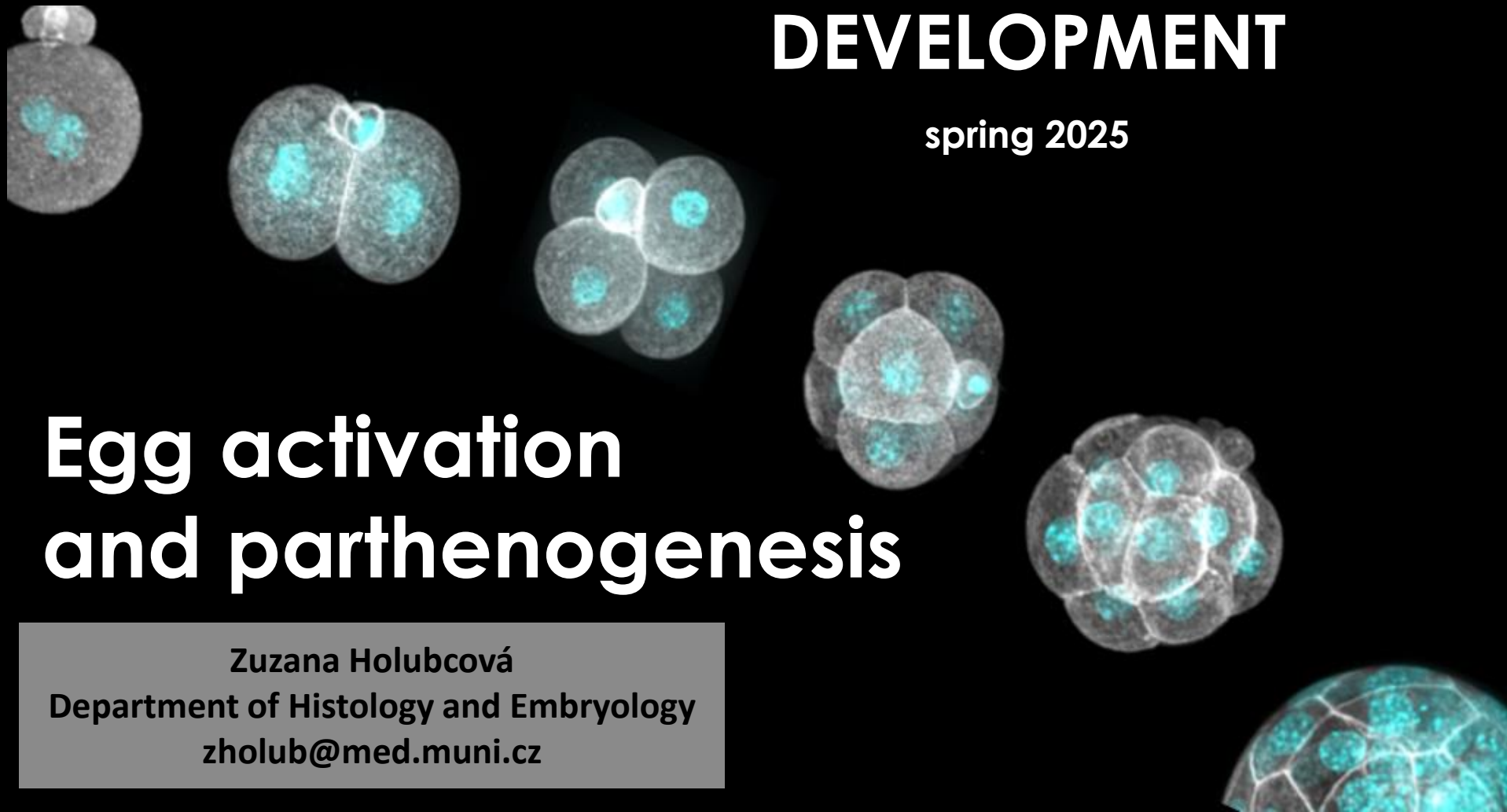
MUNI MED

# Embryology II

# PREIMPLANTATION DEVELOPMENT

spring 2025



# Egg activation and parthenogenesis

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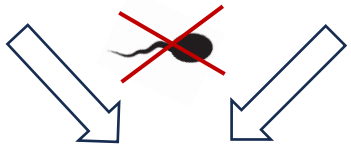
# Egg activation

(A) Physiological stimulus
- induced by sperm entry

(B) Arteficial
- physical treatment
- chemical treatment
- modulation of key proteins

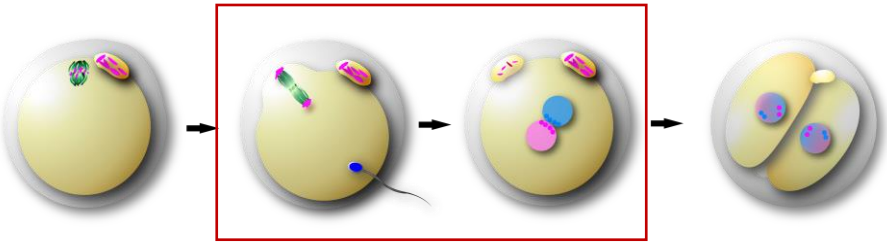
(C) Spontaneous
- no stimulus



**FERTILIZATION**

PARTHENOGENESIS

- development of haploid embryo without fertlization

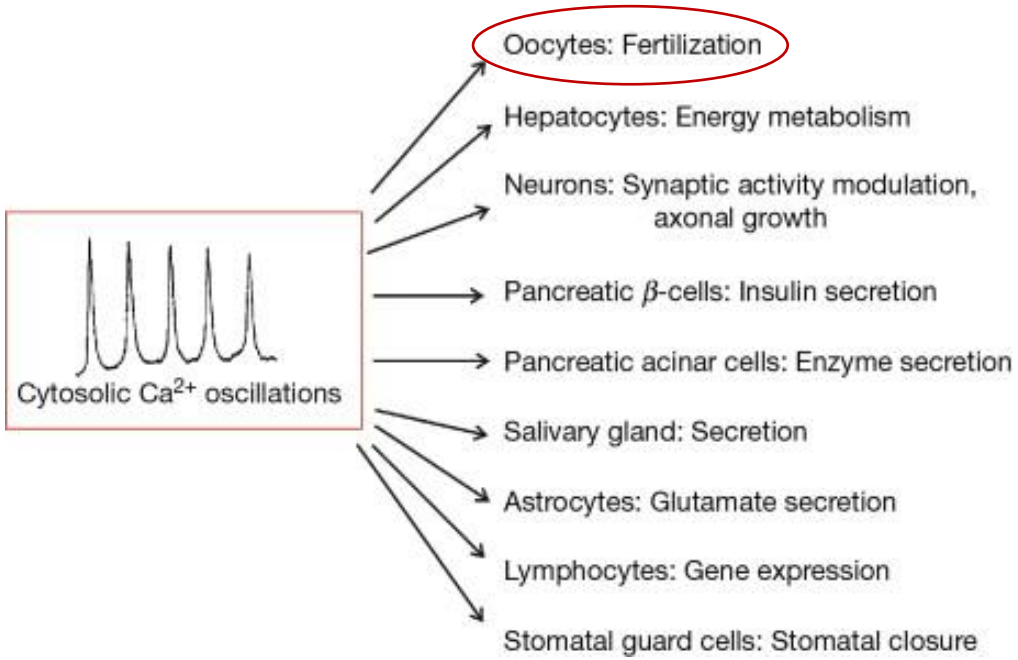


gamete-to-embryo transition

(1) release from MII arrest
(2) polyspermy block
(3) change in mRNA synthesis and protein expression

# Calcium signalling

Extracellular (e.c.) / Intracellular (i.c.) [Ca2+] gradient 2mM / 100nM

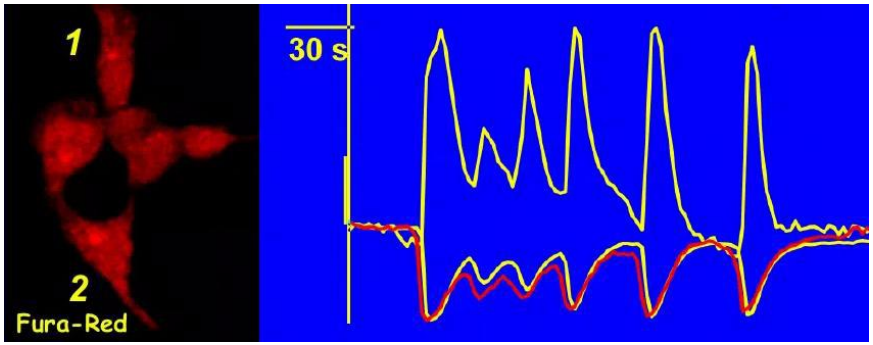



Ca2+ signalling „toolkit“

- Ca2+ mobilizing signals
- i.c. stores - ER, Mt, lysosomes
- Ca2+ influx channels
- pumps and exchanges removing Ca2+ from cytoplasm

**↑ i.c [Ca2+]**

downstream effectors of Ca2+ signalling pathway

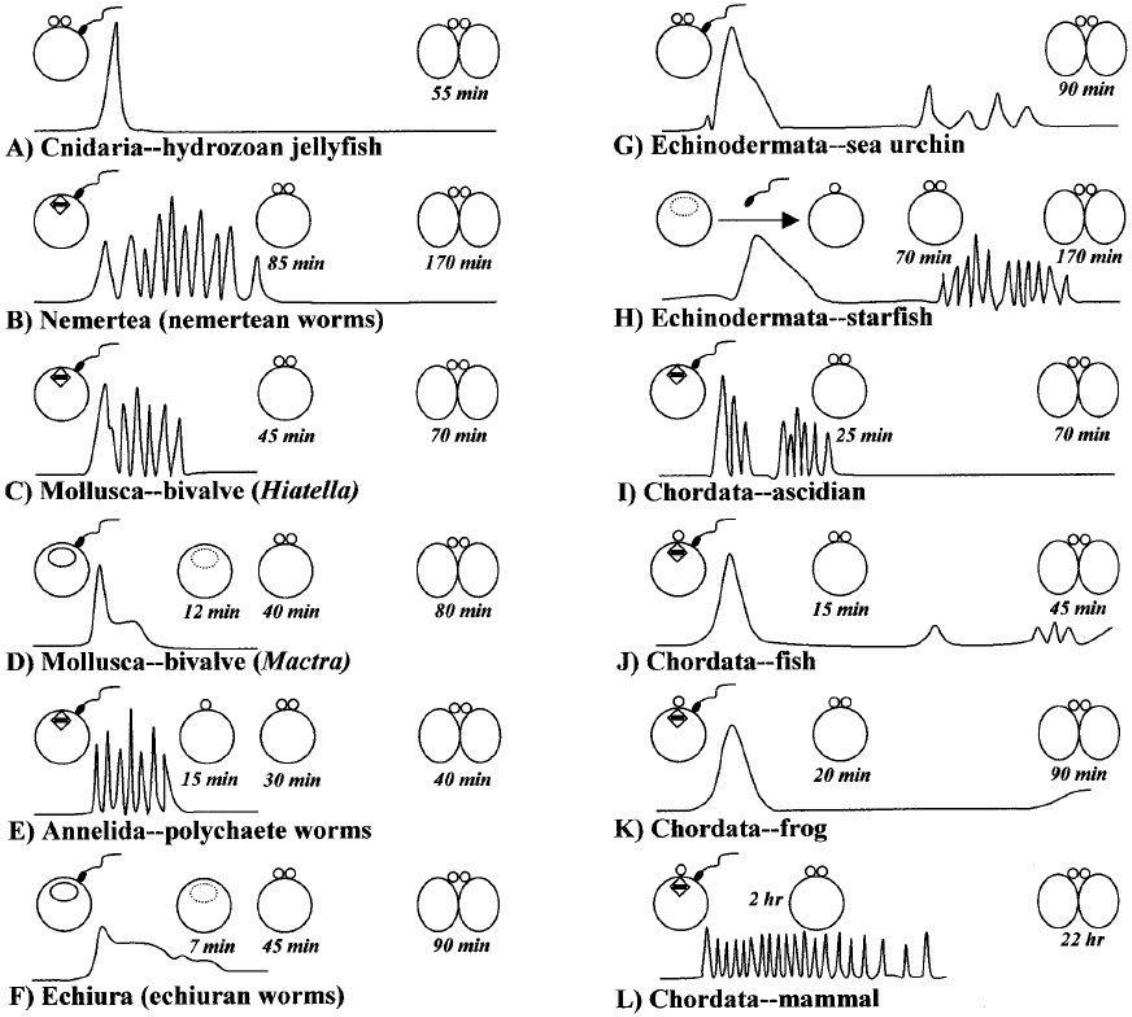



## ❖Ca2+ oscillations

- series of measurable Ca2+ spikes
- periodic increase and fall of cytosolic [Ca2+]

# Calcium signalling at fertilization

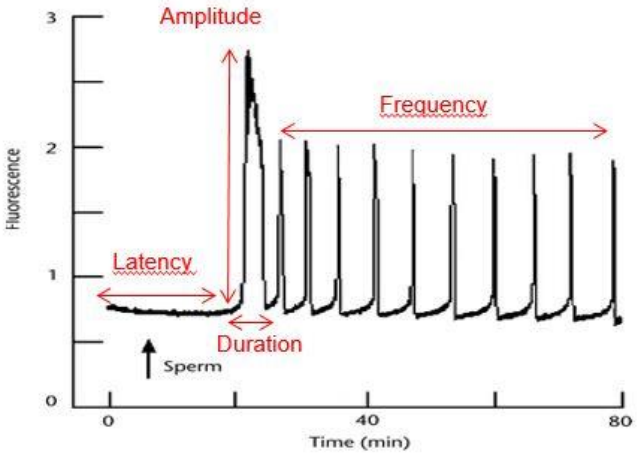
- universal hallmark of egg activation in all sexually reproducing animal species (and flowering plants)

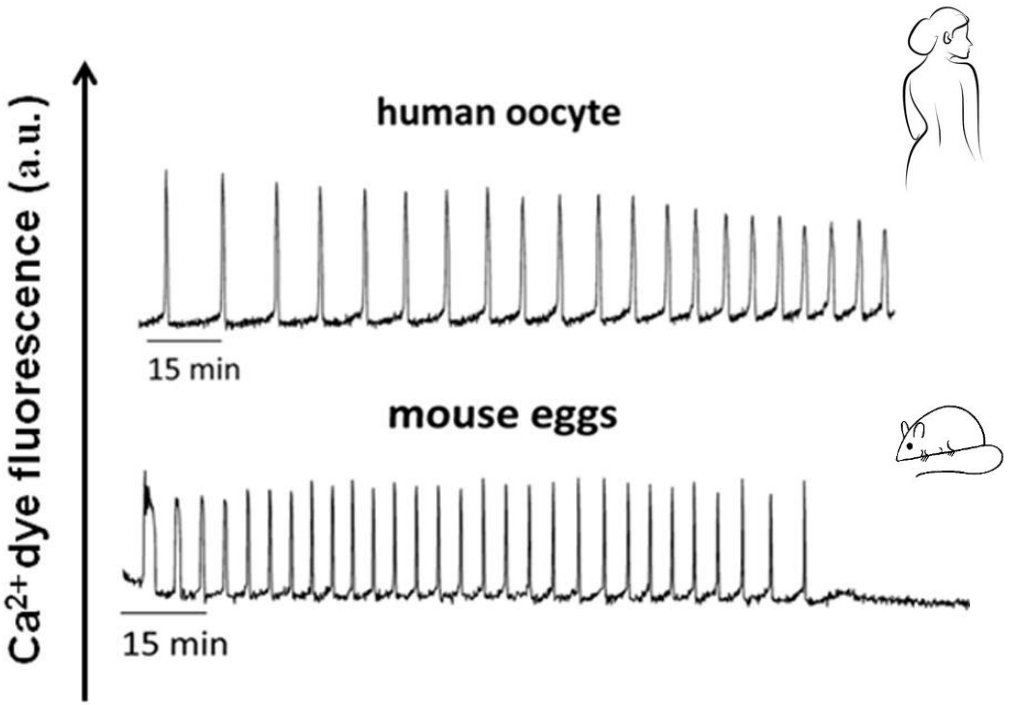


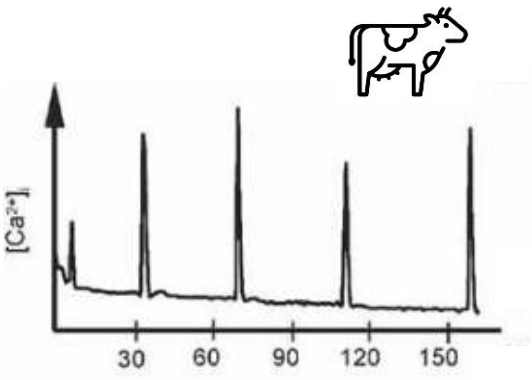

*Stricker at al. 1999*

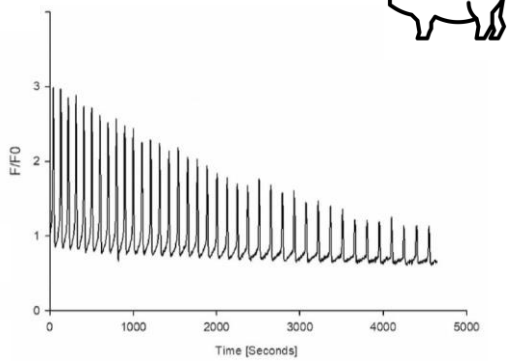
# Calcium signalling at fertilization

- in mammals, species-specific differences in latency, duration, frequency and amplitude of Ca2+ oscillations



