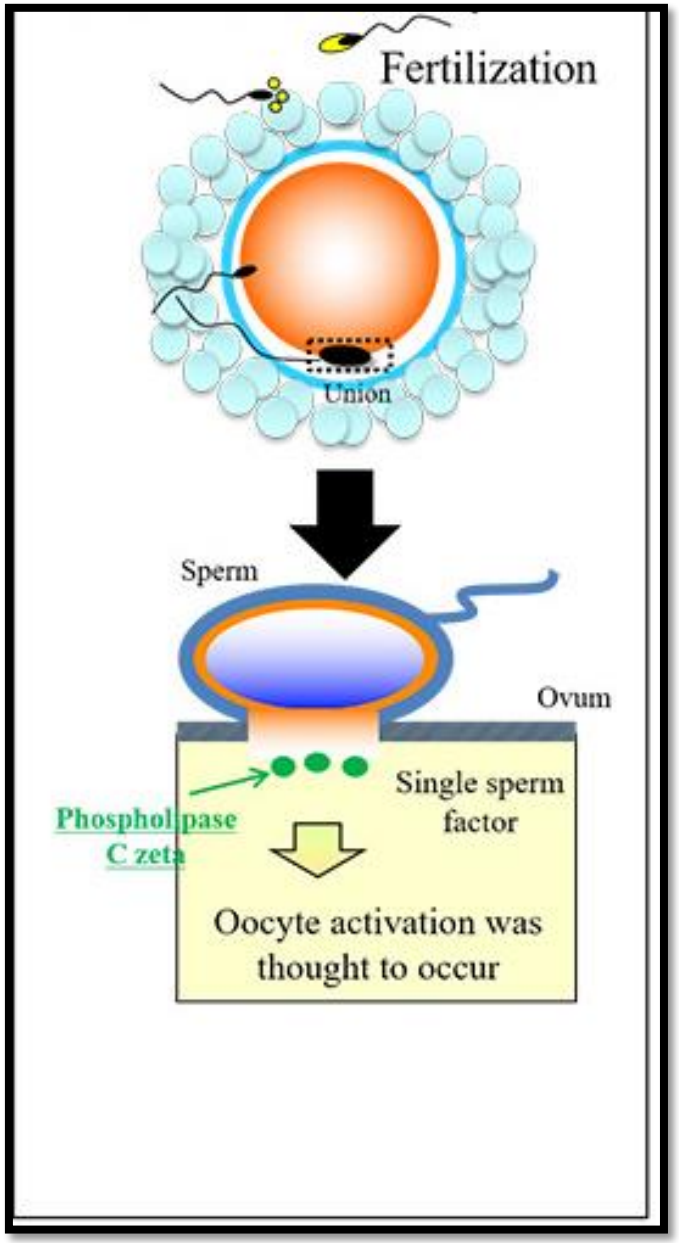
- **Mouse oocyte activation tests can exclude sperm deficiency. Yet, in cases of oocyte deficiency, it remains challenging to conclude on which level in the cascade there is a failure.**

**Third:** Calcium oscillations in fertilized oocytes have been investigated in TFF cases because of their importance in oocyte development and activation leading up to successful fertilizations.