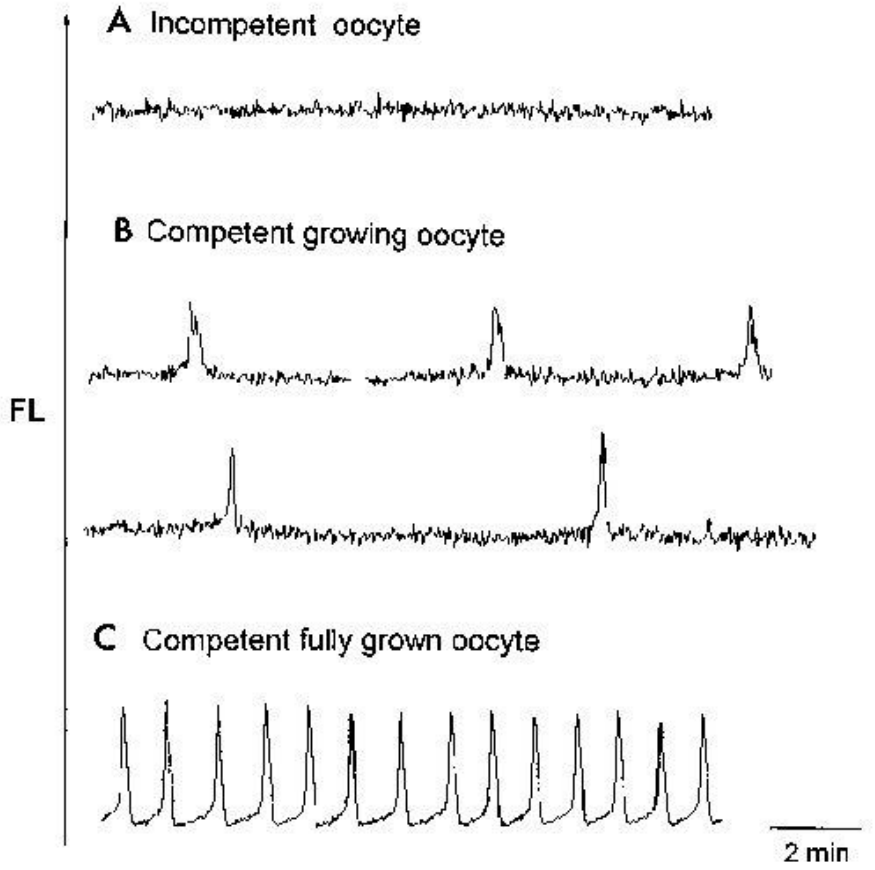



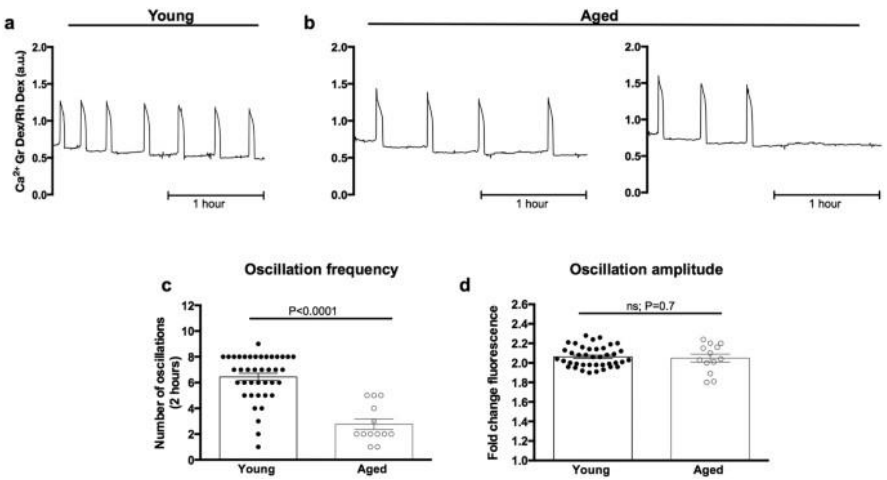



# Calcium signalling at fertilization

- Ca2+ oscillation pattern related to egg/embryo developmental competence




Carrol et al. 1995




*Haverfiled et al. 2016*

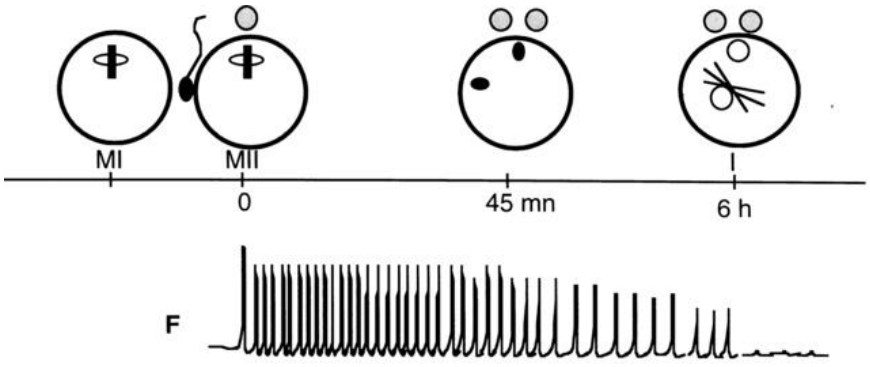
- age-related alterations of oscilation patttern

# Calcium signalling at fertilization

- Ca2+ oscillations triggered by fertilizing sperm

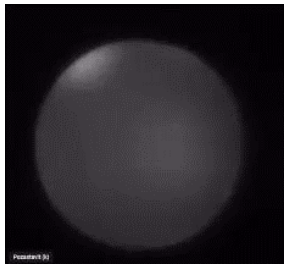
- lasts several hours until PN formation

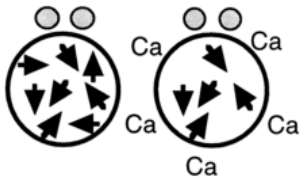
- first Ca2+ wave begins at sperm entry site and propagades radially to opposite pole



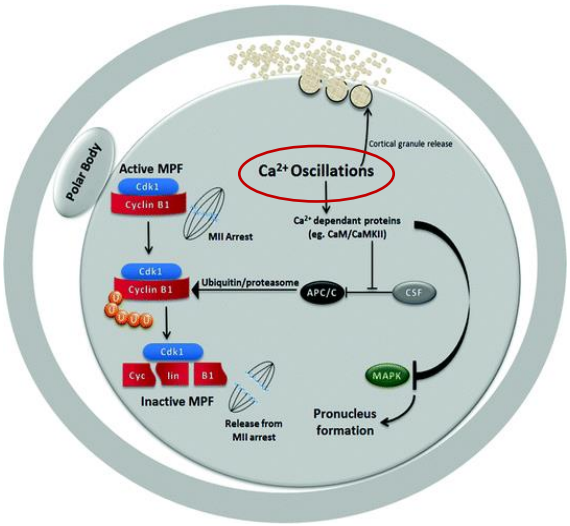

**→ GCs exocytosis**
**→ release from MII arrest**
**→ cytoplasmic movements**
**→ PN formation**

-directional



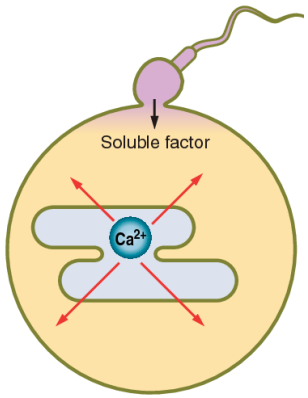



-faster, nondirectional

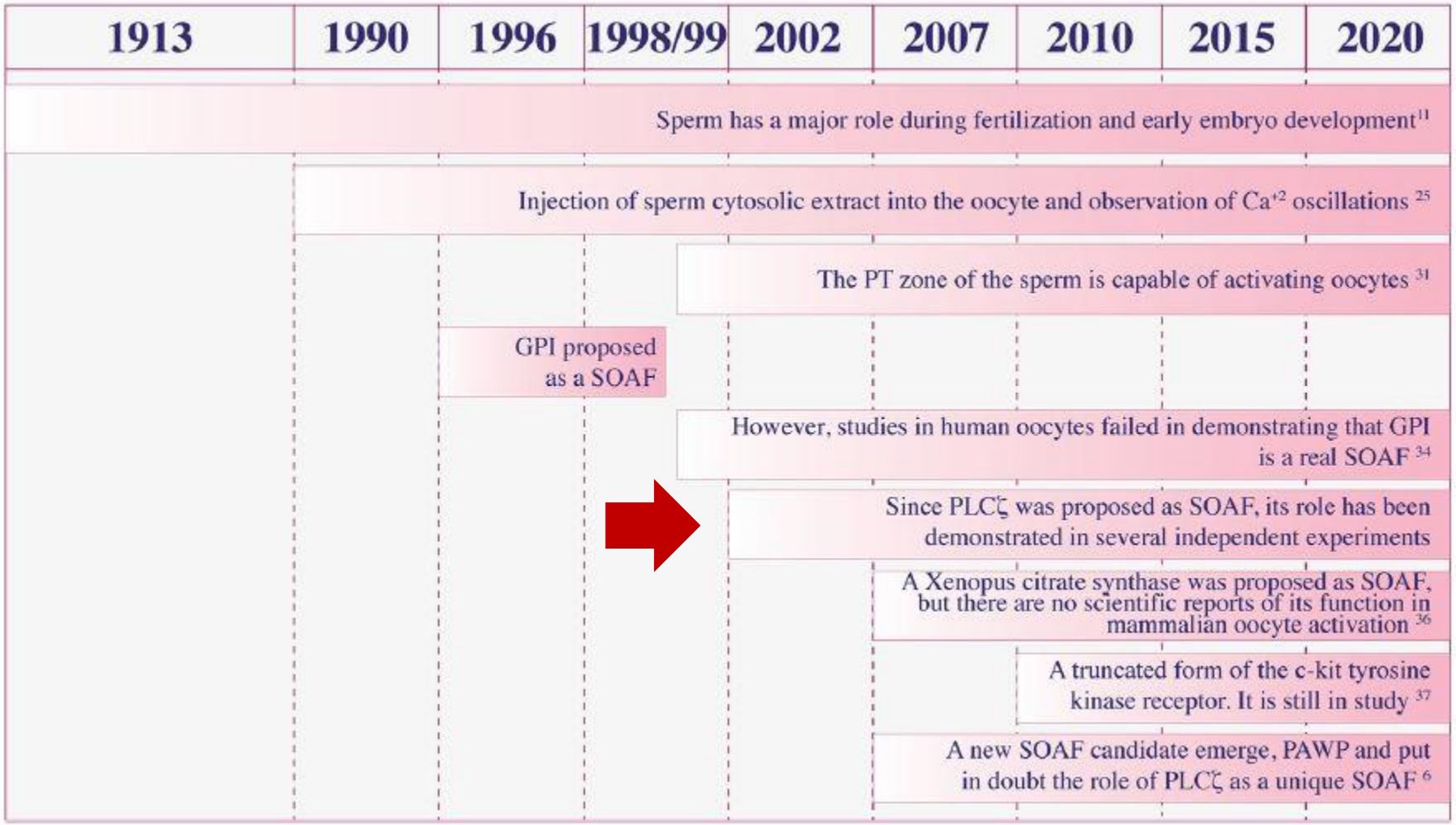



- Ca2+ oscillations triggered also by ICSI and sperm extract injection

→ role of soluble **sperm-delivered oocyte activation factor** (SOAF)



# Quest for SOAF




*Zafar et al. 2021*

# PLC zeta (PLCζ)

*Saunders et al 2002*

- testis-specific isoform of phosfolipase C
- delivered to the oocyte by fertilizing sperm
- localized to post-acrosomal region of sperm head where gamete fusion starts

- after sperm penetration, PLCζ diffuses into the ooplasm, binds to i.c. vesicles containing PIP2 and hydrolyses PIP2 to IP3 and DAG

- generated IP3 then binds to IP3 receptor, which forms a Ca2+ channel in ER membrane

- the openning of the channel release Ca2+ from i.c. store to cytosol initiating Ca2+ oscillations

Development 129, 3533-3544 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
DEV7973

**PLCζ: a sperm-specific trigger of $Ca^{2+}$ oscillations in eggs and embryo development**

**Christopher M. Saunders[1], Mark G. Larman[2], John Parrington[3], Llewellyn J. Cox[1], Jillian Royse[1], Lynda M. Blayney[1], Karl Swann[2] and F. Anthony Lai[1,*]**

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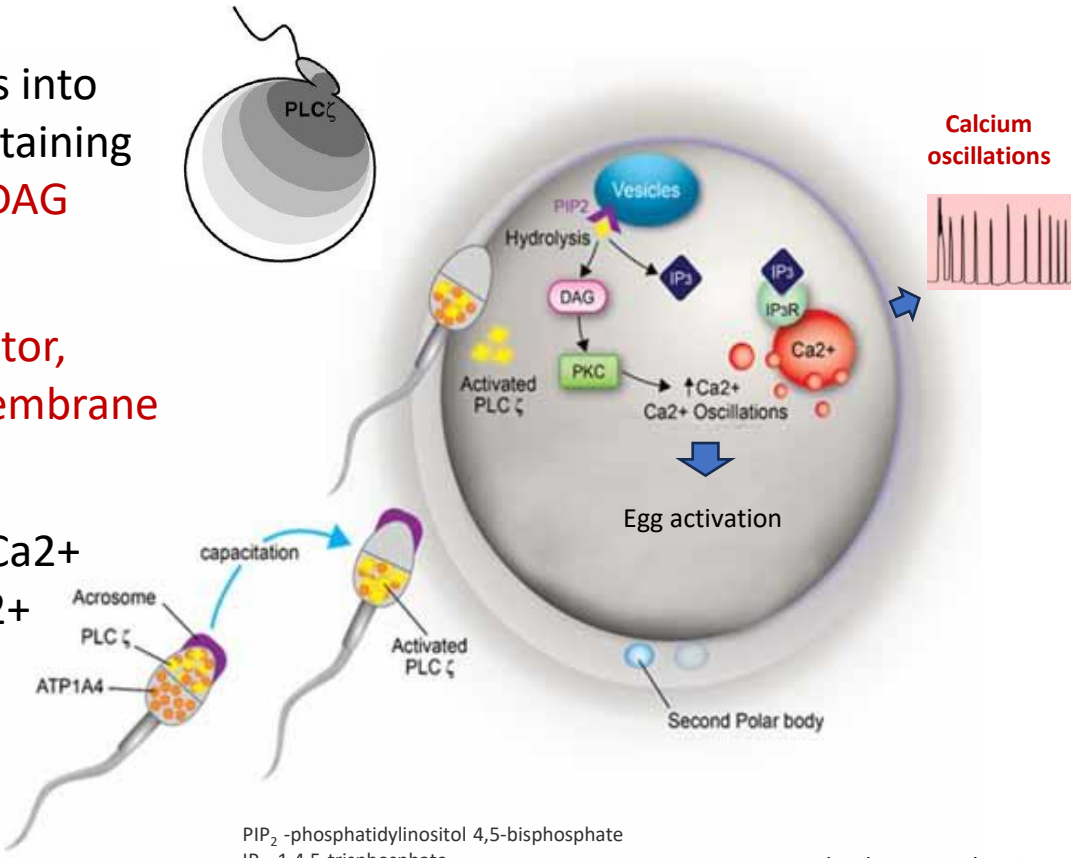