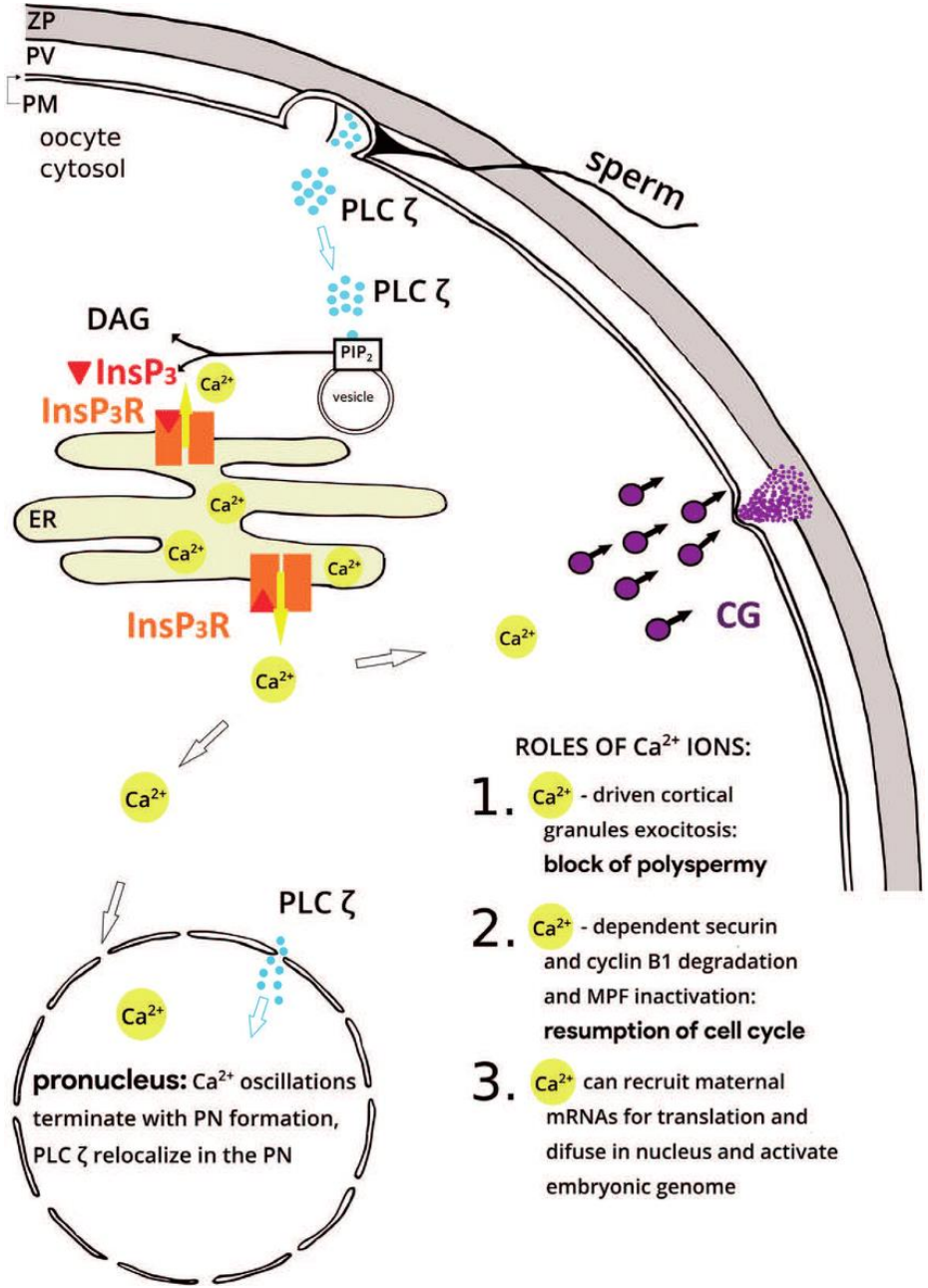


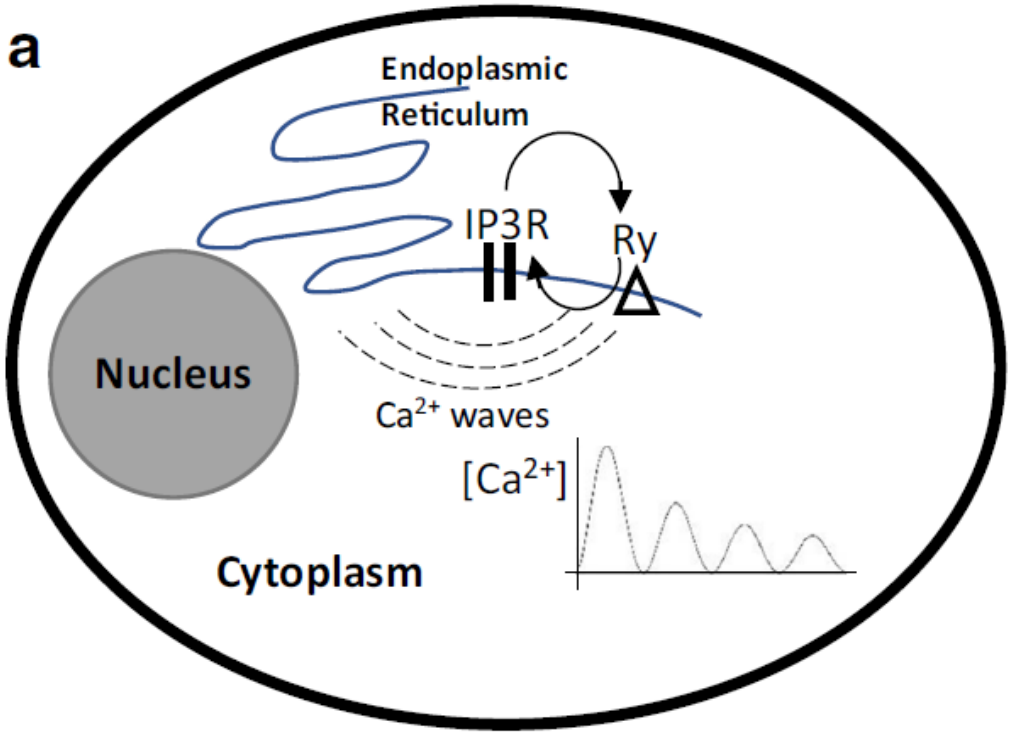
Sun B et al., 2021




Fertilization

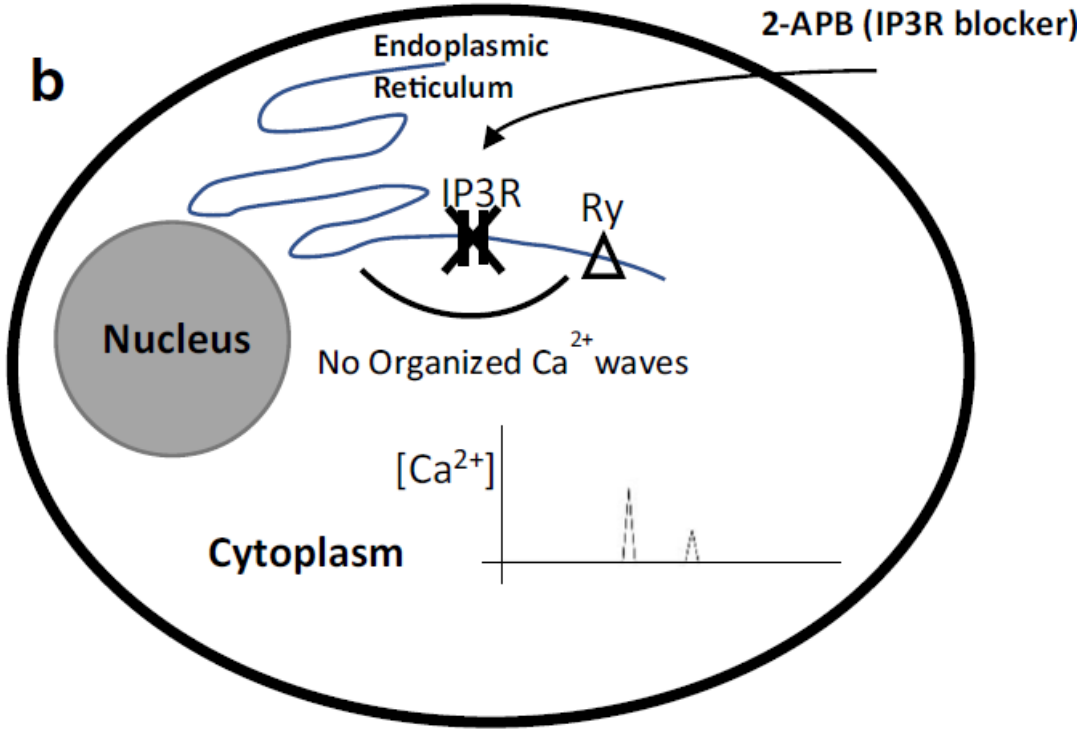



Oocyte activation mechanism and its consequences




Generation of two types of calcium waves. Inositol triphosphate receptors (IP3Rs) and ryanodine receptors (RyRs) are two main receptors in cells that mediate the generation of Ca2+ waves.

**a:** IP3Rs initiate and maintain both spontaneous and Ca2+-induced Ca2+ waves. Crosstalk between IP3Rs and RyRs shape the characteristics of Ca2+ waves.



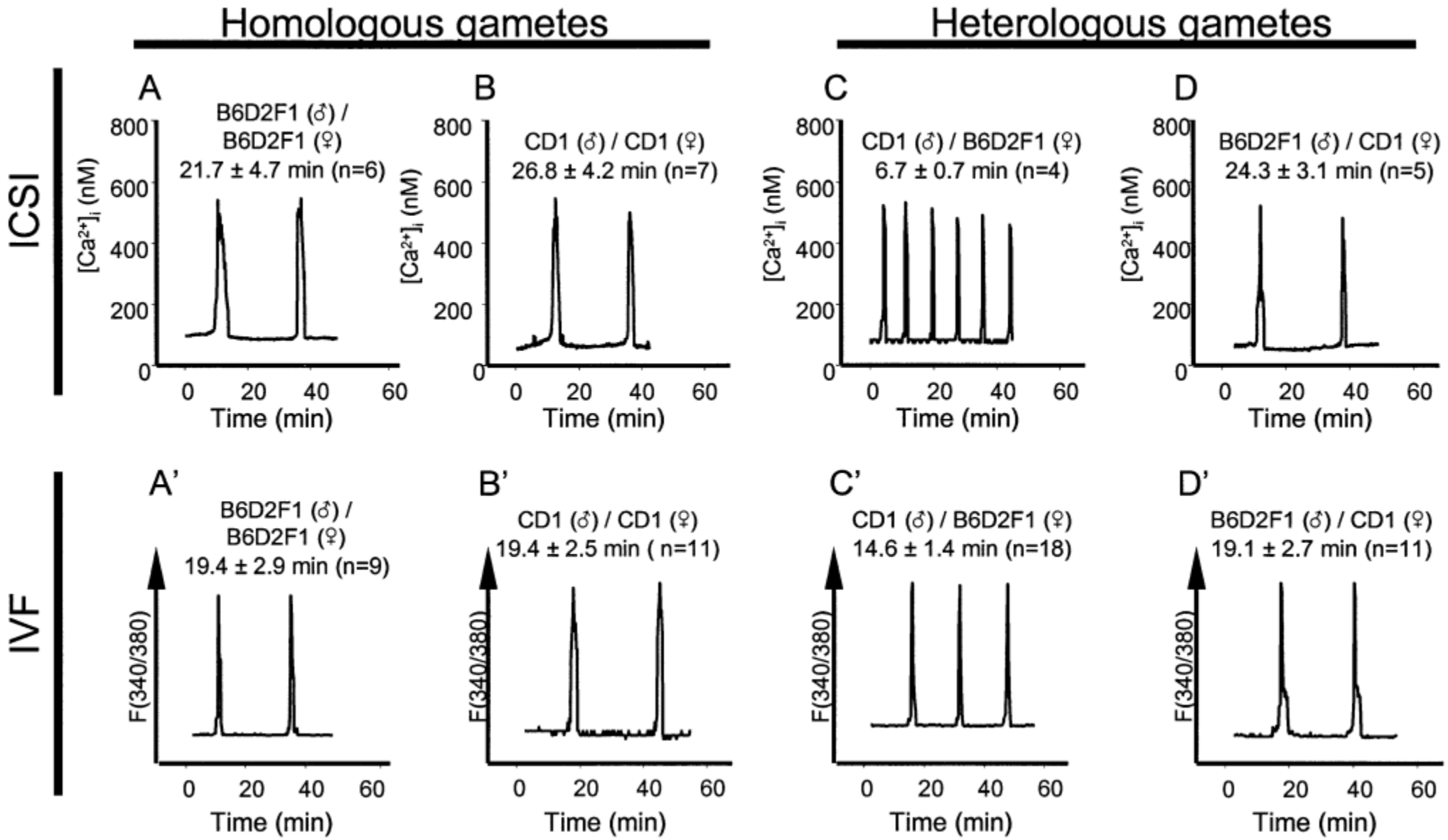

**a** Blocking IP3Rs results in loss of high-amplitude, organized Ca2+ waves. Localized low-amplitude Ca2+ spikes were observed, likely mediated by RyRs.

Processing of oocytes in assisted reproductive technology (ART) alters natural Ca2+ oscillatory patterns post fertilization. **These alternations might be due to the speed at which sperm factors are released into oocytes, with the effects modified by different immobilization and injection techniques.**

1: Different oscillation patterns have been observed in human in vitro fertilization (IVF) and ICSI protocols.
2: Calcium oscillation patterns also differ significantly from species to species.
3: Also, oocyte responses to such oscillations alter as a process of **aging**.

Whether differences in oscillation patterns hold clinical significance remains unclear.

Sun B et al., 2021




Manabu Kurokawa and Rafael A.Fissore., 2003

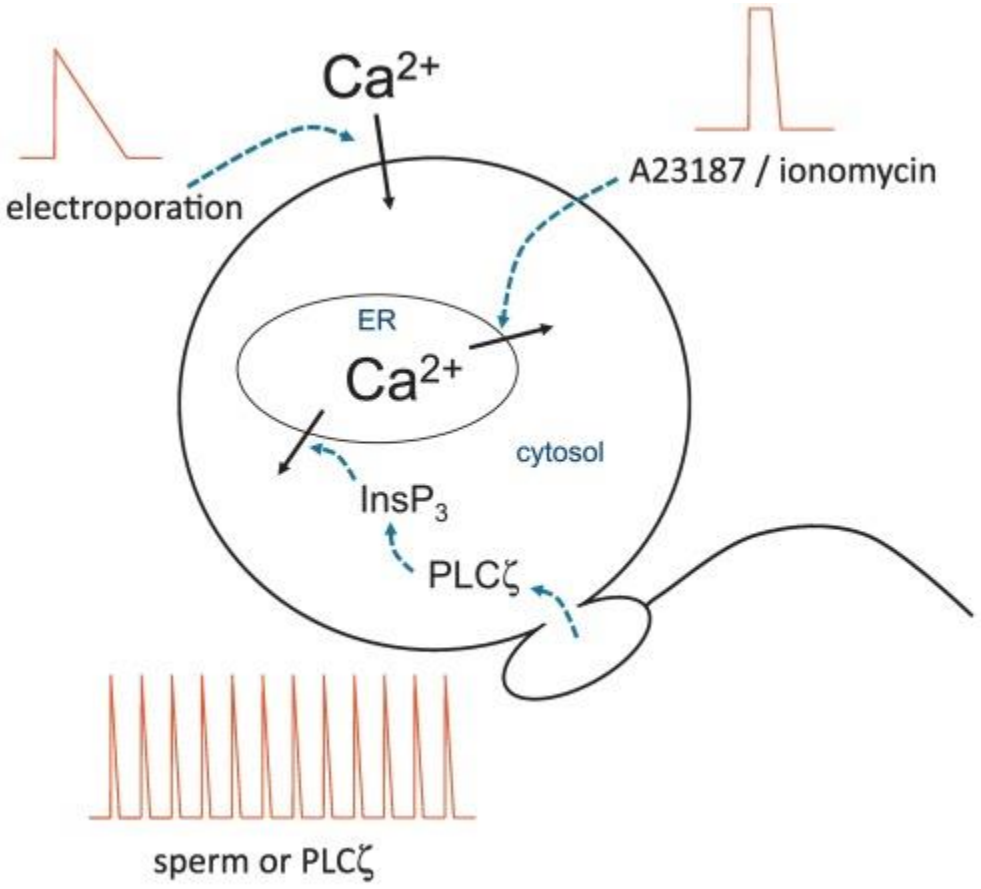
The importance of calcium signaling in TFF can be seen in the development of various artificial oocyte activation (AOA) methods. These methods attempt to raise oocyte cytoplasmic calcium level to achieve a higher fertilization rate in cases of TFF where oocyte activation failure is suspected.