$PIP_2$ -phosphatidylinositol 4,5-bisphosphate
$IP_3$- 1,4,5-trisphosphate
DAG - dyacyl glycerol

*Unnikrishnan et al 2021*

# Calcium signaling homeostasis

## ❑ elevation of i.c. [Ca2+]

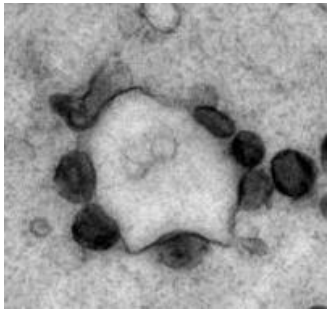
← Ca2+ release from ER stores

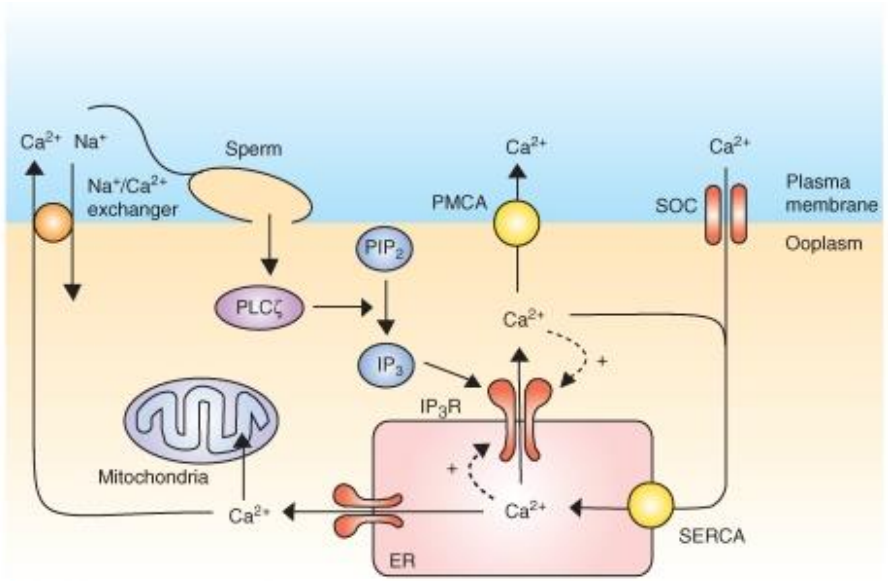
IP3-mediated Ca2+ release and subsequent
Ca2+- mediated Ca2+ release through a Ca2+ sensitive
IP3 receptor channel (allosterically modulated by Zn)

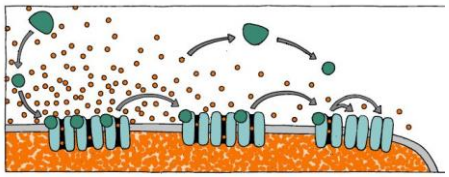
← Ca2+ influx accross plasma membrane

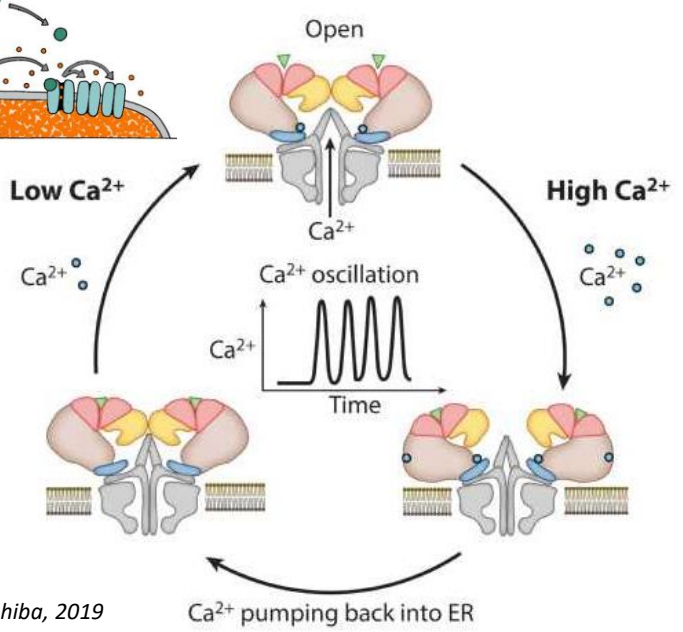
SOCE (store operated $Ca^{2+}$ entry)

SER-Mt „necklace“ complexes








## ❑ return to baseline level of i.c. [Ca2+]

←removal from the cell

PMCA (plasma membrane $Ca^{2+}$ pump)
NCX ($Na/Ca^{2+}$ exchanger)

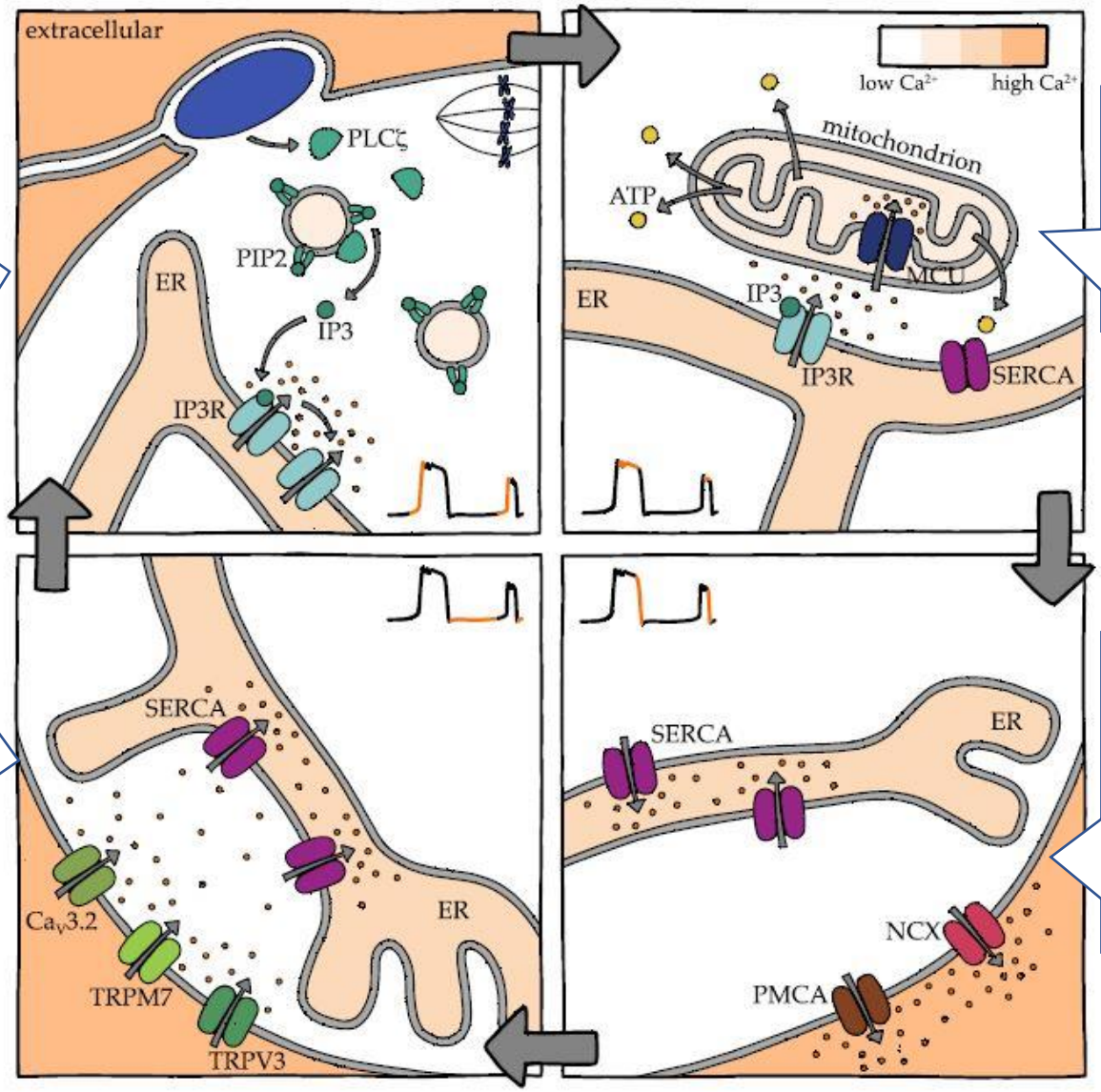
← uptake into i.c. Stores

SERCA (sarcoendoplasmic reticulum $Ca^{2+}$ATPase)

*Hamada and Mikoshiba, 2019*

# Cycle of Ca2+ transient generation

Sperm PLCζ acts on PIP2 in intracellular vesicles to generate IP3, which stimulates IP3R-mediated Ca2+ release and subsequent Ca2+-induced Ca2+ release




Ca2+ stimulates mitochondrial ATP production; ATP is required for SERCA pump activity.

Ca2+ flows into the cytoplasm through TRMP7, CaV3.2 and TRPV3 channels and is then available for SERCA pumps to replenish ER Ca2+ stores in preparation for the next Ca2+ release event

Ca2+ is pumped back into the ER through SERCA pumps and out of the egg through PMCA pumps and NCX

*Stein et al 2020*

# Calcium signaling effectors

- ↑$[Ca^{2+}]$ → modulation of activity of $Ca^{2+}$-sensitive enzymes

❖ CaMKII (calmodulin-dependent protein kinase II)
- phosphorylates Emi2 promoting its interaction with Plk1
- Plk1-induced phosphorylation of Emi2 leads to its ubiquitination and destruction by proteasome
- degradation of Emi2 releases a brake on the APC
- APC activity degrades cycB

exit form MII arrest

❖ PKC (protein kinase C)
- DAG-induced relocation to plasma membrane
- regulation of Ca2+ influx
- phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS) → actin reorganization – GCs exocytosis

❖ TCA enzymes

→ activation of mitochondrial metabolism

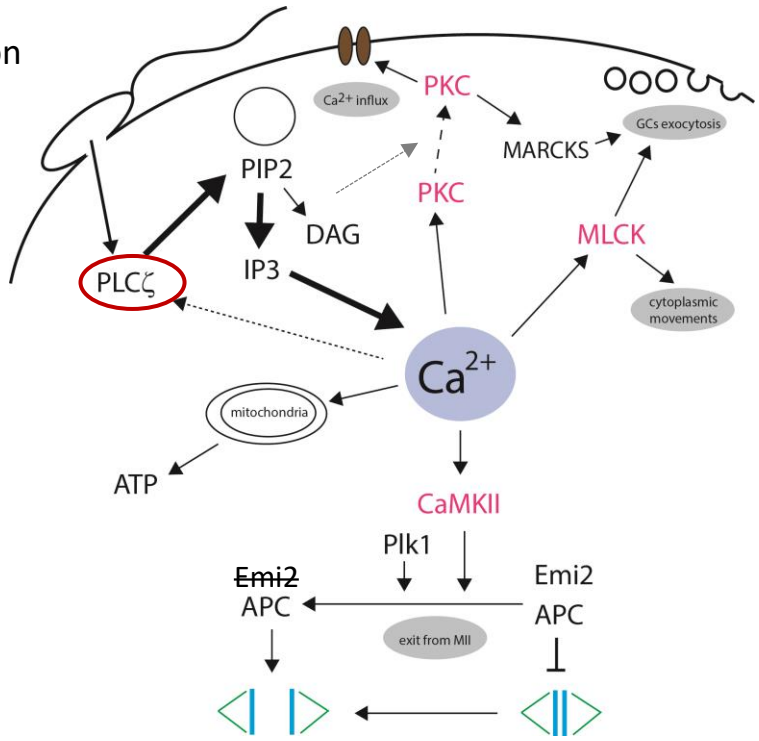
→ ↑ATP supply → maintainance of Ca2+ waves

❖ MLCK (myosin light chain kinase)

→ exocytosis of CGs → polyspermy block

→ cytoplasmic movements

❖ PLCζ
- positive feedback loop

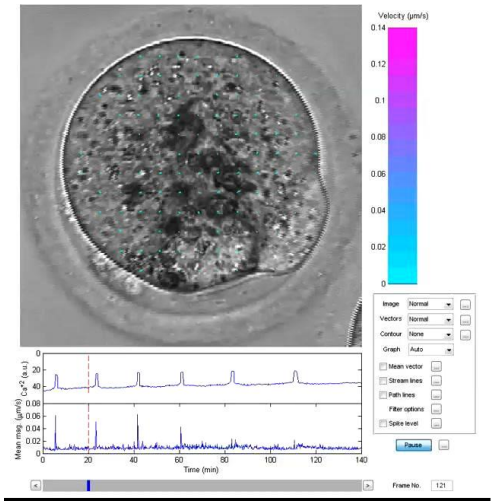


# Activation-induced cytoplasmic movements

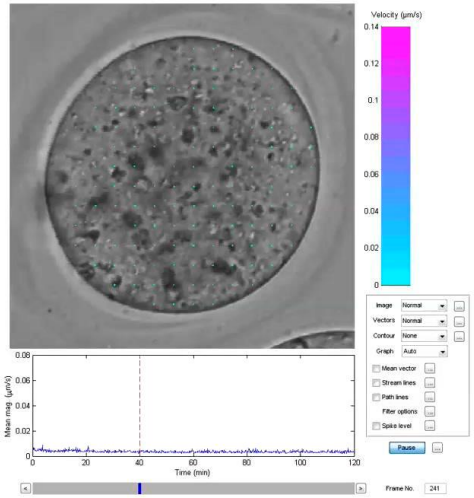
- rhythmic cytoplasmic movement  triggered by Ca2+ oscilations
- actomyosin-mediated spasms detectable by particle image vector analysis
- non-invasive prediction of embryo viability?

*Ajduk et al 2011*

fertilized egg



unfertilized egg



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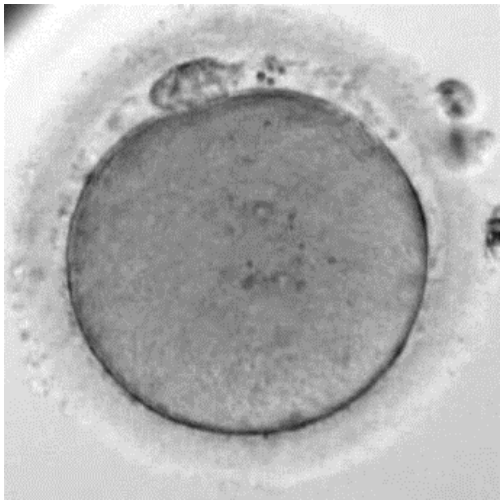
## Rhythmic actomyosin-driven contractions induced by sperm entry predict mammalian embryo viability

Anna Ajduk[1], Tagbo Ilozue[1], Shane Windsor[2], Yuansong Yu[3], K. Bianka Seres[1,4], Richard J. Bomphrey[2], Brian D. Tom[5], Karl Swann[3], Adrian Thomas[2], Chris Graham[2] & Magdalena Zernicka-Goetz[1]

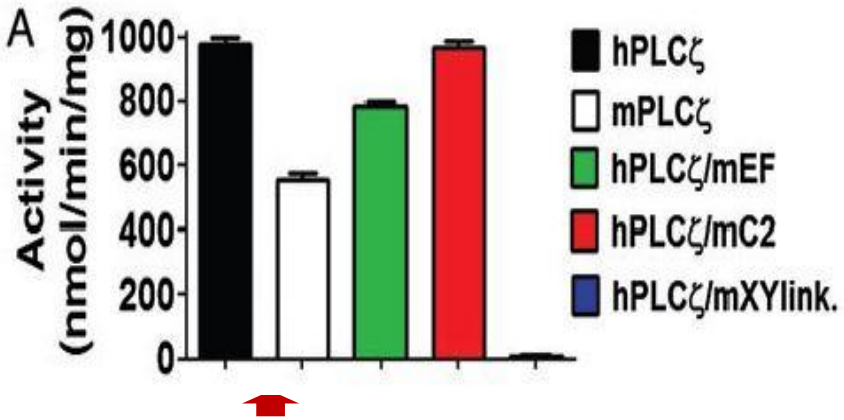


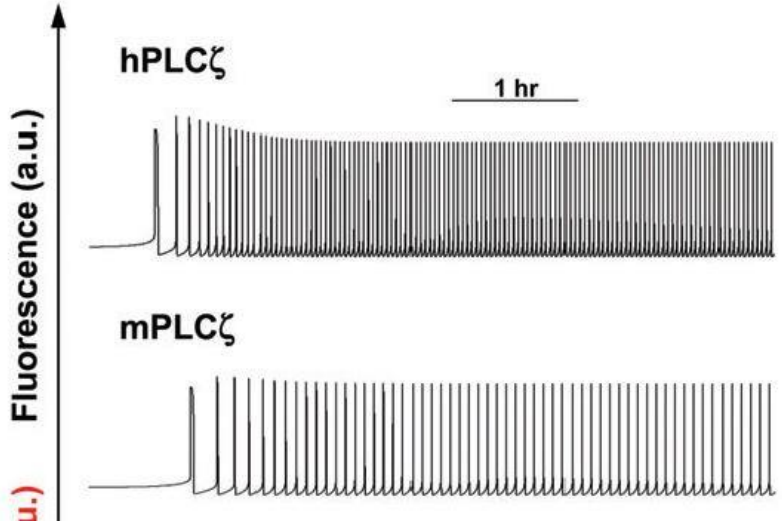
Fertilized human oocytes exhibit
a coordinated cyclic movement of cytoplasm („swirl“, „twirl“)
before PBE2 is visible in transmitted light

# PLCζ activity

- **PLCζ** active only in oocytes
- existence of oocyte-specific activator?