**Natural activators:** Phospholipase C-zeta (PLCζ)

**Non-natural activators:**

- Ca+2 ionophore A23187 (calcimycin);
- Ionomycin
- Strontium chloride

AND electrical




George Anifandis et al., 2018

# Natural activator: Phospholipase C-zeta (PLCζ)

www.nature.com/scientificreports

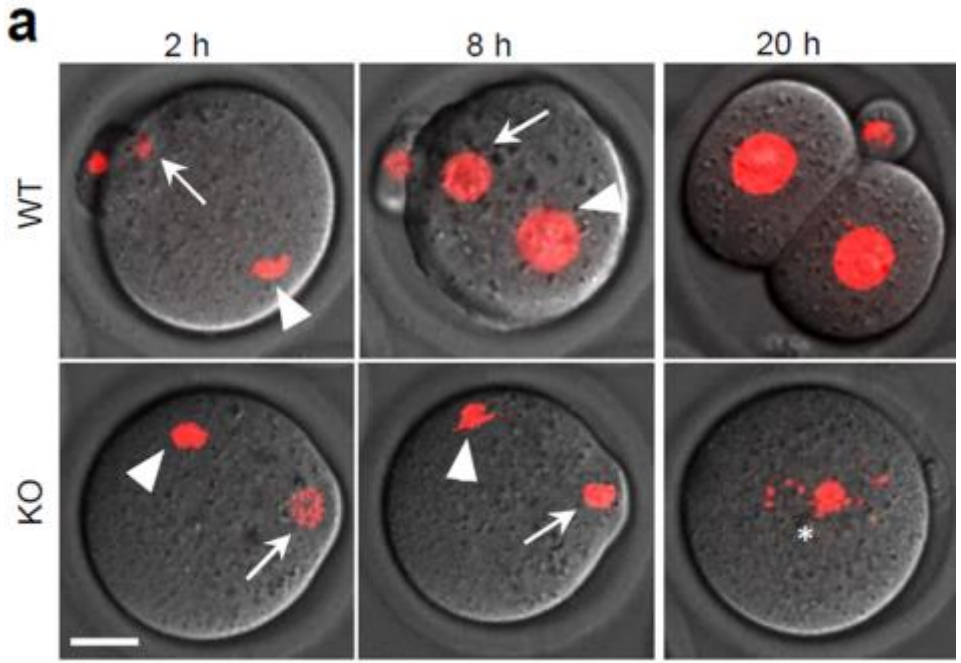
OPEN

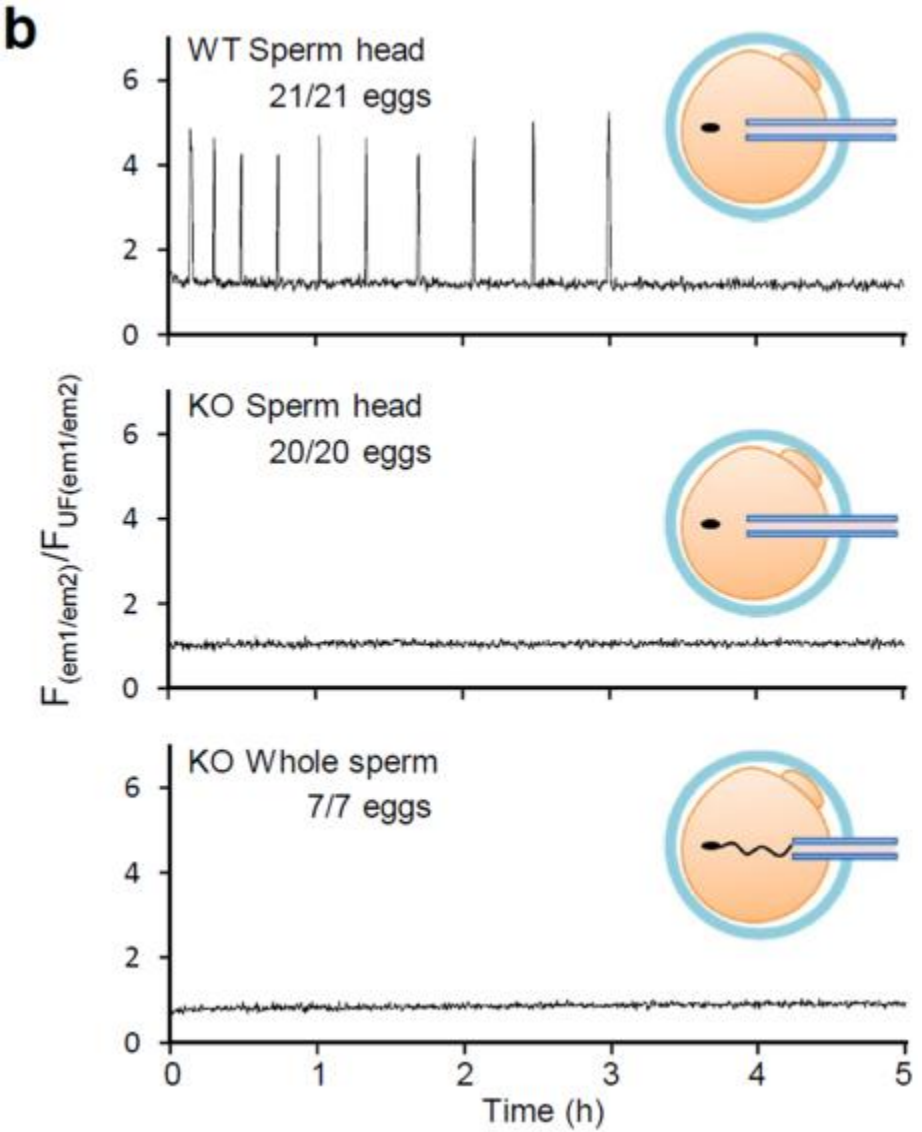
# Sperm-borne phospholipase C zeta-1 ensures monospermic fertilization in mice

Received: 1 September 2017
Accepted: 3 January 2018
Published online: 22 January 2018

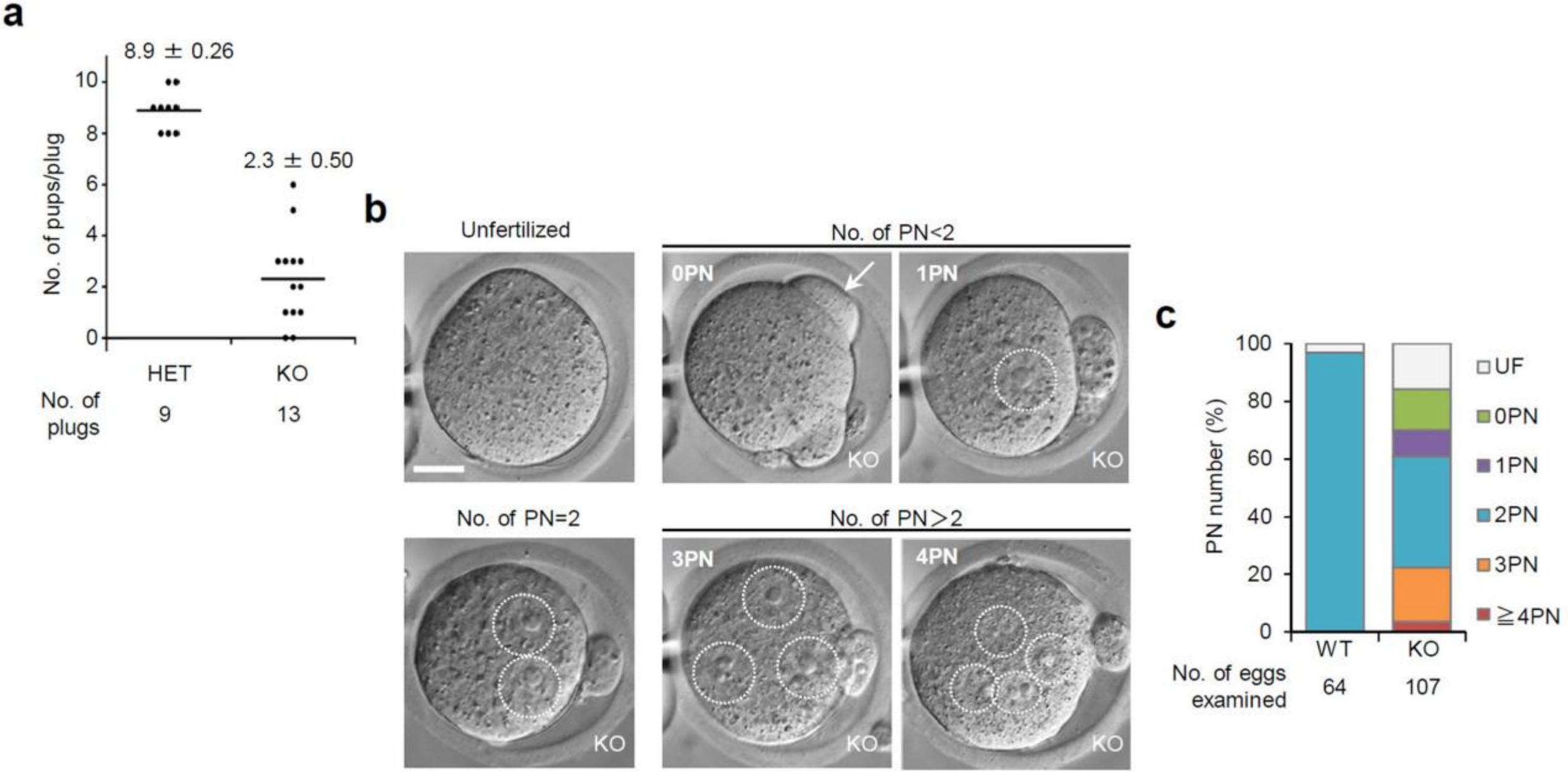
Kaori Nozawa[1,2], Yuhkoh Satouh[1], Takao Fujimoto[1,3], Asami Oji[1,3] & Masahito Ikawa[1,2,3,4]