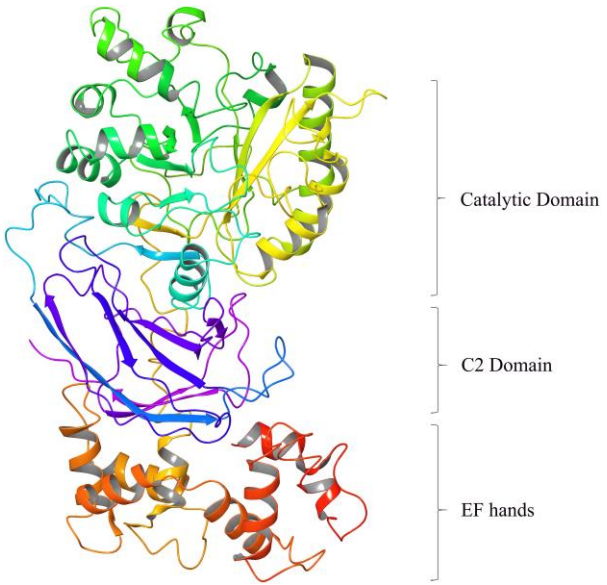
- human PLCζ has higher Ca2+ signalling potency than mouse PLCζ

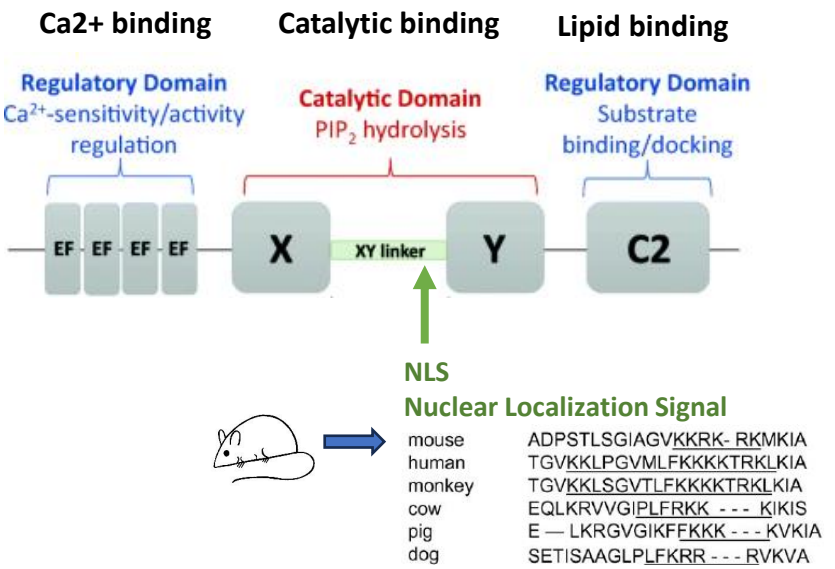


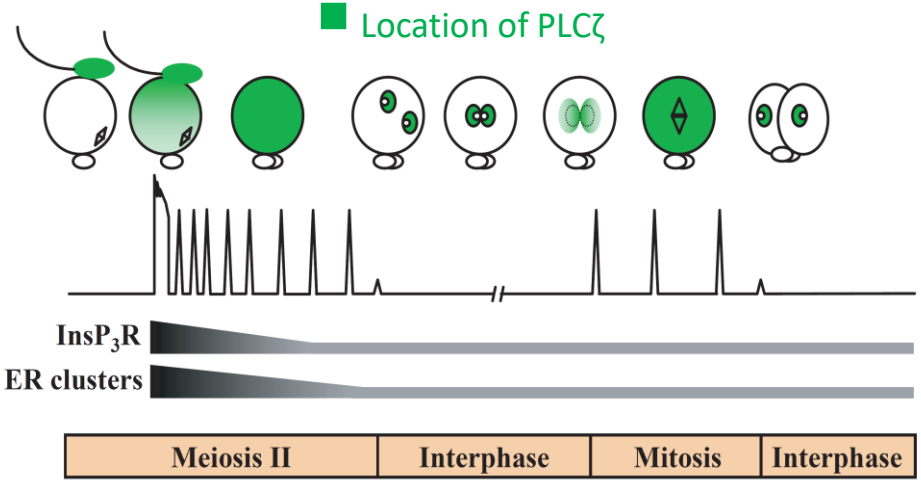

*Nomikos et al. 2010*

# PLCζ activity




- in mouse, Ca2+ oscillations temporarily ceases with PLCζ being sequestered into newly formed pronuclei
- single $Ca^{2+}$ peak observed shortly before every mitotic division
- sinusoidal fluctuations disappear progressively in arrested human embryos
- mitosis can be stopped using Ca2+ chelators

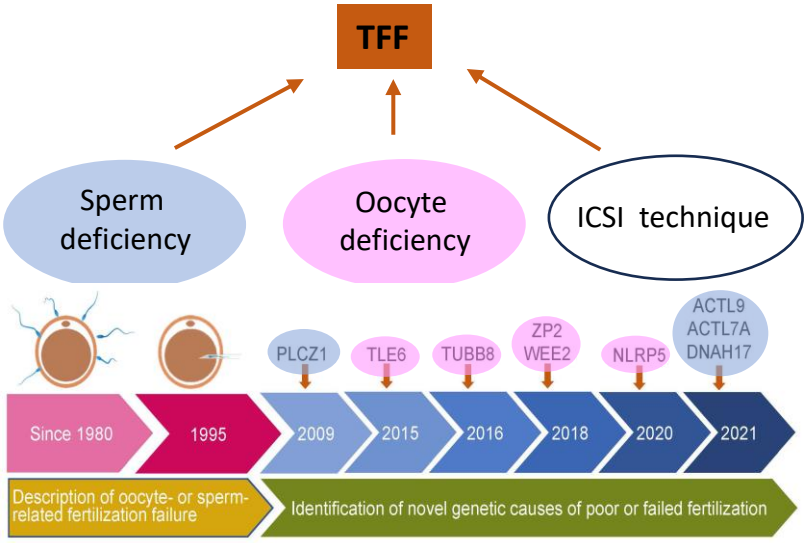


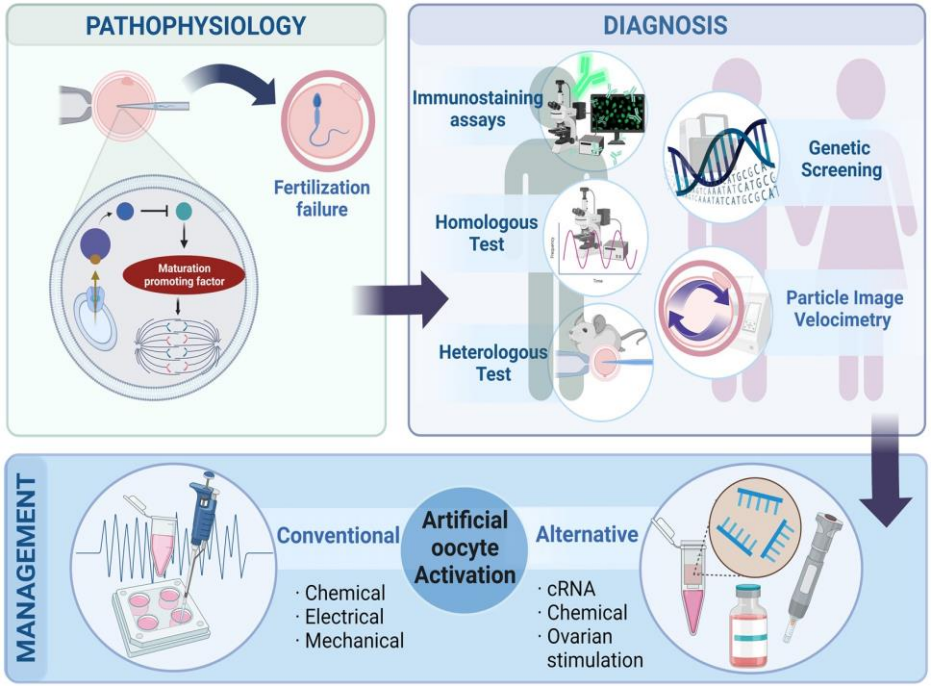

*Larman et al., 2004*

# Clinical implications

## ❖Total fertilization failure (TFF)

- unexpected low fertilization efficiency (<25% FR)
- normal spermiogram and apparently normal oocyte morphology
- oocyte activation deficiency (OAD)
- failure of sperm chromatin decondensation
- conventional IVF failure can be bypassed by ICSI
- rare (1-3%) in ICSI cycles
- recurrence 30-50%
- premature termination of treatment
- distressful for patients and personell

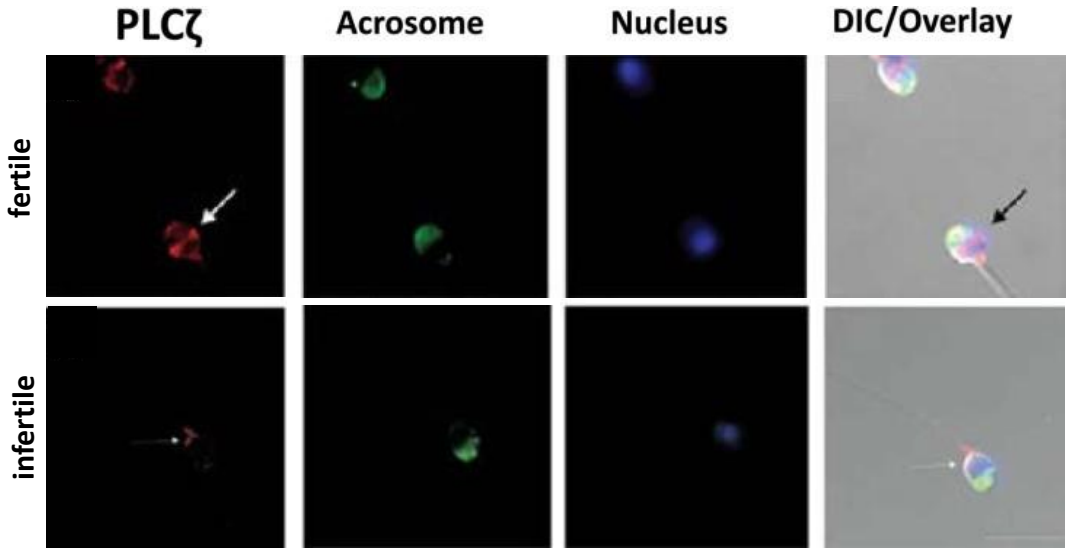

*Xue et al 2022*

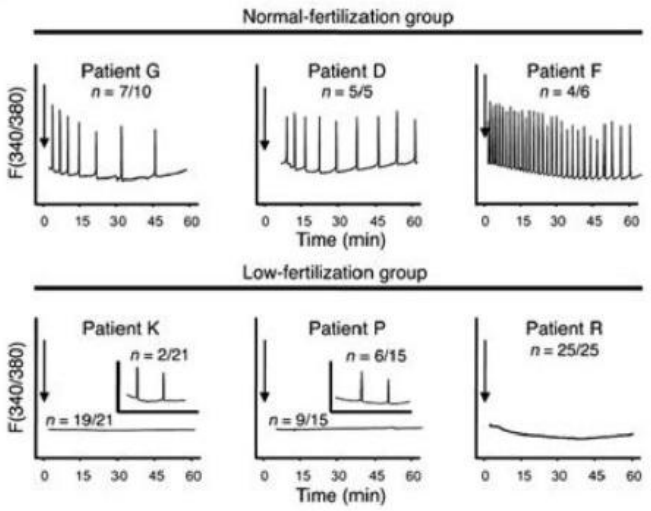
*Campos et al. 2023*

# Clinical implications

- **PLCζ**

- mutation/impaired expression of PLCζ is related to recurrent fertilization failure and male/idiopathic infertility
- PLC **ζ** deficiency in sperm correlates with altered Ca2+ oscillation pattern
- diagnostical screening for PLCζ genetic profile and/or immunostaining pattern in infertile patients for tailored treatment and informed consulting



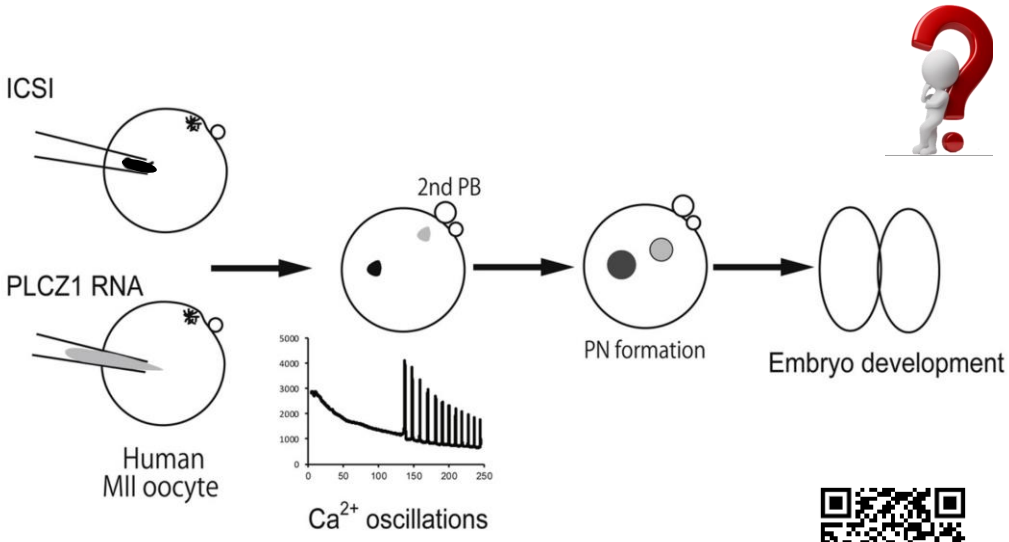

*Ramadan et al 2012*

# Clinical implications

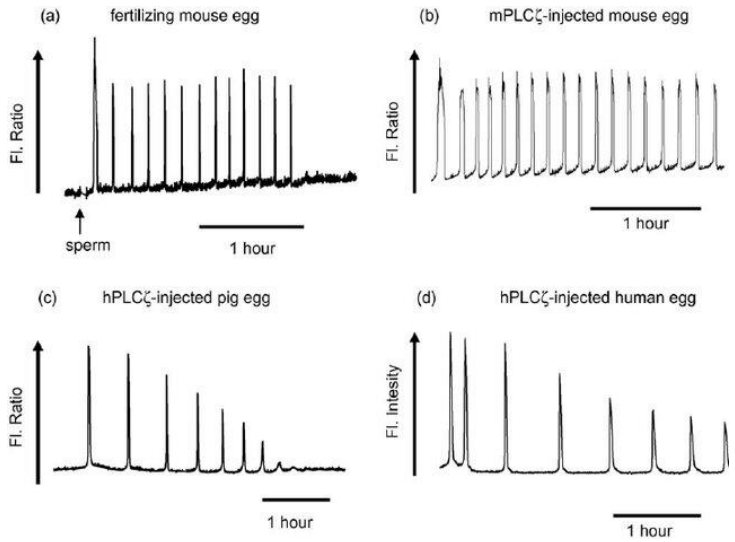
- **PLCζ**

- injection of mouse/human PLCζ cRNA/protein induces Ca2+ oscillation similar to those seen during fertilization and triggers parthenogenesis

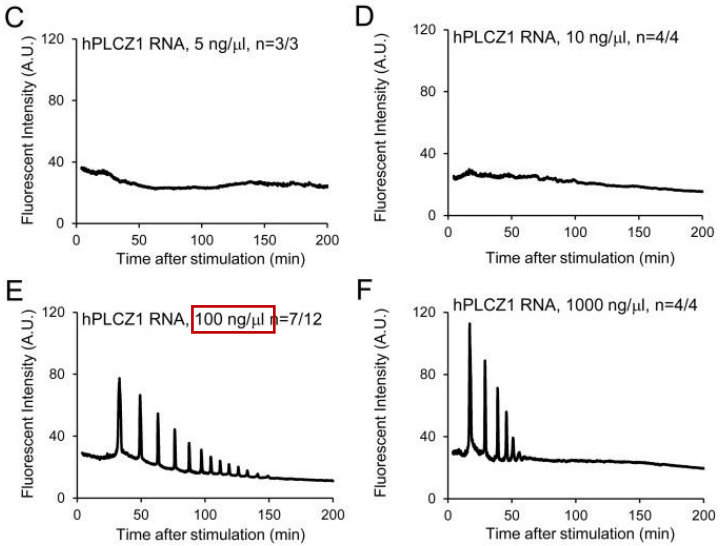
- rescue by (co)injection of PLCζ along with defective sperm?