Sperm entry in mammalian oocytes triggers intracellular $Ca^{2+}$ oscillations that initiate resumption of

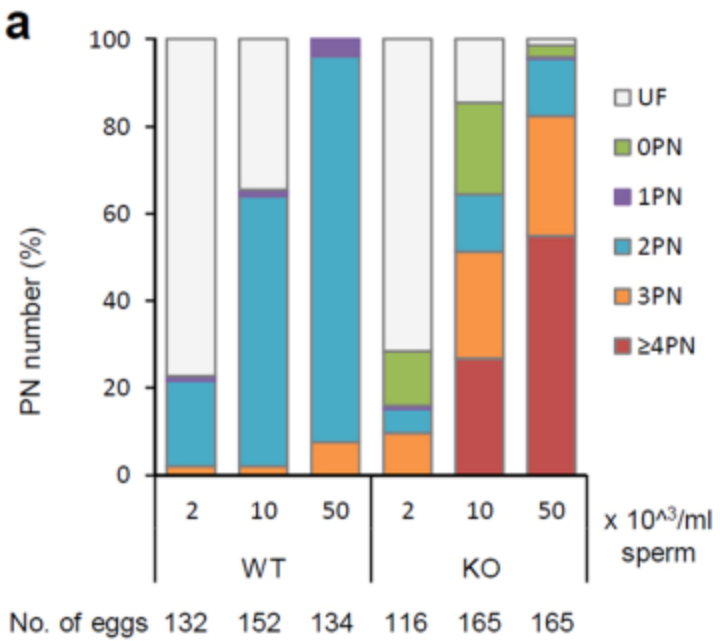



Kaori Nozawa et al., 2018




Kaori Nozawa et al., 2018

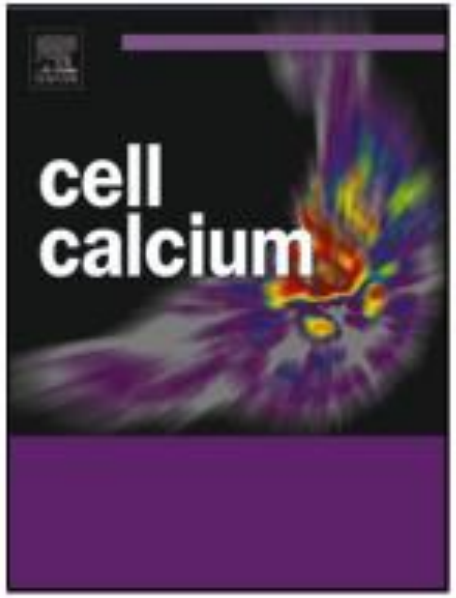



These results suggest that mammalian spermatozoa have a primitive oocyte activation mechanism and that PLCζ1 is a SOAF that ensures oocyte activation steps for monospermic fertilization in mammals.

**Conclusion:** Although ICSI ensures monospermic fertilization, oocyte activation failures still occur and have been reported as a cause of unexplained infertility. As we observed polyspermy in Plcz1 mutant mice, such patients might have polyspermy in vivo as well as oocyte activation failure following ICSI. If it is caused by male infertility, this form could possibly be treated by a combination of ICSI and PLCζ1 complementation in vitro.

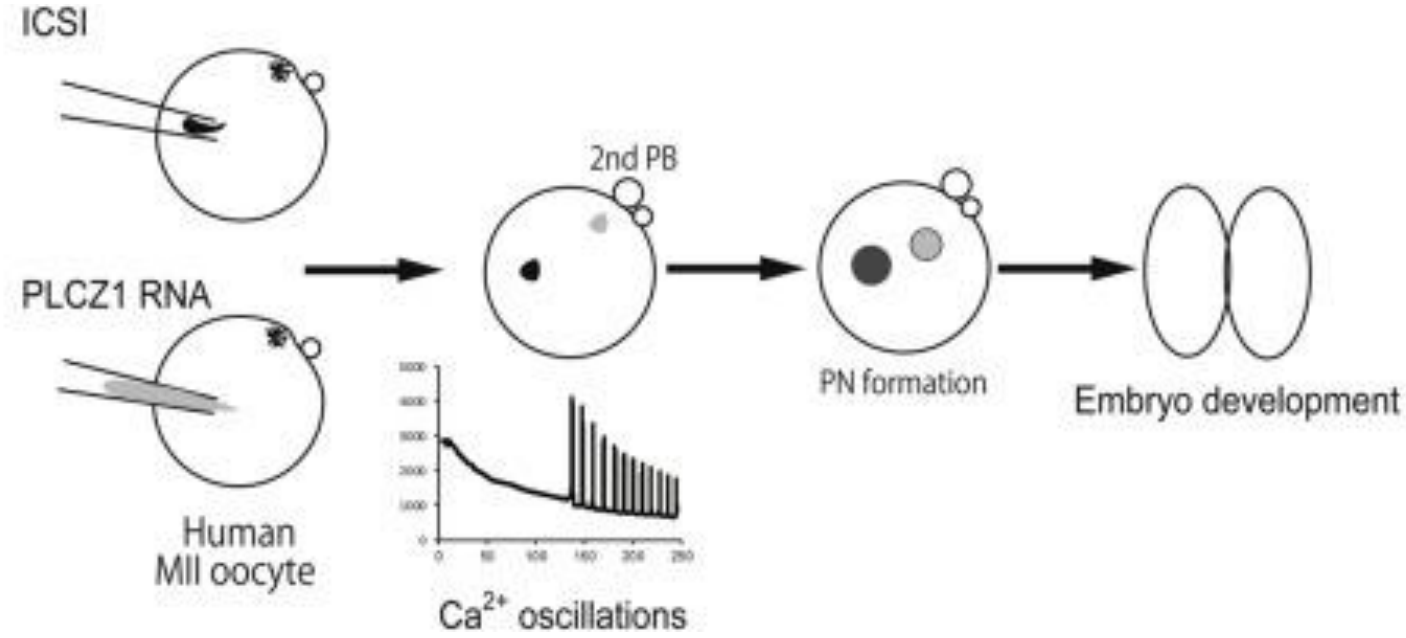
Kaori Nozawa et al., 2018

# The establishment of appropriate methods for egg-activation by human PLCZ1 RNA injection into human oocyte




**Authors:** Takashi Yamaguchi, Masahiko Ito, Keiji Kuroda, Satoru Takeda, Atsushi Tanaka

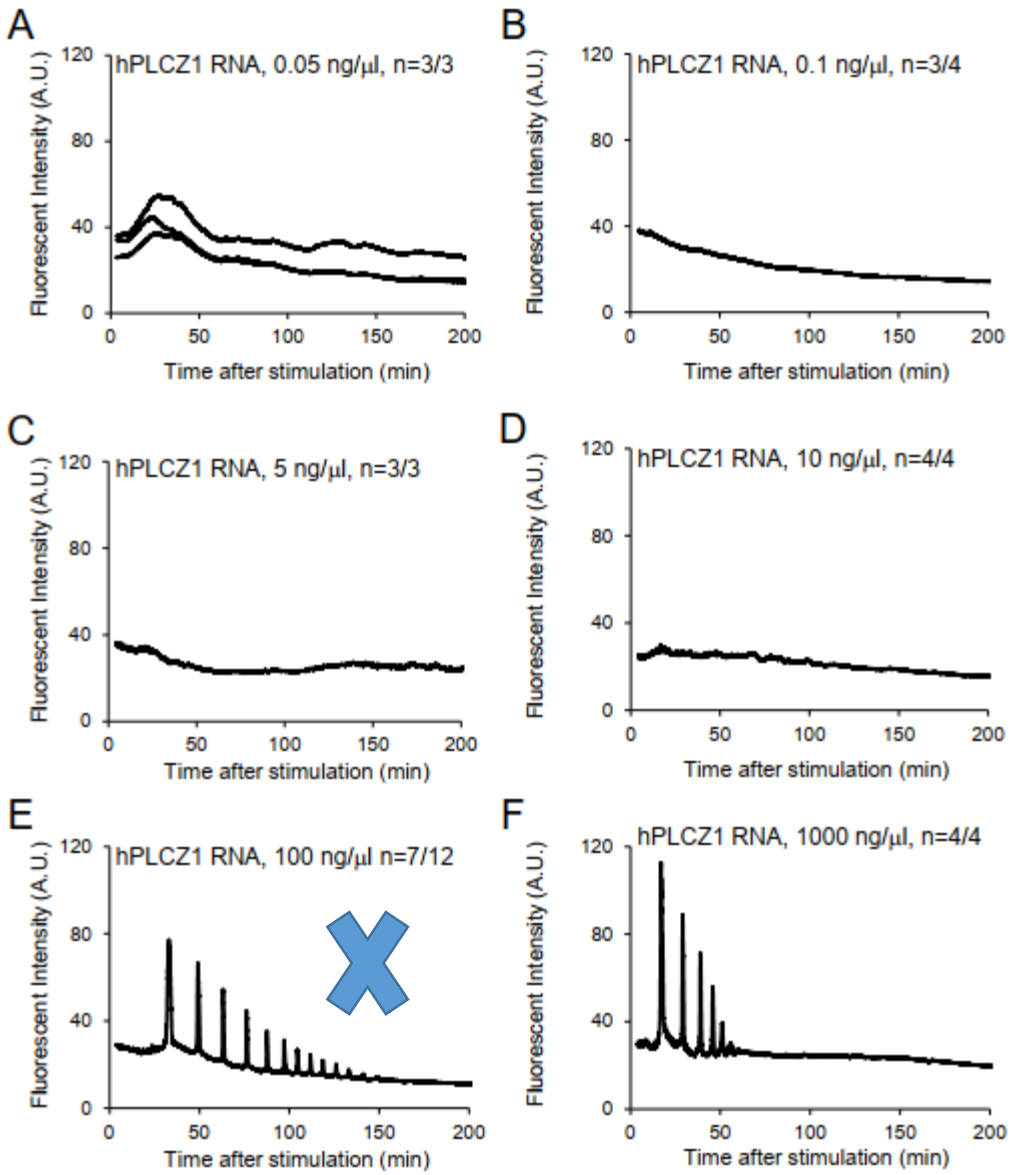
PII: S0143-4160(16)30191-9
DOI: http://dx.doi.org/doi:10.1016/j.ceca.2017.03.002
Reference: YCECA 1839
To appear in: Cell Calcium
Received date: 8-11-2016
Revised date: 3-3-2017
Accepted date: 3-3-2017