*Nokimos et al 2013 (mouse)*
*McCarter et al, ESHRE 2024, conference abstract (human)*




*Swann et al 2006*




*Yamaguchi et al 2017*

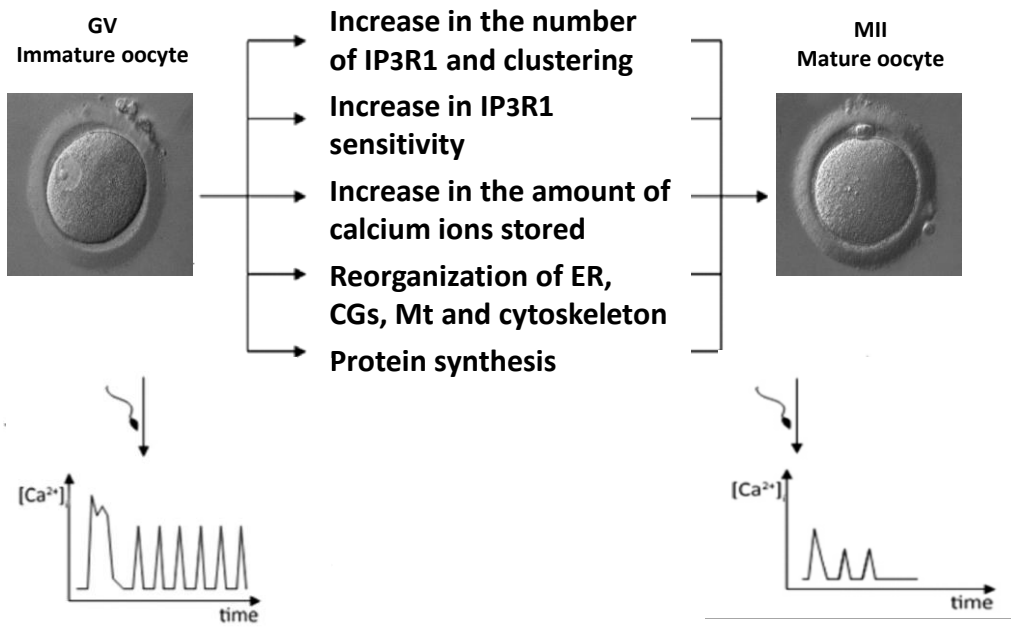
# Clinical implications

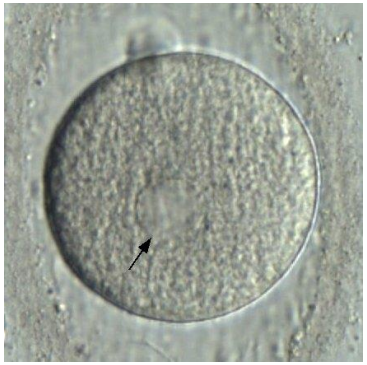
- **Oocyte deficiency**

- deficiency in PLC ζ-triggered activation signalling cascade

- activation capacity and Ca2+ oscillation adversely affected by

  - incomplete/aberrant cytoplasmic maturation
  - oocyte in vitro maturation
  - cryopreservation
  - reproductive aging
  - in vitro postovulatory aging



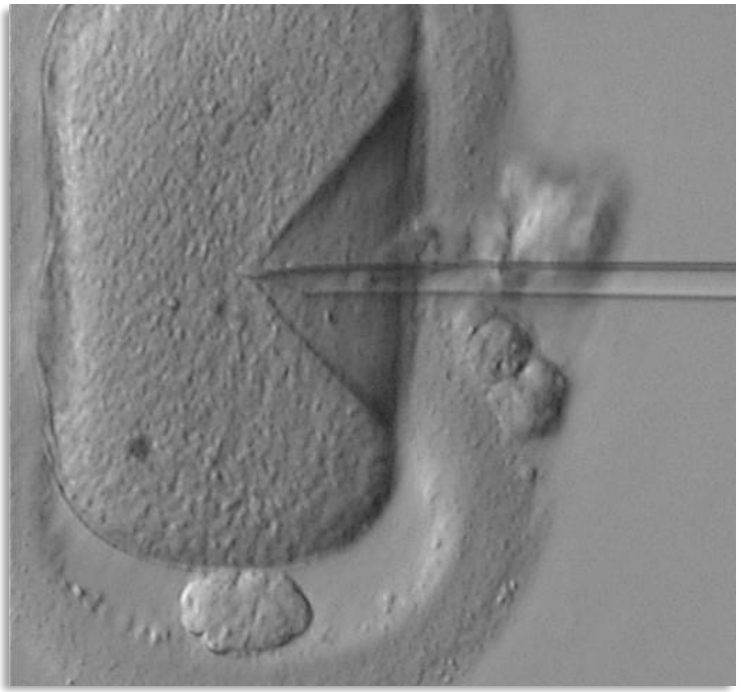



sER+ oocytes

- abnormal disribution of IP3 receptors at SER
- release more Ca2+ over a period of time?

# Clinical implications

- **ICSI technique**



Aspiration

Human Reproduction Vol.16, No.2 pp. 306–312, 2001

**Embryo development and chromosomal anomalies after ICSI: effect of the injection procedure***

John C.M.Dumoulin[1,3], Edith Coonen[1], Marijke Bras[1], J.Marij Bergers-Janssen[1], Rosie C.M.Ignoul-Vanvuchelen[1], Lucie C.P.van Wissen[1], Joep P.M.Geraedts[2] and Johannes L.H.Evers[1]

Puncture

FERTILITY AND STERILITY®
VOL. 73, NO. 1, JANUARY 2000


Human Reproduction vol.11 no.3 pp.540–547, 1996

**Two essential steps for a successful intracytoplasmic sperm injection: injection of immobilized spermatozoa after rupture of the oolema**

P.Vanderzwalmen[1], G.Bertin, B.Lejeune, M.Nijs, B.Vandamme and R.Schoysman

Deposition

**Sperm deposition site during ICSI affects fertilization and development**

*Marlena Blake, B.Sc., John Garrisi, Ph.D., Giles Tomkin, and Jacques Cohen, Ph.D.*

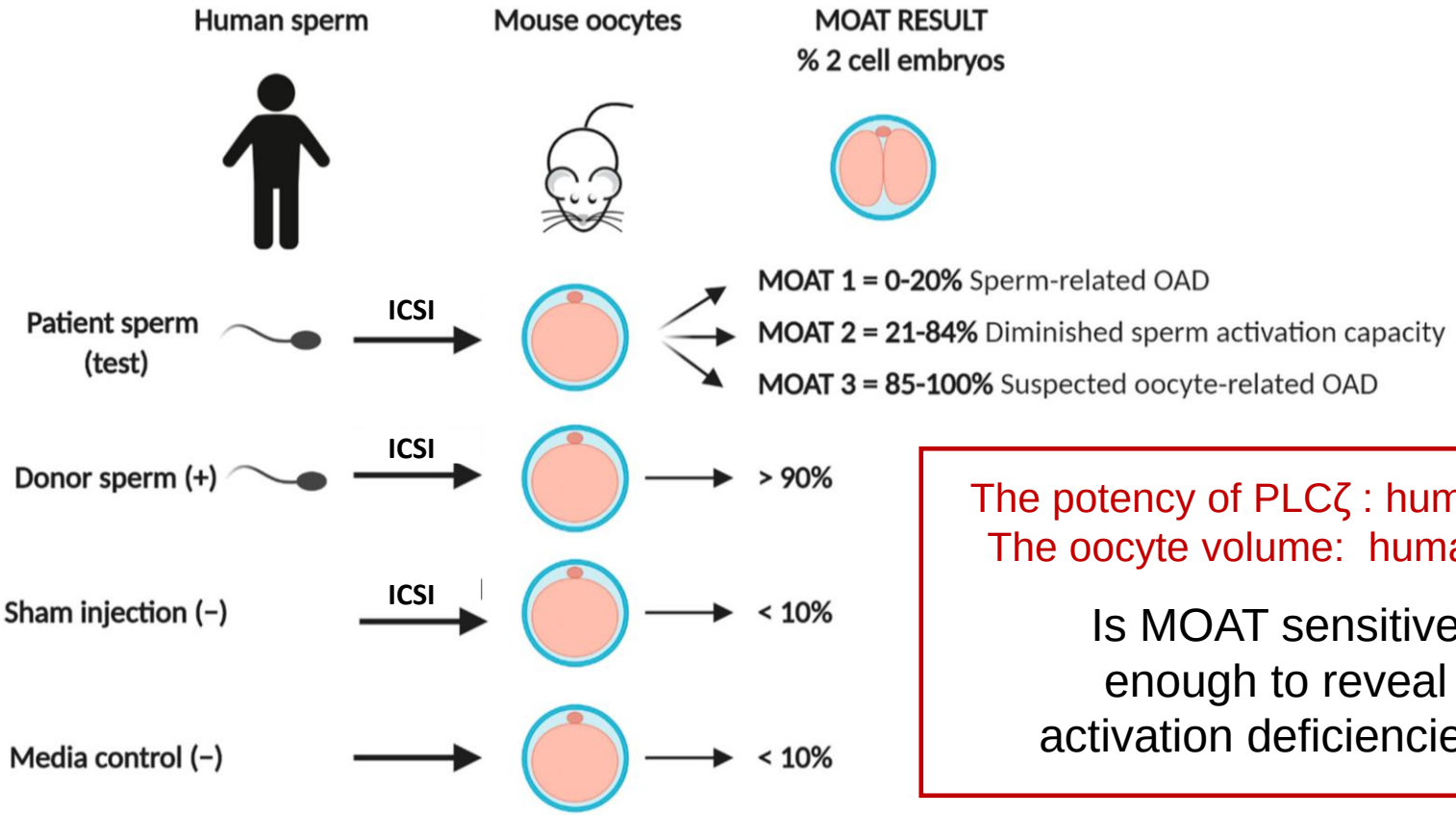
*The Institute for Reproductive Medicine and Science of Saint Barnabas, West Orange, New Jersey*

# Clinical implications

## ❖Diagnosis of activation problems

Björn Heindryckx

- Mouse oocyte activation test (MOAT)
  - heterologous ICSI assay (human sperm + mouse egg)
  - evaluation of fertilization outcome




The potency of PLCζ : human > mouse
The oocyte volume: human > mouse

Is MOAT sensitive enough to reveal activation deficiencies?

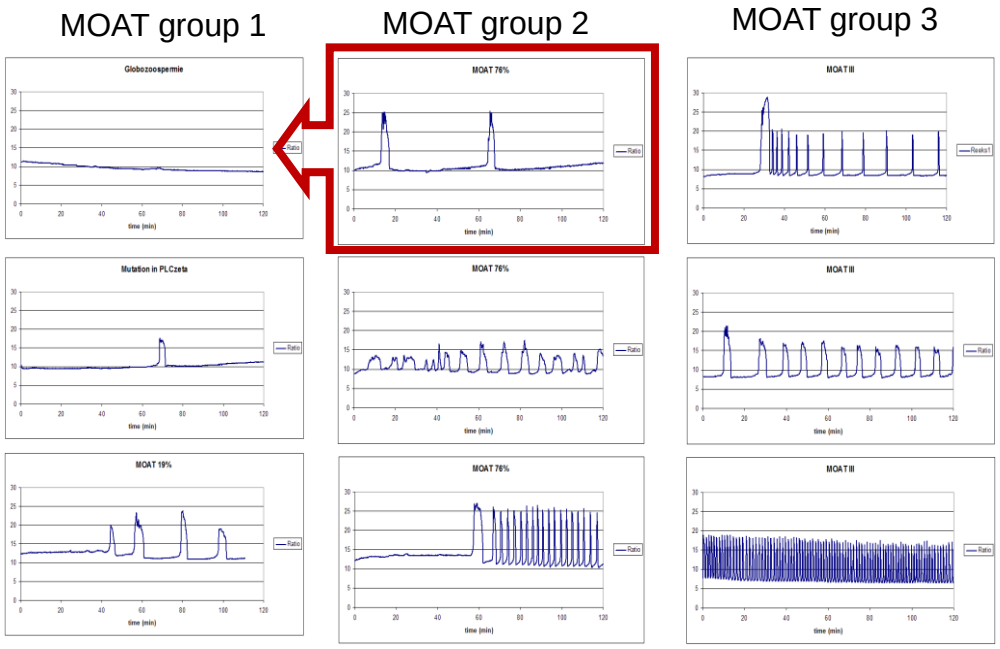
*Barberán et al 2020*

# Clinical implications

## ❖Diagnosis of activation problems

Björn Heindryckx

- Mouse oocyte calcium analysis (MOCA)
  - heterologous ICSI assay (human sperm + mouse egg)
  - measurement of Ca2+ oscillations amplitude and frequency




Courtesy of B. Heindryckx

Interpretation of results:

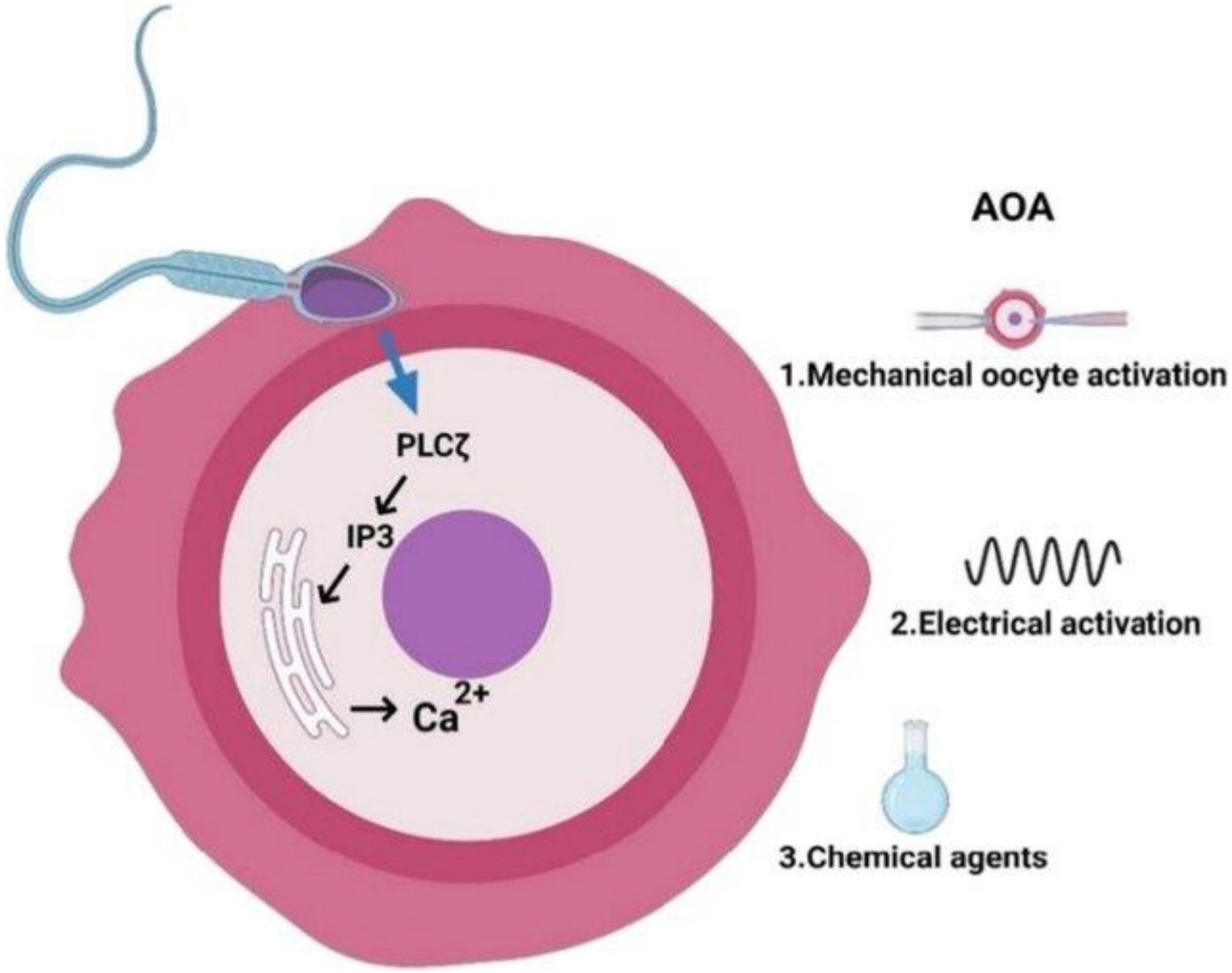
| MOCA > 9 | MOCA < 9 |
|---|---|
| Group A | Group B |
| **Normal sperm factor activity** | **Possible sperm factor deficiency** |

- more sensitive than MOAT but can´t be directly extrapolated to human model

- Human oocyte activation test (HOCA)
  - homologous ICSI assay (human sperm + human egg)
  - analysis of Ca2+ oscillations

# Clinical implications

## ❖Artificial oocyte activation (AOA)



# Clinical implications

## ❖Artificial oocyte activation (AOA)

- Mechanical activation
  - hypo/hypertonic solution
  - hydrostatic pressure
  - modified ICSI technique

Thomas Ebner

Human Reproduction Vol.19, No.8 pp. 1837–1841, 2004
Advance Access publication May 27, 2004

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

T.Ebner[1,2], M.Moser[1], M.Sommergruber[1], K.Jesacher[1] and G.Tews[1]

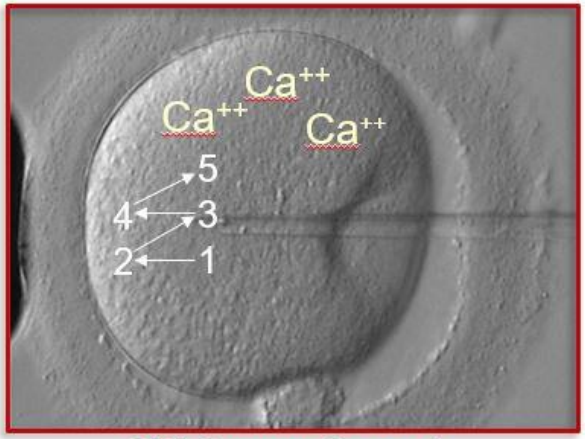
[1]Women's General Hospital, IVF-Unit, Lederergasse 47, A-4010 Linz, Austria

FERTILITY AND STERILITY®
VOL. 78, NO. 3, SEPTEMBER 2002

**Use of a modified intracytoplasmic sperm injection technique to overcome sperm-borne and oocyte-borne oocyte activation failures**

Jan Tesarik, M.D., Ph.D.,[a,b] Laura Rienzi, B.Sc.,[c] Filippo Ubaldi, M.D.,[c] Carmen Mendoza, Ph.D.,[a] and Ermanno Greco, M.D.[c]

MAR&Gen Molecular Assisted Reproduction and Genetics, Granada, Spain; Laboratoire d'Eylau, Paris, France; and Centre for Reproductive Medicine, European Hospital, Rome, Italy




21% degeneration rate!

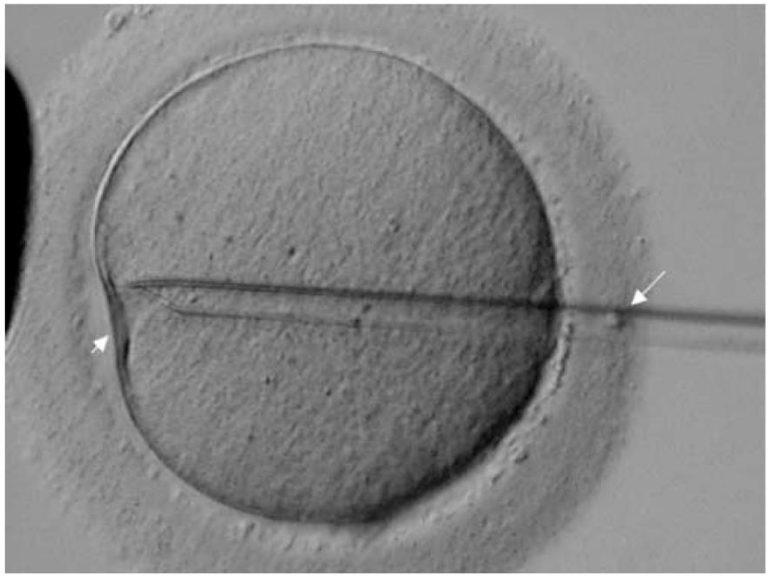


**Figure 2.** Culmination of the aspiration phase. During aspiration, the opposite membrane responds with a slight invagination (small arrow). The large arrow (10 μm) indicates the spermatozoon.

- ~50% FR

BUT no improvement if used as a routine technique!

# Clinical implications

## ❖Artificial oocyte activation (AOA)

- Electrostimulation

- direct current voltage causes rearrangement of cell membrane proteins leading to the formation of pores allowing for the influx of $Ca^{2+}$ ions
- ms/µs/ns pulses

✓ Single pulse of 1.5 kV/cm for 100 µs

✓ nanosecond pulsed electric fields (nsPEFs)
✓ 10 ns stimulation

Fertility and Sterility® Vol. 94, No. 3, August 2010

**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; [b] Gen Art Woman Health and Reproductive Biotechnology Center; 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* value |
|---|---|---|---|
| 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 2010.*

- risk of large pore opening leading to cell death !

PHYSICAL REVIEW APPLIED 11, 024001 (2019)

Editors' Suggestion

**Communicating with Mouse Oocytes via Regulating Calcium Oscillation Patterns by Nanosecond Pulsed Electric Fields**

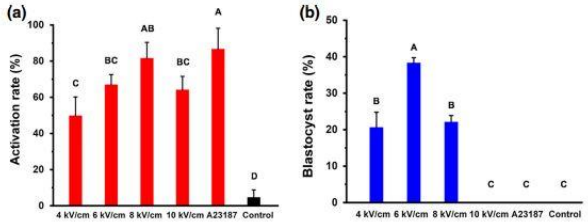
Jiahui Liu,[1] Qun Lu,[2] Rong Liang,[2] Jinsong Guo,[3] Kaile Wang,[1] Feihong Dong,[1] Jianliu Wang,[4,*] Jue Zhang,[1,3,†] and Jing Fang[1,3]

[1] *Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, People's Republic of China*
[2] *Center of Reproductive Medicine, Peking University People's Hospital, Beijing 100044, People's Republic of China*
[3] *College of Engineering, Peking University, Beijing 100871, People's Republic of China*
[4] *Department of Obstetrics and Gynecology, Peking University People's Hospital, Beijing 100044, People's Republic of China*

(Received 26 February 2018; revised manuscript received 7 November 2018; published 1 February 2019)

Oocyte activation deficiency is considered to be the principal factor underlying fertilization failure after intracytoplasmic sperm injection (ICSI), and consequently exhibits a lack of cytosolic calcium ($Ca^{2+}$) oscillations in oocytes. Traditional artificial activation methods are usually proposed to induce an intracellular $Ca^{2+}$ transient in oocytes. However, these current methods either cannot mimic the precise physiological processes of mammalian oocyte activation or there still exists controversy concerning their activation mechanisms. Here, we present a report on the potential of using nanosecond pulsed electric fields (nsPEFs) with a 10-ns duration not only to achieve a high activation rate of mouse oocytes, but also to significantly improve the developmental ability of parthenogenetic embryos. More importantly, in the exploration of activation mechanisms, we find that nsPEFs can regulate three different patterns of $Ca^{2+}$ oscillation via mobilizing both $Ca^{2+}$ influx from the outside and efflux from the endoplasmic reticulum (ER), and can even induce spontaneous $Ca^{2+}$ oscillations. Furthermore, a nsPEFs-mediated $Ca^{2+}$-channel kinetic model is introduced, and the simulation results agree well with our experiments. Our model predicts that nsPEFs could regulate $Ca^{2+}$ oscillations via the direct effects on the openness of $Ca^{2+}$ channels by simultaneously mobilizing extracellular and intracellular $Ca^{2+}$. Therefore, as an effective and controllable method, nsPEFs provide a valuable tool for potential artificial activation treatment in the future.

DOI: 10.1103/PhysRevApplied.11.024001




- stimulate Ca2+ eflux from ER and spontaneous Ca2+ oscillations while maintaining plasma membrane integrity

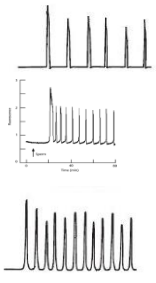
- high activation rate
- not tested in humans

# Clinical implications

## ❖Artificial oocyte activation (AOA)

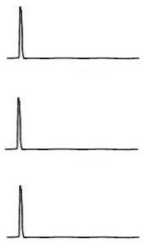
- Chemical activation

Multiple peaks



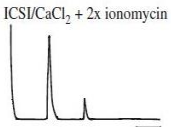
- ✓ Strontium chloride ($SrCl_2$)*
- ✓ Phorbol esters
- ✓ Thiomersal**

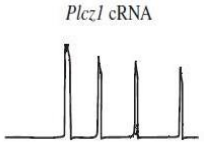
Single peak



- ✓ Ethanol
- ✓ Puromycin
- ✓ Calcium ionophore
  - ionomycin
  - calcimycin (A23187)

- Molecular targetting
  - injection of calcium (0.1 M CaCl2)
  - injection of sperm extract***
  - PLCζ cRNA/recombinant protein – experimental***



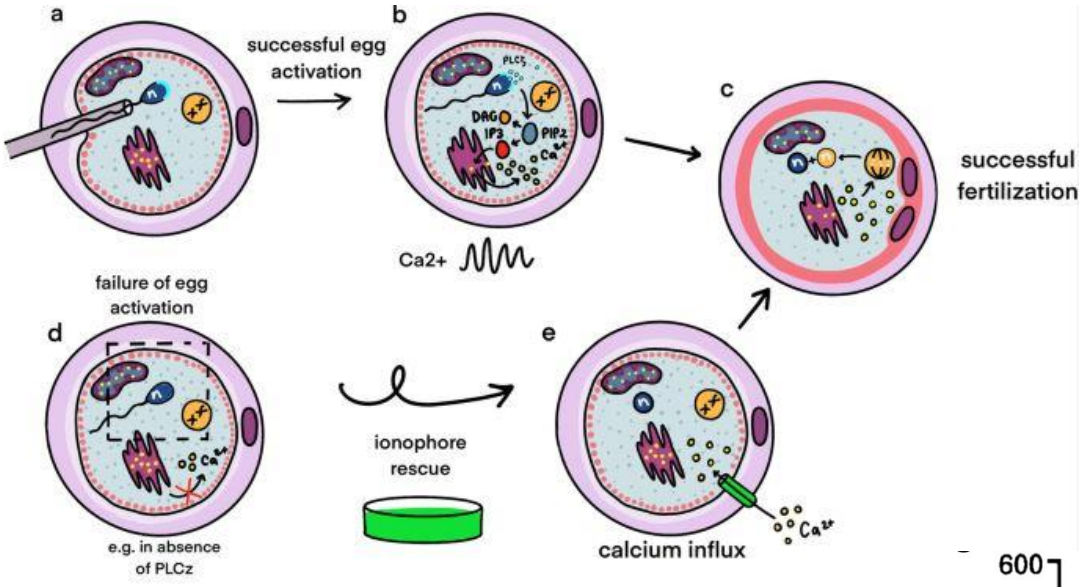

* Work only in rhodents!
** Impairs spindle
*** Regulatory issues

# Clinical implications

## ❖Artificial oocyte activation (AOA)

- **Calcium ionophores**

- highly selection ion carriers that form stable complex with Ca2+ and pass through the cell membrane
- causes transient release of Ca2+ from i.c. stores and enhance Ca2+ influx

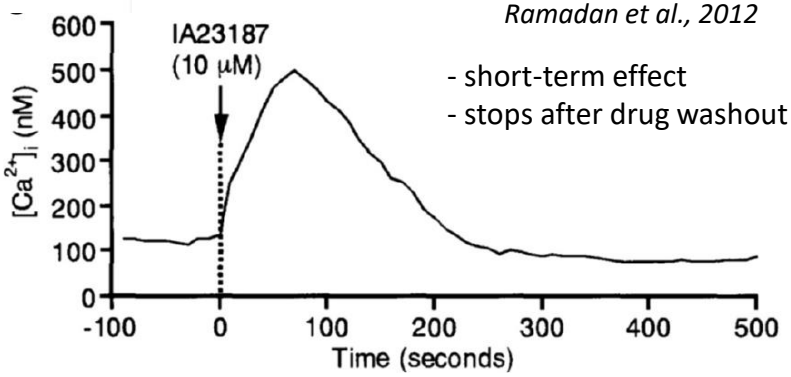



**Mammalian oocytes appear to be tolerant to pertubations in $Ca^{2+}$ oscillation pattern as long as the total amount of $Ca^{2+}$ release is uncompromised and passes a critical threshold**

*(Markoulaki et al., 2003, Johnson et al., 1998, Nikiforaki et al., 2014, Ebner and Montag 2016)*

**↑ i.c [Ca2+]**

different Ca2+ oscillation pattern than IVF/ICSI/PLCζ!




*Ramadan et al., 2012*

- short-term effect
- stops after drug washout

# Clinical implications

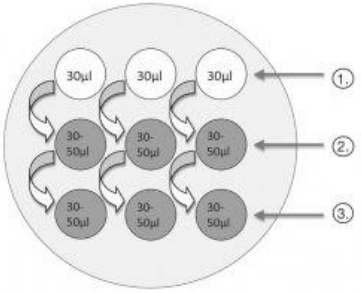
## ❖Artificial oocyte activation (AOA)

### - Calcium ionophore treatment

- occasional clinical use in specific cases

- nonstandartized

- supplementation of culture media with chemical compounds OR commertially available ready-to-use medium GM508 CultActive containing calcimycin




15 min


1. Aktivierung - GM508 CultActive - 30 µl
2. Waschschritt 1 - z.B. GM501 Cult - 30-50 µl
3. Waschschritt 2 - z.B. GM501 Cult - 30-50 µl

- ionomycin appears to be more efficient than calcimycin in both mouse and human eggs

*(Nikiforaki et al 2016)*

**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<br>Amorphous heads ($n$ = 18)<br>Tapered heads ($n$ = 23)<br>Bent necks ($n$ = 15) | <br>36.7<br>39.3<br>49.4 | <br>82.7<br>81.7<br>48.2 | <br>0.0008<br>0.005<br>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<br>14.3 (1–33)<br>47 (34–65)<br>85.8 (66–100) | 57.8<br>58.3<br>63.4<br>77.9 | S<br>S<br>S<br>NS |
| Borges et al. (2009a) | Single exposure to 5 µM calcimycin for 30 min, immediately following ICSI | ICSI with spermatozoa from:<br>TESE NOA ($n$ = 29)<br>TESE OA ($n$ = 24)<br>PESE OA ($n$ = 49) | <br>44.0<br>65.2<br>65.8 | <br>44.7<br>55.0<br>67.0 | <br>NS<br>NS<br>NS |
| Borges et al. (2009b) | Single exposure to 5 µM calcimycin for 30 min immediately following ICSI | ICSI with spermatozoa from:<br>Ejaculated ($n$ = 46)<br>Epididymal ($n$ = 41)<br>Testicular ($n$ = 70) | <br>76.2<br>66.6<br>56.1 | <br>69.4<br>48.9<br>50.6 | <br>NS<br>NS<br>NS |
| Montag et al. (2012) | Single exposure to 10 µmol/l calcimycin for 15 min immediately following ICSI | ICSI with previous:<br>Failed fertilization ($n$ = 27)<br>Low fertilization ($n$ = 38)<br>Very low fertilization ($n$ = 24) | <br>0<br>19.3 (0–29)<br>36.8 (30–50) | <br>41.6<br>44.4<br>56.1 | <br><0.05<br><0.001<br><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:<br>Failed fertilization ($n$ = 5)<br>Low fertilization ($n$ = 7) | <br>25.0<br>60.4 | <br>72.7<br>75.0 | <br><0.001<br>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 |

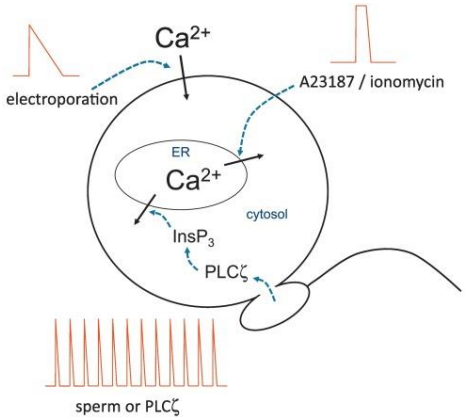
*Vanden Meerschaut et al 2014*

# Clinical implications

## ❖Artificial oocyte activation (AOA)

- **Indications**
  - ✓ Complete fertilization failure in previous cycle (Ebner & Montag, 2012)
  - ✓ Less than 30% fertilization rate in previous cycle (Montag et al., 2012)
  - ✓ Severe male factor (TESE, PESA, globozospermia)
  - ✓ Developmental problems in previous cycle (Ebner et al., 2015)
    - ✓ Low blastulation rate (<15%)
    - ✓ Developmental arrest
    - ✓ Developmental delay (-24h)
    - ✓ Arrest at 2Pn stage (Darwish & Magdi, 2015)

- **Contraindication**
  - ✓ SER+ eggs ?
  - ✓ Immature eggs?