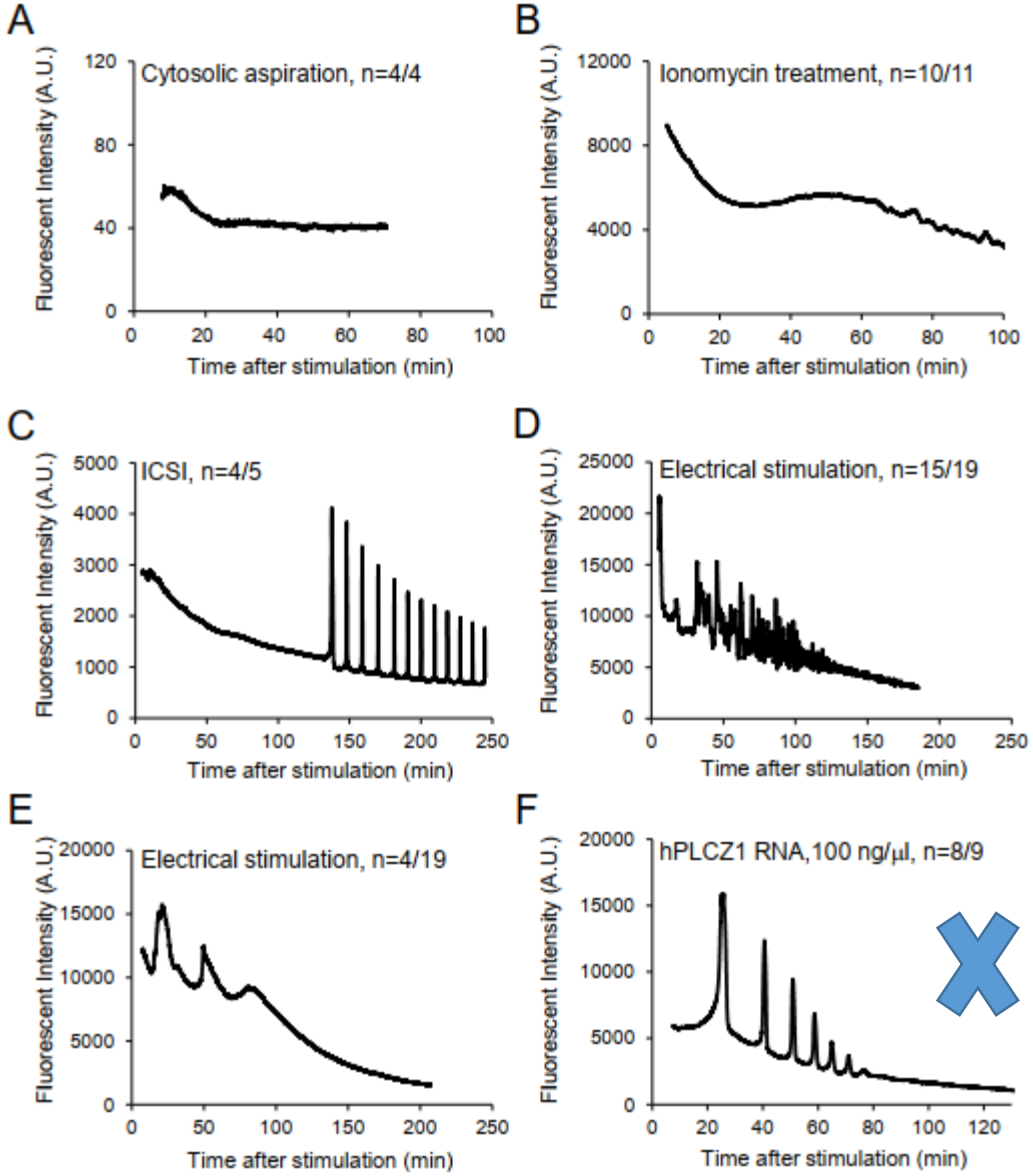

For the application of PLCZ1 **to clinical use**, the pattern of Ca2+ responses and developmental rate by comparing PLCZ1 RNA injection methods with the other current methods, such as cytosolic aspiration, electrical stimulation and ionomycin treatment in human oocytes.

I. The pattern of Ca2+ oscillations after PLCZ1 RNA injection exhibited similar characteristics to that after ICSI treatment.
II. Optimal concentration of human PLCZ1 RNA to activate the human oocytes was determined.
III. Human PLCZ1 RNA is a better therapeutic agent to rescue human oocytes from failed activation, leading to normal and efficient development.

Takashi Yamaguchi et al., 2017




Optimal concentration of human PLCZ1 RNA




Takashi Yamaguchi et al., 2017

# Conclusion

Human PLCZ1 RNA is a better therapeutic agent to rescue human oocytes from failed activation, leading to normal and efficient development.

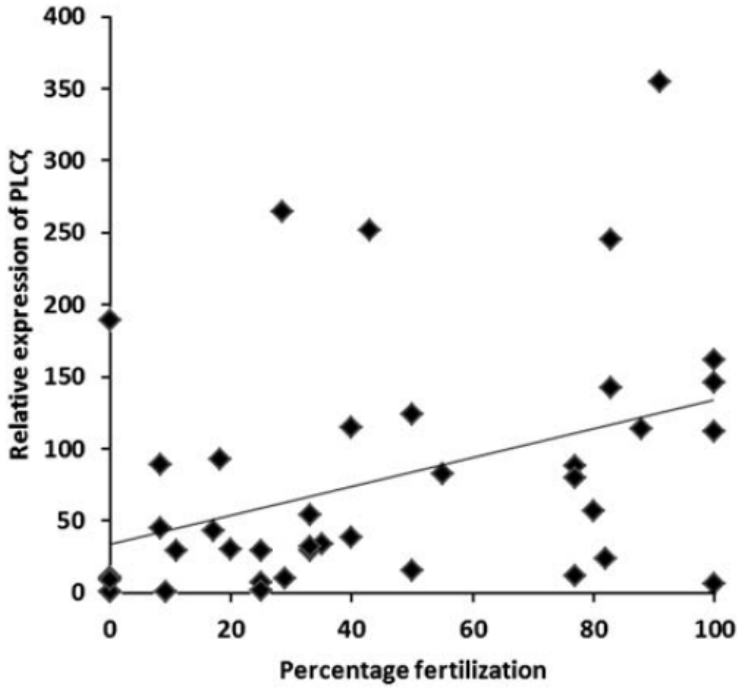
Takashi Yamaguchi et al., 2017

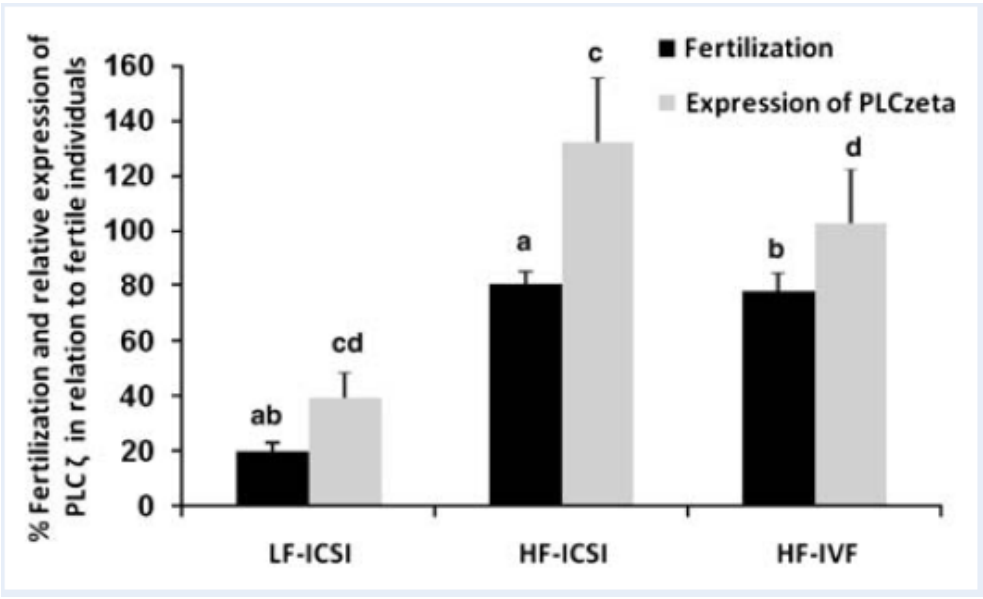
Hum. Reprod. Advance Access published September 6, 2011
Human Reproduction, Vol.0, No.0 pp. 1–7, 2011
doi:10.1093/humrep/der285

human reproduction ORIGINAL ARTICLE *Andrology*

# Quantitative expression of phospholipase C zeta, as an index to assess fertilization potential of a semen sample

S. Aghajanpour[1,2], K. Ghaedi[1,2], A. Salamian[1], M.R. Deemeh[1,3], M. Tavalaee[1], J. Moshtaghian[2], J. Parrington[4], and M.H. Nasr-Esfahani[1,3,5,*]



ISSN: 2047-2919



ORIGINAL ARTICLE

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**Keywords:**
fertilization, globozoospermia, *PAWP*, *PLCζ*, pregnancy, *TR-KIT*

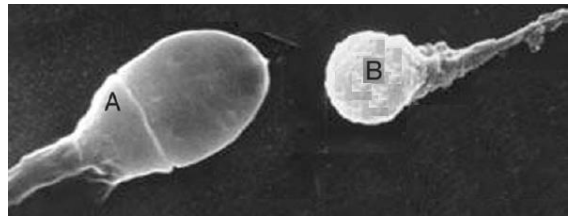
Received: 17-Sep-2015
Revised: 1-Feb-2016
Accepted: 3-Feb-2016

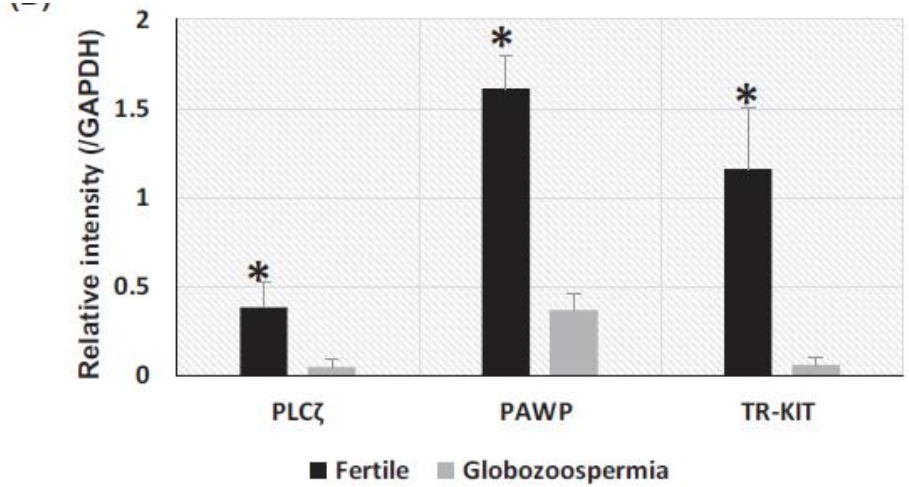
doi: 10.1111/andr.12179

# Expression profile of *PLCζ*, *PAWP*, and *TR-KIT* in association with fertilization potential, embryo development, and pregnancy outcomes in globozoospermic candidates for intra-cytoplasmic sperm injection and artificial oocyte activation

[1]M. Tavalaee and [1,2]M. H. Nasr-Esfahani

[1]Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran, and [2]Isfahan Fertility and Infertility Center, Isfahan, Iran



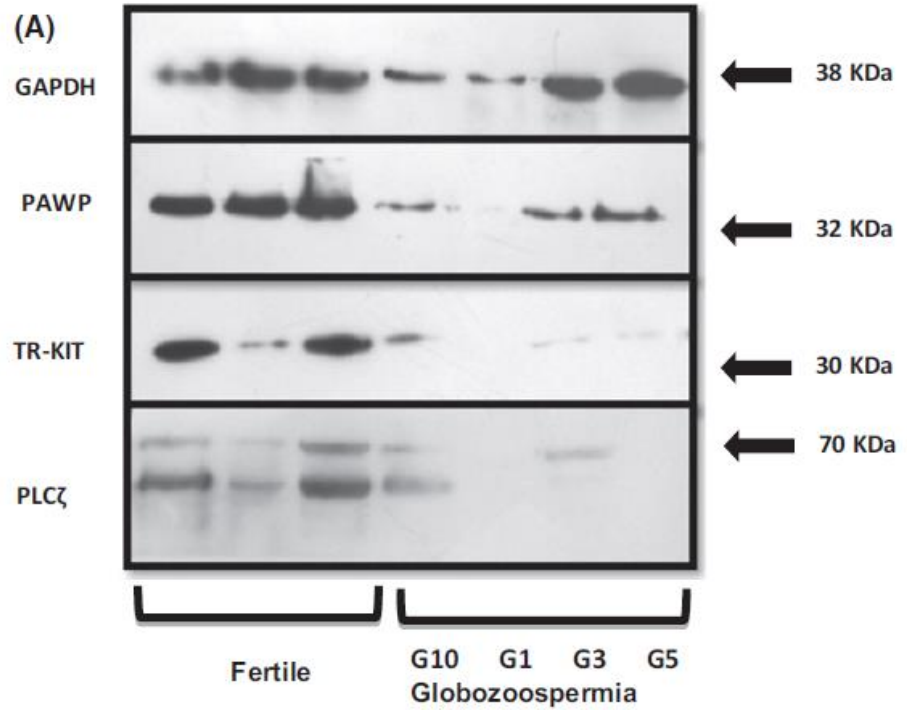



Table 3 Characteristics of each globozoospermic cases and their ICSI-AOA clinical outcomes

| | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Male age (years) | 28 | 36 | 35 | 36 | 32 | 37 | 44 | 23 | 26 | 35 | 35 | 35 |
| Female age (years) | 26 | 33 | 28 | 23 | 35 | 30 | 41 | 24 | 22 | 28 | 32 | 32 |
| No. oocytes retrieved | 4 | 13 | 12 | 23 | 30 | 13 | 3 | 12 | 8 | 7 | 8 | 10 |
| No. MII oocytes | 2 | 9 | 7 | 19 | 26 | 11 | 2 | 11 | 8 | 6 | 8 | 6 |
| No. fertilized oocytes | 2 | 8 | 6 | 14 | 16 | 6 | 1 | 5 | 3 | 2 | 2 | 1 |
| Fertilization rate (%) | 100 | 89 | 85.7 | 73.7 | 61.5 | 54.54 | 50 | 45.46 | 37.5 | 33.34 | 25 | 17 |
| Cleavage rate (%) | 100 | 62.5 | 100 | 50 | 18.7 | 66 | 100 | 40 | 66 | 100 | 100 | 100 |
| No. embryos transferred | 2 | 2 | 3 | 2 | 2 | 2 | 1 | 3 | 3 | 2 | 2 | 1 |
| Clinical pregnancy | – | + | + | – | – | + | – | + | – | + | – | – |
| Sac number | | 1 | 1 | | | 2 | | 1 | | 2 | | |
| Implantation rate (%) | 00 | 50 | 33.4 | 00 | 00 | 100 | 00 | 33.4 | 00 | 100 | 00 | 00 |
| Live birth | | 1 M | 1 FM | | | 00 | | 1 | | 2 FM | | |
| Abortion rate (%) | | | | | | 2 FM | | | | | | |

M, Male; FM, Female; G, globozoospermia.

**High fertilization (56.06%) and pregnancy (41.7%) rates accomplished in this study following ICSI-AOA indicated that expression profiles of PLCf and PAWP were low in globozoospermic individuals, and ICSI combined with artificial oocyte activation could mimic physiological calcium changes which occur during fertilization.**

Title: Expression of sperm PLCζ and clinical outcomes of ICSI-AOA in men affected by globozoospermia due to DPY19L2 deletion.

Author: Marziyeh Tavalaee, Michail Nomikos, F. Anthony Lai, Mohammad Hossein Nasr-Esfahani.

PII: S1472-6483(17)30708-3
DOI: https://doi.org/10.1016/j.rbmo.2017.12.013
Reference: RBMO 1872
To appear in: Reproductive BioMedicine Online
Received date: 24-6-2017
Revised date: 28-11-2017
Accepted date: 7-12-2017

The expression of PLCzeta at RNA and protein levels in globozoospermic men with DPY19L2 deletion was significantly lower compared with fertile men ($n$ = 32).

Fertilization rate in globozoospermic couples following ICSI-AOA was significantly lower compared with fertile men (53.14% versus 87.64%, $P < 0.001$).

However, **implantation** (26.2%) and **pregnancy** (53.8%) rates were not jeopardized by DPY19L2 deletion in these couples.

## Non-natural activator:

- Electrical

FERTILITY AND STERILITY®
*VOL. 72, NO. 3, SEPTEMBER 1999*

Published by Elsevier Science Inc.
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REPRODUCTIVE BIOLOGY

# Electrical activation and in vitro development of human oocytes that fail to fertilize after intracytoplasmic sperm injection

Electric field induces micropores in gamete membranes that facilitate Ca2+ influx.
One group found that single or multiple electrical pulse(s) was able to activate oocytes that showed no evidence of fertilization 16–24 h after ICSI.

In this study, human oocytes that displayed two pronuclei and extruded a second polar body within 16 h were considered to have fertilized normally.

- One electrical pulse fertilized oocyte at a rate of 70%;
- Three electrical pulses fertilized oocytes at a rate of 78%.

**Despite the relatively high fertilization rate, however, only 11% of the oocytes fertilized with the assistance of three electrical pulses developed to early blastocyst stage.**

The study did not analyze calcium transients induced by electrical pulses or correlate the difference in fertilization rate with corresponding calcium responses.

John Zhang et al., 1999

## Non-natural activators:

- Chemical

Chemical agents such as calcium ionophores are generally assumed to directly facilitate the transport of Ca2+ across the plasma membrane.