- not beneficial for all patients!
- experimental, considered as an „adds-on treatment“ by ESHRE

# Clinical implications

## ❖Artificial oocyte activation (AOA)

- **Safety**

  - no increase in meiosis II segragation errors
  - healthy children born from AOA cycles

  - appears to be safe BUT lack of data

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

**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]

**Table 2** Treatment outcome in the study group with a ready-to-use ionophore (rate in per cent).

| | |
|---|---|
| Started cycle | 101 |
| Cycles with at least one fertilization | 100 |
| Pregnancy | 37 (37) |
| Ectopic pregnancy | 2 |
| Clinical pregnancy | 35 (35) |
| Multiple pregnancy | 10/35 (28.6) |
| Vanishing twins | 3 |
| Spontaneous abortion | 7 (20.0) |
| Implantation rate | 47/185 (25.4) |
| Live birth per started cycle | 28 (27.7) |
| Children born from singleton pregnancy | 18 |
| Children born from twin pregnancy | 17 |
| Malformation | 1 (2.9) |

anal atresia

*Ebner et al., RBM 2015*

✓ Language skills
✓ Motor skills
✓ Cognition

were found to be within expected ranges

Reproductive BioMedicine Online (2014) 28, 54–63

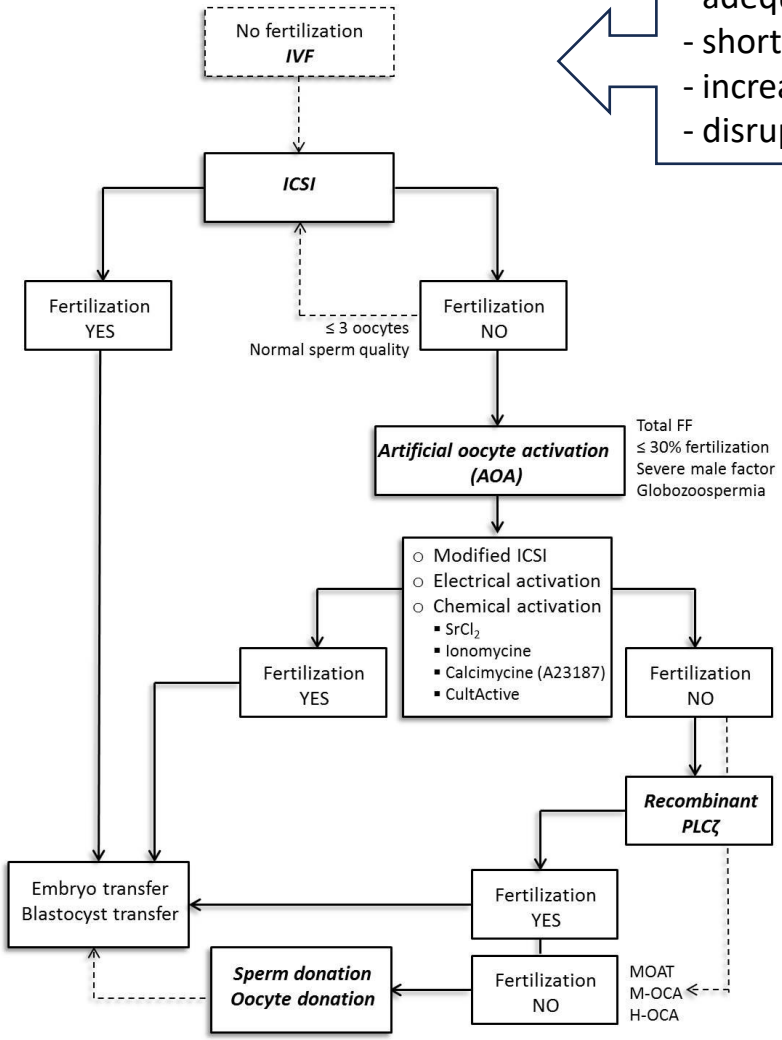
**Neonatal and neurodevelopmental outcome of children aged 3–10 years born following assisted oocyte activation**

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]

# Clinical implications

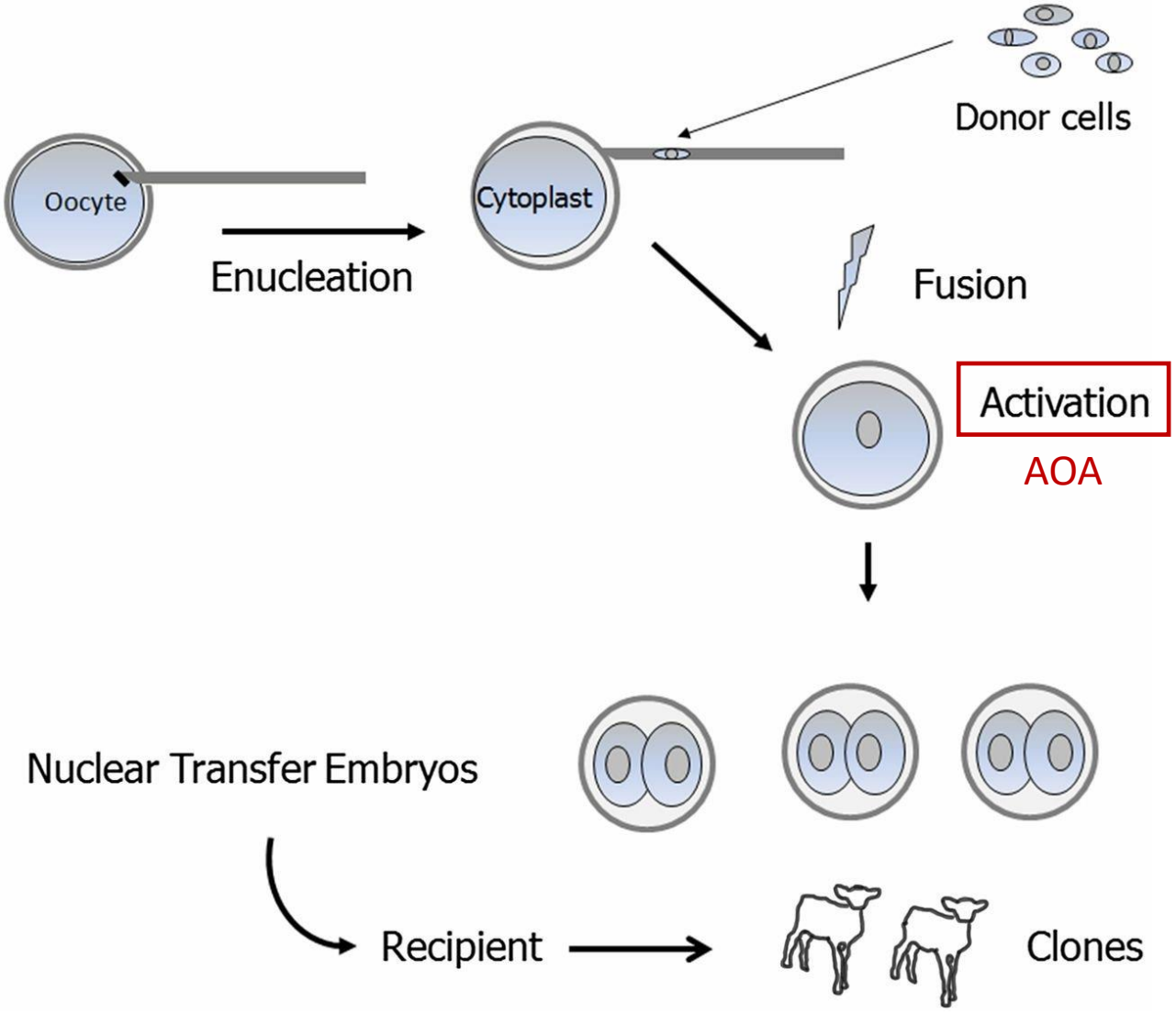
## ❖Management of fertilization failure

- adequate patient selection
- short gamete co-incubation (1h)
- increase sperm number
- disrupt COC




Courtesy of T. Ebner

# Egg activation and SCNT

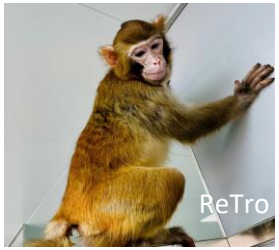



1962

1996


2018


2024



# Parthenogenesis

- asexual reproduction
- „virgin birth“

- an egg can develop without being fertilized by a sperm

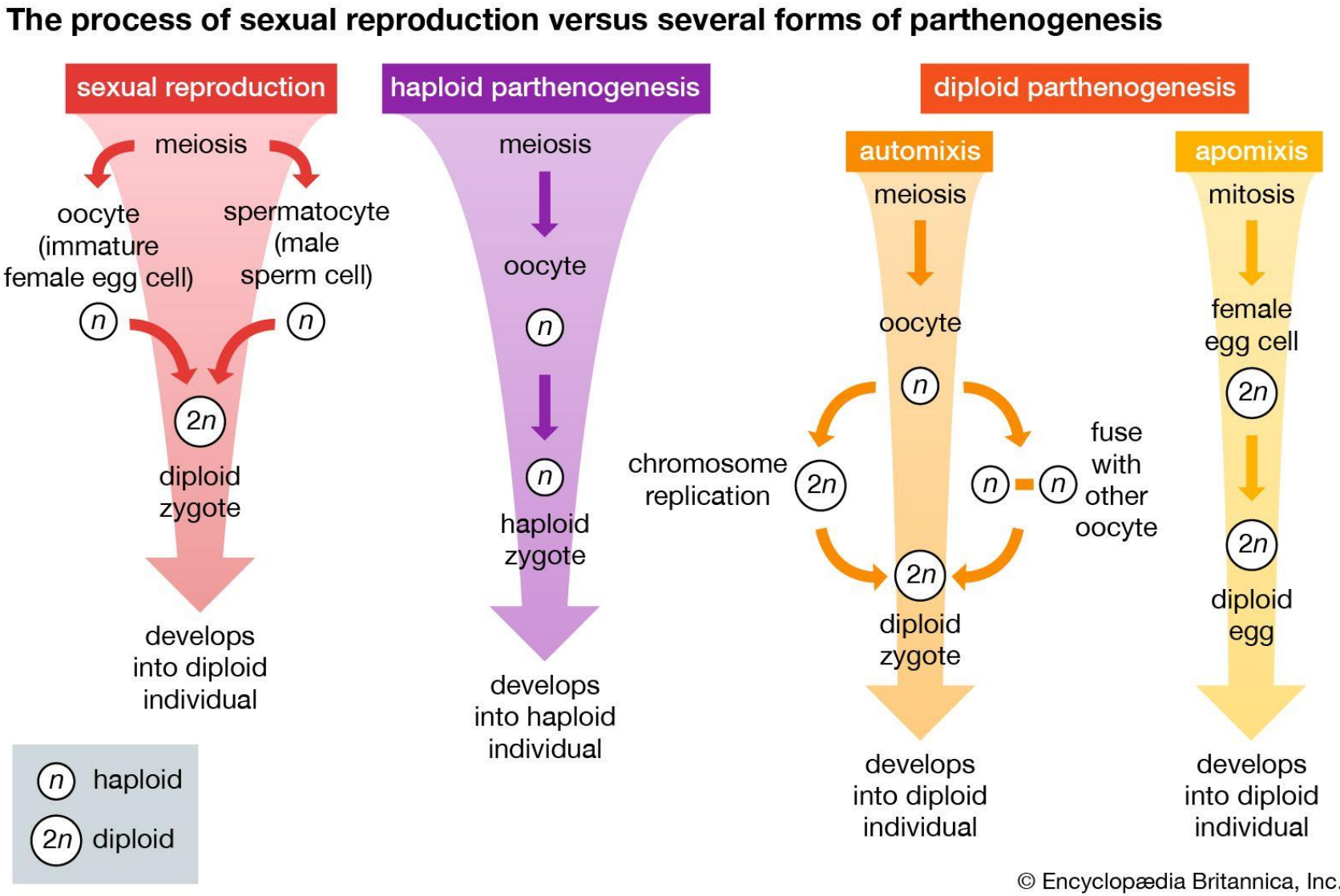
- occasional reproduction manner in lower species

- in mammals, parthenogensis can be induced by AOA

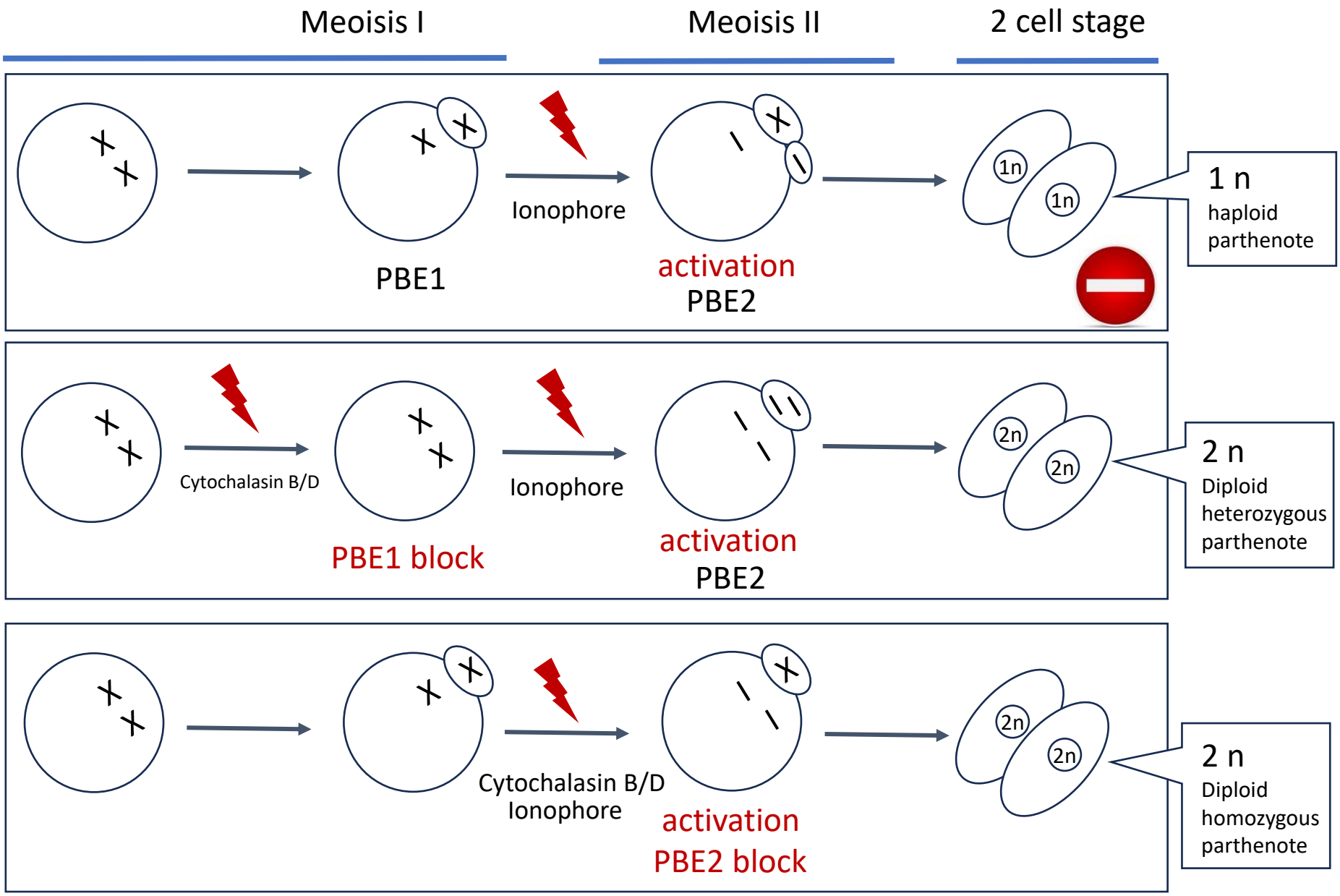
- parthenotes can develop to different stages but not to term