**Table 2** Results of assisted oocyte activation (AOA) on human oocyte activation rate and rate to blastocyst formation

| Activation agent | $Ca^{2+}$ response | Protocol | Oocyte activation rate | Rate to blastocyst | Study |
|---|---|---|---|---|---|
| Ionomycin | Single transients | 10 μM for 10 min (2 exposures, 30-min interval); human sperm with low or complete failed fertilization | 74.2% | N/A | [16] |
| Calcium ionophore A23187 | Single transients | 5% for 5 min followed by 2 mM 6-DMAP incubation<br>Three-day-old oocytes that failed to activate after IVF/ICSI | 75.4% | 9.1% | [62] |
| $SrCl_2$ | Oscillatory signaling | 10 mM for 20 min followed 2.5 mM 6-DMAP incubation<br>Three-day-old oocytes that failed to activate after IVF/ICSI | 46.8% | 4.8% | |
| Ionomycin<br>Calcimycin | Single transients | 10 μM for 10 min (2 exposures, 30 min interval) + 0.1 mol/L $CaCl_2$; human globozoospermic sperm ready to use for 15 min (1 exposure); human globozoozpermic sperm | 30.0%<br>11.8% | N/A | [15] |
| Electric field | Single transients | 1.36–1.50 kV/cm for 40–60 ms (1 pulse) | 70% | 8% | [63] |
| | Oscillatory signaling | 1.36–1.50 kV/cm for 40–60 ms (3 pulses, 15–20-min interval) | 78% | 11% | |
| $SrCl_2$ | Oscillatory signaling | 10 mM for 1 h (1 exposure); human sperm with low or complete failed fertilization | 61.7% | 25.7% | [64] |

Sun B et al., 2021

# Meta analysis studies

Hum. Reprod. Advance Access published June 16, 2015

**Human Reproduction, Vol.0, No.0 pp. 1–11, 2015**

doi:10.1093/humrep/dev136

human reproduction

META-ANALYSIS *Embryology*

# Artificial oocyte activation to improve reproductive outcomes in women with previous fertilization failure: a systematic review and meta-analysis of RCTs

**Ioannis A. Sfontouris[1], Carolina O. Nastri[2], Maria L.S. Lima[2], Eisa Tahmasbpourmarzouni[3], Nick Raine-Fenning[4,5], and Wellington P. Martins[2,*]**

There is insufficient evidence available from the currently available RCTs to judge the efficacy or safety of ICSI-AOA on key reproductive outcomes in couples with previous fertilization failure. Such interventions should be further examined by well-designed RCTs before the introduction of ICSI-AOA as a standard treatment.

VOL. 108 NO. 3 / SEPTEMBER 2017

ORIGINAL ARTICLES: ASSISTED REPRODUCTION

# Does the use of calcium ionophore during artificial oocyte activation demonstrate an effect on pregnancy rate? A meta-analysis

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We performed a standard meta-analysis to determine the value of calcium ionophore treatment as a method of AOA.

This meta-analysis included a total of **1,521 ICSI cycles spread over 14 studies.**

Focusing on the **primary outcomes**, calcium ionophore use after ICSI treatment significantly increases:

- **Overall pregnancy rate per ET**
- **Live-birth rate per both ET and treatment cycle;**
- **Multiple pregnancy rate per ET.**

Focusing on the **secondary outcomes**, calcium ionophore use after ICSI treatment significantly increases:

- **Fertilization rate,**
- **Cleavage,**
- **Blastocyst formation,**
- **Implantation rate**


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Journal of Obstetrics and Gynaecology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ijog20

# A retrospective analysis of artificial oocyte activation in patients with low or no fertilisation in intracytoplasmic sperm injection cycles

Kevin K. W. Lam, Jacki Y. Y. Wong, Tak-Ming Cheung, Raymond H. W. Li, Ernest H. Y. Ng & William S. B. Yeung

**To cite this article:** Kevin K. W. Lam, Jacki Y. Y. Wong, Tak-Ming Cheung, Raymond H. W. Li, Ernest H. Y. Ng & William S. B. Yeung (2021): A retrospective analysis of artificial oocyte activation in patients with low or no fertilisation in intracytoplasmic sperm injection cycles, Journal of Obstetrics and Gynaecology, DOI: 10.1080/01443615.2021.1922878

**To link to this article:** https://doi.org/10.1080/01443615.2021.1922878

# Infertile couples with severe male infertility, poor fertilization

Table 1. Laboratory and clinical data of AOA and non-AOA cycles.

| | AOA group ($n = 34$) | Non-AOA group ($n = 34$) | $p$ Value |
|---|---|---|---|
| Female age (years)[a] | 36.4 ± 3.3 | 36.1 ± 3.7 | NS |
| Number of oocytes per retrieval[a] | 10.2 ± 4.9 | 7.7 ± 4.0 | **.030** |
| Number of metaphase II oocyte injected per cycle[a] | 7.8 ± 4.1 | 5.7 ± 3.6 | **.026** |
| Total metaphase II oocyte number | 265 | 188 | – |
| Total normally fertilised oocytes number | 138 | 26 | – |
| Number of normally fertilised oocytes per cycle[a] | 4.1 ± 2.8 | 0.8 ± 1.0 | **<.001** |
| Normal fertilisation rate (%)[b] | 52.1% | 13.8% | **<.001** |
| Total number of failed fertilisation cycles (%)[c]/ number of oocytes injected for these cycles (median ± standard deviation) | 4 (11.8%) / 3.5 ± 1.3 oocytes injected | 17 (50%) / 3.7 ± 2.2 oocytes injected | **<.001** |
| Total cleaved embryos number (%) | 136 (98.6%) | 25 (96.1%) | NS |
| Total usable embryos number (%) | 98 (72.1%) | 22 (88.8%) | NS |
| Total top quality embryos number (%) | 28 (20.6%) | 4 (16.0%) | NS |
| Number of fresh embryo transfer | 17 | 8 | – |
| Number of clinical pregnancy per transfer (%) | 5 (29.4%) | 1 (12.5%) | NS |
| Number of clinical pregnancy from subsequent FET cycles | 11 | 0 | – |
| Cumulative clinical pregnancy rate[c] | 47.1% | 2.9% | **<.001** |
| Number of live births | 10 | 1 | – |
| Cumulative live birth rate[c] | 29.4% | 2.9% | **.003** |

[a]Data expressed as mean ± standard deviation.
[b]Per injected oocytes.
[c]Per oocyte retrieval cycle.
NS: not significant; FET: frozen thawed embryo transfer.
Bold values denote statistical significance at the $p < .05$.

Table 2. Laboratory and clinical data of AOA cycles using A23187 only and concomitant injection of calcium chloride.

| | A23187 group ($n = 9$) | A23187 + calcium chloride co-treatment group ($n = 25$) | $p$ Value |
|---|---|---|---|
| Female age (years)[a] | 38.6 ± 0.6 | 35.7 ± 0.7 | .021 |
| Number of oocytes per retrieval[a] | 7.8 ± 4.4 | 11.1 ± 4.9 | NS |
| Number of metaphase II oocyte injected per cycle[a] | 6.1 ± 3.5 | 8.4 ± 4.2 | NS |
| Total metaphase II oocyte number | 55 | 210 | – |
| Total normally fertilised oocytes number | 29 | 109 | – |
| Normally fertilised oocytes (%)[b] | 52.7% | 51.9% | NS |
| Total number of failed fertilisation cycles (%)[c] | 2 (22.2%) | 2 (8.0 %) | NS |
| Total cleaved embryos number (%) | 29 (100%) | 107 (98.2%) | NS |
| Total usable embryos number (%) | 22 (75.9%) | 76 (71.0%) | NS |
| Total top quality embryos number (%) | 3 (10.3%) | 25 (23.4%) | NS |
| Number of fresh embryo transfer cycles | 5 | 12 | – |
| Number of clinical pregnancies per embryo transfer (%) | 2 (40%) | 3 (25%) | NS |
| Cumulative clinical pregnancy rate (%)[c] | 44.4% | 40.0% | NS |

[a]Data expressed as mean ± standard deviation.
[b]Per injected oocytes.
[c]Per oocyte retrieval cycle.
NS: not significant.

**Table 3.** Obstetrics data of the pregnant patients in the AOA group.

| Cases | Gender | Birth weight (g) | Gestation week (weeks + days) | Congenital abnormalities (yes/no) |
|---|---|---|---|---|
| Patient 1 | F (Twins) | 2290 | 33 + 6 | No |
| | F (Twins) | 2290 | 33 + 6 | No |
| Patient 2 | M | 2300 | 37 | No |
| Patient 3 | M | 3110 | 38 + 2 | No |
| Patient 4 | M (Twins) | 2345 | 37 + 3 | No |
| | F (Twins) | 2020 | 37 + 3 | No |
| Patient 5 | M | 3010 | 39 | No |
| Patient 6 | Miscarriage | / | / | / |
| Patient 7 | Miscarriage | / | / | / |
| Patient 7 | M | 3500 | 40 | No |
| Patient 8 | Miscarriage | / | / | / |
| Patient 9 | Miscarriage | / | / | / |
| Patient 10 | Miscarriage | / | / | / |
| Patient 10 | F | 3000 | 39 | No |
| Patient 11 | Miscarriage | / | / | / |
| Patient 12 | F | 2550 | 38 + 3 | No |
| Patient 13 | F | 2800 | 39 + 4 | No |
| Patient 14 | M | 3380 | 39 | No |

Journal of Assisted Reproduction and Genetics
https://doi.org/10.1007/s10815-021-02338-3

EMBRYO BIOLOGY

# Ionophore application for artificial oocyte activation and its potential effect on morphokinetics: a sibling oocyte study

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Received: 21 July 2021 / Accepted: 1 October 2021

One of the new technologies in the field of medically assisted reproduction from the last decade is time-lapse technology (TLT).


**First**, time-lapse incubators guarantee the safest and most stable embryo culture environment available by simply avoiding extensive exposure outside the incubator.

**Second,** continued embryo monitoring that allows for a 24-h surveillance of the oocytes, zygotes, and embryos facilitates the detection or further investigation of previously unknown phenomena in preimplantation development.

The good practice recommendation paper for the use of TLT by the ESHRE working group on time-lapse technology further highlighted that TLT allows for a more flexible and better management of a laboratory workload.

**Finally,** TLT is an important tool within the framework of existing quality management systems. In fact, it may be used o train embryologists and assess intra- and inter-operator variability with respect to the annotation of morphokinetic parameters.

More importantly, TLT can also be used to identify and monitor in vitro stress that may arise from suboptimal culture conditions. In particular, a large number of consumables or new culture media for testing could interfere with anticipated cleavage timings.

Although subtle changes may have no clinical consequences larger deviations in morphokinetic behavior may reflect severe physiological problems.

**Purpose** To evaluate whether ionophore application at the oocyte stage changes the morphokinetics of the associated embryos in cases of artificial oocyte activation.

**Methods** In a prospective sibling oocyte approach, **78 ICSI patients** with suspected fertilization problems had **half of their MII-oocytes** treated with a **ready-to-use ionophore** (calcimycin) immediately following ICSI (study group). Untreated ICSI eggs served as the control group. Primary analyses focused on morphokinetic behavior and the presence of irregular cleavages. The rates of fertilization, utilization, pregnancy, and live birth rate were also evaluated.

**Results** Ionophore-treated oocytes showed a significantly earlier formation of pronuclei (t2PNa) and a better synchronized third cell cycle (s3) ($P < .05$). The rate of irregular cleavage was unaffected ($P > .05$). **Ionophore treatment significantly improved the overall rates of fertilization ($P < .01$) and blastocyst utilization ($P < .05$).**

**Conclusion** Ionophore application does not negatively affect cleavage timing nor is it associated with irregular cleavage.

NIH Public Access
Author Manuscript
*Reprod Biomed Online*. Author manuscript; available in PMC 2012 August 1.

Published in final edited form as:
*Reprod Biomed Online*. 2011 August ; 23(2): 234–244. doi:10.1016/j.rbmo.2011.04.007.

# Methodology matters: IVF versus ICSI and embryonic gene expression

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**In blastocysts derived by:**

- ICSI versus IVF: the expression of 197 genes differed ($P < 0.01$).
- ICSI-A versus IVF: the expression of 132 differed ($P < 0.01$).
- ICSI-A versus ICSI: the expression of 65 genes differed ($P < 0.01$).

| Technique | Fertilized oocytes cultured | 2-cell embryos | Blastocysts |
|---|---|---|---|
| IVF | 108 | 101 (93.52) | 79 (78.22) |
| ICSI | 130 | 111 (85.38) | 57 (51.35) |
| ICSI-A | 80 | 74 (92.50) | 40 (54.05) |

Data are the sum results from four independent experiments. Values are *n* or *n* (%).

Bridges et al. 2011

Reproductive BioMedicine Online (2015) **30**, 323–324

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EDITORIAL

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


Jonathan van Blerkom, Jacques Cohen, Martin Johnson

Long *et al. Reproductive Biology and Endocrinology* (2020) 18:123
https://doi.org/10.1186/s12958-020-00680-2

Reproductive Biology and Endocrinology

REVIEW Open Access

# Risk of birth defects in children conceived by artificial oocyte activation and intracytoplasmic sperm injection: a meta-analysis

Rui Long, Meng Wang, Qi Yu Yang, Shi Qiao Hu, Li Xia Zhu* and Lei Jin*

Five studies were included in the final analysis. Compared with conventional ICSI, ICSI-AOA did not increase the birth defects rate (RR = 1.27, 95%CI 0.70–2.28) of children.

**Table 1** Characteristics of included studies of birth defects in babies from conventional ICSI and ICSI-AOA pregnancies

| Author(s) Publication year | Location | Study design | Time-period | Length of follow-up | Included study population | Methods of oocyte activation | Conventional-ICSI | | ICSI-AOA | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | No. of children (singletons/multiples)[a] | Children with birth defects | No. of children (singletons/multiples)[a] | Children with birth defects |
| Deemeh (2015) [18] | Iran | Historical cohort study | 2008–2010 | 1–30 months | Live births | ionomycin | 89 (67/22) | 2 (2.2%) | 79 (68/2019) | 0 (0%) |
| Nakajo (2016) [16] | Japan | Retrospective cohort study | 1995–2014 | 6 years | Live births | $Ca^{2+}$ ionophore/$SrCl_2$ | 1978 (1640/338) | 75 (3.8%) | 62 (51/11) | 2 (3.2%) |
| Miller (2016) [15] | Israel | Retrospective cohort study | 2006–2014 | Birth | Live births and TOP | $Ca^{2+}$ ionophore | 426 (315/111) | 26 (6.1%) | 62 (51/11) | 6 (9.7%) |
| Li B (2019) [17] | China | Retrospective cohort study | 2011–2016 | Birth | Live births | ionomycin | 2442 (1504/938) | 31 (1.3%) | 95 (59/36) | 2 (2.1%) |
| Kobayashi (2013) [28] | Japan | Retrospective cohort study | 2006–2012 | Not known | Live births | ionomycin | 571 (Not known) | 8 (1.4%) | 18 (Not known) | 1 (5.5%) |
| Total | | | | | | | 5506 | 142 (2.6%) | 316 | 11 (3.5%) |

*ICSI* intracytoplasmic sperm injection, *AOA* artificial oocyte activation, *TOP* terminal of pregnancy

[a]Multiples including twins and triplets

**Table 2** Number of birth defects in specific organ system from included studies

| Author(s) | Specific organ system | Circulatory system | Genitourinary system | Musculoskeletal system | Digestive system | Nervous system | Face | Beckwith-Weidemann syndrome | Poland syndrome |
|---|---|---|---|---|---|---|---|---|---|
| **Deemeh** [18] | **AOA** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **ICSI** | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **Miller** [15] | **AOA**[a] | 4 | 2 | 2 | 0 | 0 | 0 | 1 | 0 |
| | **ICSI**[b] | 8 | 5 | 3 | 2 | 3 | 1 | 0 | 0 |
| **Li B** [17] | **AOA** | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **ICSI** | 20 | 1 | 3 | 0 | 0 | 3 | 0 | 1 |

*ICSI* intracytoplasmic sperm injection, *AOA* artificial oocyte activation

[a]In the AOA group, one case with several structural defects (dysplastic kidney, reflux, ventricular septal defect) and another case with ventricular septal defect, interrupted inferior vena cava and short thumb with low insertion of right hand

[b]In the ICSI group, one case with both hypospadias and ventricular septal defect

Furthermore, in a subgroup analysis, birth defects were classified into two types (chromosomal aberrations and non-chromosomal aberrations) in four studies and no statistical difference were revealed.

**Table 3** Number of children with types of birth defects from included studies

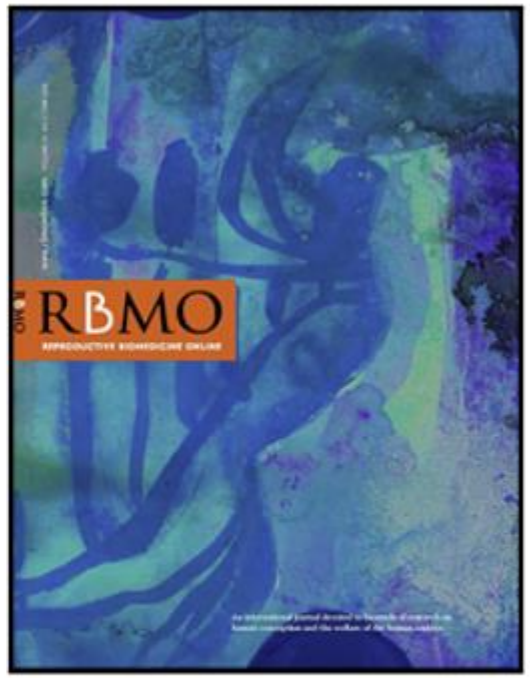
| Author(s) | Total number of children | | Non-chromosomal aberrations | | Chromosomal aberrations | |
|---|---|---|---|---|---|---|
| | ICSI | AOA | ICSI | AOA | ICSI | AOA |
| Deemeh [18] | 89 | 79 | 2 | 0 | 0 | 0 |
| Nakajo [16] | 1978 | 62 | 67 | 1 | 8 | 1 |
| Miller [15] | 426 | 62 | 19 | 6 | 7 | 0 |
| Li B [17] | 2442 | 95 | 30 | 2 | 1 | 0 |

*ICSI* intracytoplasmic sperm injection, *AOA* artificial oocyte activation

Journal Pre-proof

# Artificial oocyte activation to improve reproductive outcomes in couples with various reproductive problems: a retrospective cohort study

Mingrong Lv , Dan Zhang , Xiaojin He , Beili Chen , Qiang Li , Ding Ding , Yan Hao , Rufeng Xue , Dongmei Ji , Weiwei Zou , Huijuan Zou , Yajing Liu , Jianye Wang , Zhaolian Wei , Ping Zhou , Yunxia Cao , Zhiguo Zhang




PII: S1472-6483(20)30001-8
DOI: https://doi.org/10.1016/j.rbmo.2020.01.001
Reference: RBMO 2324

- 1261 ICSI cycles and 796 ICSI-artificial oocyte activation (ICSI-AOA) cycles.
- The rates of implantation, positive β-hCG, clinical pregnancy, and live birth were significantly improved compared **with the previous cycles.**
- Additionally, compared with previous cycles, the rates of blastulation and high-quality blastocysts were increased significantly for couples with **male factors, young patients with chronic salpingitis, and infertile couples with both factors in the ICSI-AOA cycles.**
- The miscarriage rate was decreased significantly for the couples with male factors, young patients with chronic salpingitis, and patients with polycystic ovary syndrome (PCOS) in the treatment cycles. However, no significant differences were found for fertilization rate, embryo development, or outcomes in patients with primary ovarian insufficiency between the ICSI and ICSIAOA groups.

**Conclusions AOA was able to “rescue” the poor reproductive outcomes in certain types of infertile couples with history of failure pregnancy.**

**Conclusion:** This conclusion may provide clinicians evidence-based support in patient counseling and instruction of the application and safety concern about ICSI-AOA.