- all female offsprings (XY determination) OR all male offsprings (ZW determination)

# Parthenogenesis



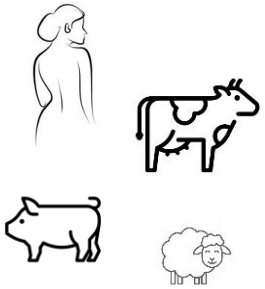
# Induction of parthenogenesis in vitro

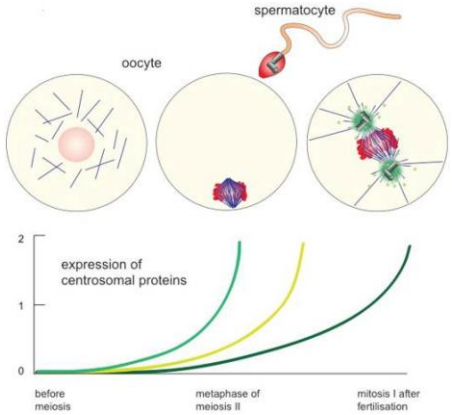


# Parthenotes vs. embryos

- uniparental origin

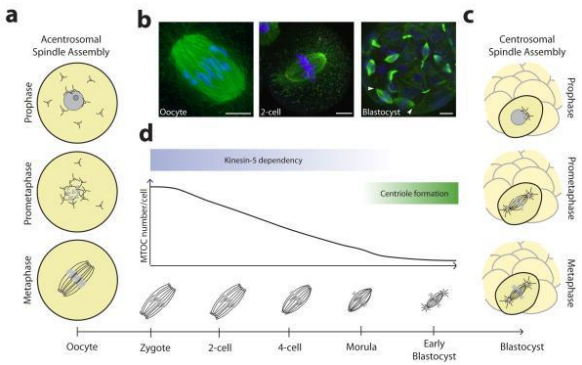
## unphysiological reconstruction of centrosome



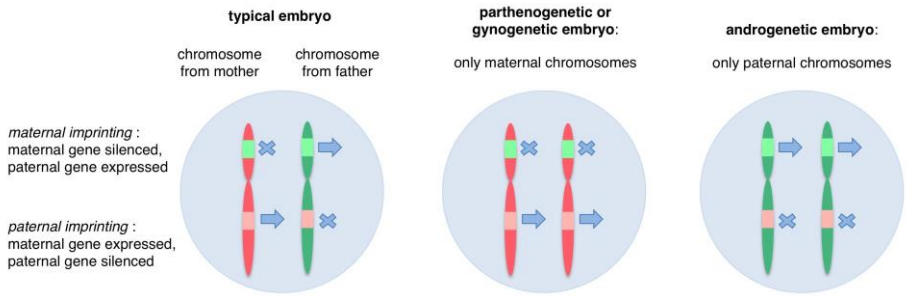


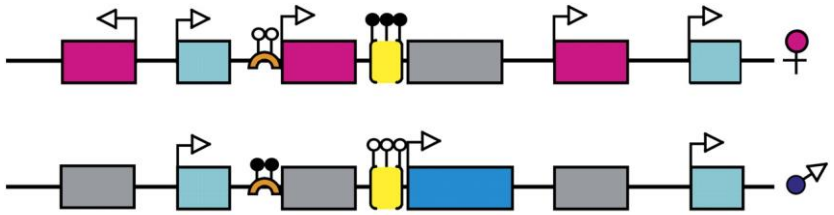

- genetic instability and aneuploidy in pathenotes

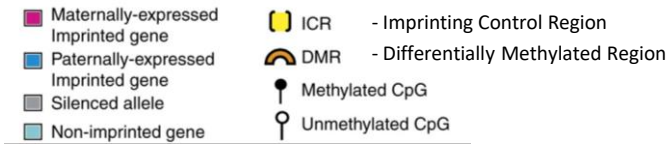
**Rodents less sensitive to the lack of parental centriole!**



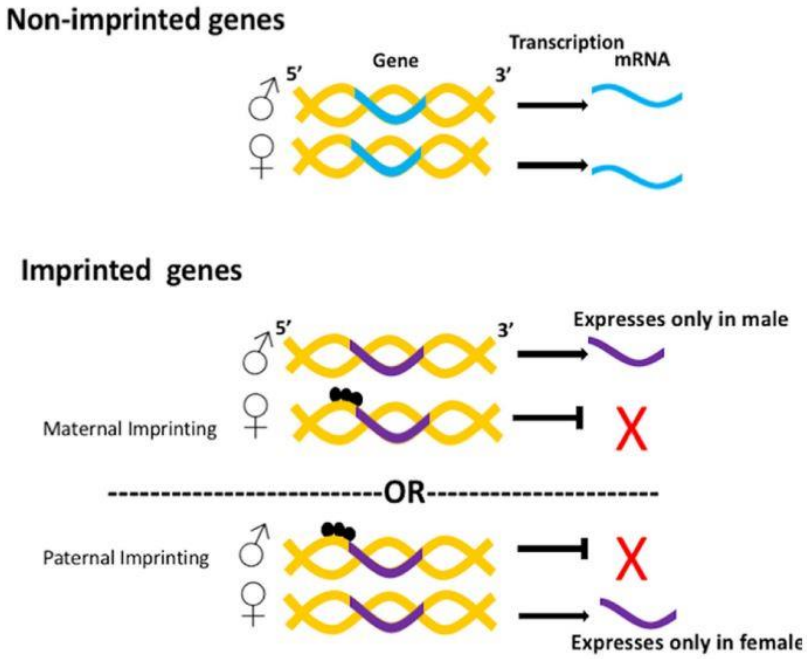

## Imprinting perturbations



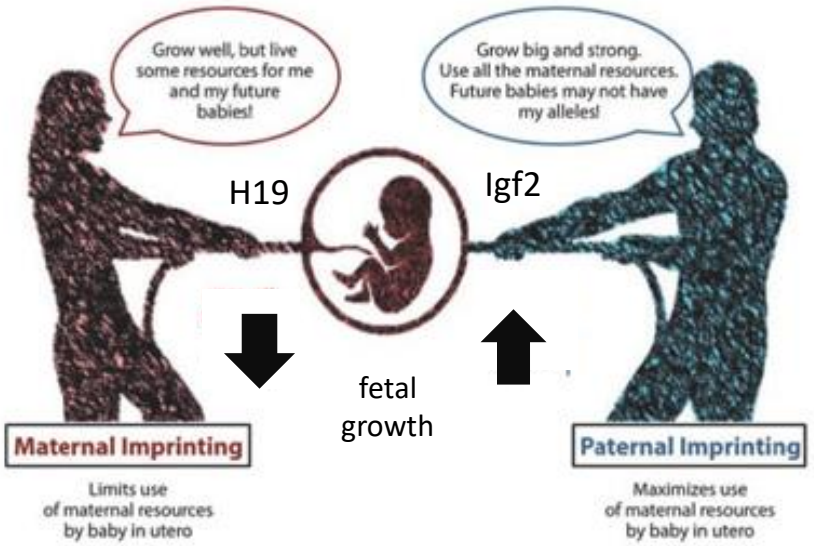





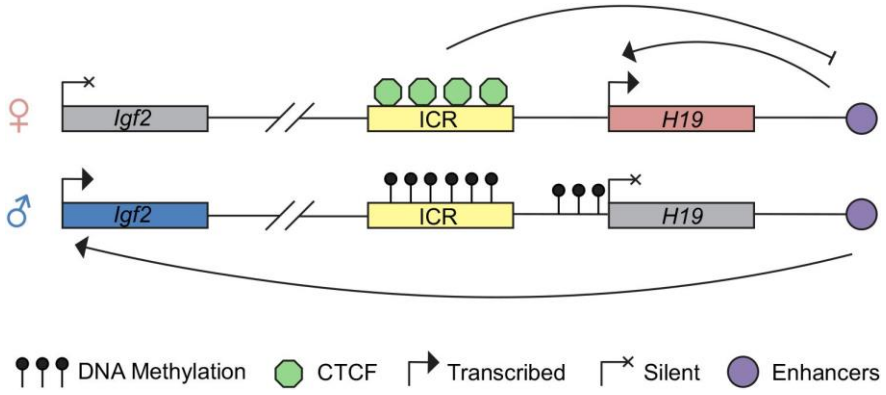
# Parental imprinting in mammals




**„parental conflict“**




imprinting-mediated balance between paternal and maternal genomes is critical for mammalian development



# Parthenogenesis in mammals

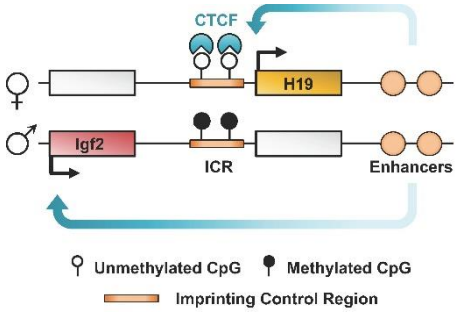
*Kono et al 2004*

nature

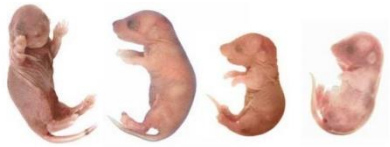
## Birth of parthenogenetic mice that can develop to adulthood

**Tomohiro Kono[1,3], Yayoi Obata[1,3], Quiong Wu[1,3], Katsutoshi Niwa[1,3], Yukiko Ono[1], Yuji Yamamoto[2,3], Eun Sung Park[4], Jeong-Sun Seo[4,5] & Hidehiko Ogawa[1,3]**

*[1]Department of BioScience, and [2]Department of Applied Science, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156-8502, Japan*
*[3]Bio-oriented Technology Research Advancement Institution (BRAIN), Minato-ku, Tokyo 105-0001, Japan*
*[4]MacroGen Inc, Chongno-Ku, Seoul 110-061, Korea*
*[5]Department of Biochemistry, Seoul National University College of Medicine, Chongno-Ku, Seoul 110-799, Korea*







- AOA of oocyte containing two sets of maternal genome reconstructed by series of nuclear transfers
- one allele derived from mouse with deleted H19 gene (loss of maternal imprints)
- the mutated parthenote developed to adulthood with the ability to reproduce

Table 1 **Development of reconstructed parthenogenetic embryos**

| Developmental progress | Number |
|---|---|
| Number of reconstructed eggs | 457 |
| Number of embryos developed to blastocysts | 417 (91.2% of reconstructed eggs) |
| Number of embryos transferred | 371 (89.0% of blastocysts) |
| Number pregnants/recipients | 24/26 |
| Number of implantation to recipients | 246 (71.7% of embryos transferred to pregnants) |
| Number of pups | 28 (8.2% of embryos transferred to pregnants) |
| Dead | 18 (5.2% of embryos transferred to pregnants) |
| Live | 8 (2.3% of embryos transferred to pregnants) |
| Survived | 2 (0.6% of embryos transferred to pregnants) |

**genomic imprinting is a barrier for parthenogenesis in mammals**

# Parthenogenesis in mammals

## Viable offspring derived from single unfertilized mammalian oocytes

Yanchang Wei[a,b,1], Cai-Rong Yang[a,b,c], and Zhen-Ao Zhao[a,b,d]

[a]Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200135, China; [b]Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai 200135, China; [c]Center for Reproductive Sciences, University of California, San Francisco, CA 94143; and [d]State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

PNAS

Zhen-Ao Zhao

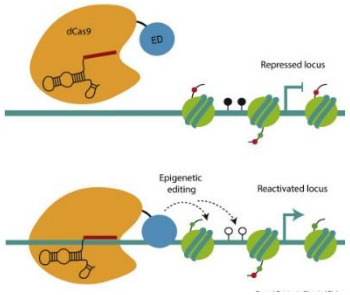
- **Targeted epigenetic editing of imprinting control regions**

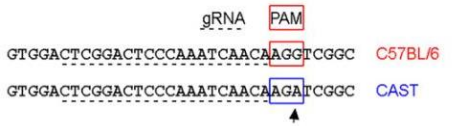
- oocyte injection with single-guide RNAs with protospacer adjacent motif (PAM) sequences matching one allele but not the other

- DNA (de)methylation modification by catalytically inactive 9 (dCas9)-Dnmt3a or dCpf1-Tet1

- transfer of mouse genome-edited parthenogenetic blastocysts to foster mothers

- significantly extended development, and viable full-term offspring



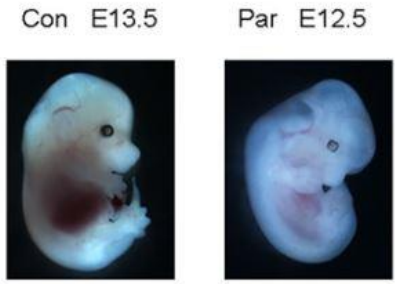



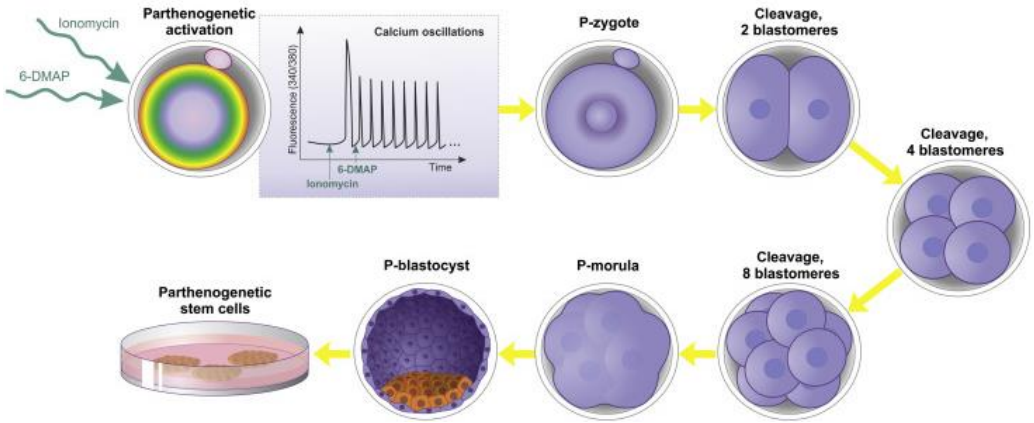

**Table 1. Development of modified parthenogenetic embryos[2+5]**

| Developmental progress | No. |
|---|---|
| No. of reconstructed oocytes | 227 |
| No. of oocytes developed to blastocyst | 192 (84.6% of reconstructed oocytes) |
| No. of embryos transferred | 192 (100% of blastocysts) |
| No. of pregnants/recipients | 14/14 |
| No. of live pups | 3 (1.6% of embryos transferred to recipients) |
| No. of survived pups | 1 (0.5% of embryos transferred to recipients) |

- low efficiency due to insufficient methylation or loss of imprinting

*Wei et al. 2022*

# Parthenogenetic stem cells (PESCs)




1n PESCs

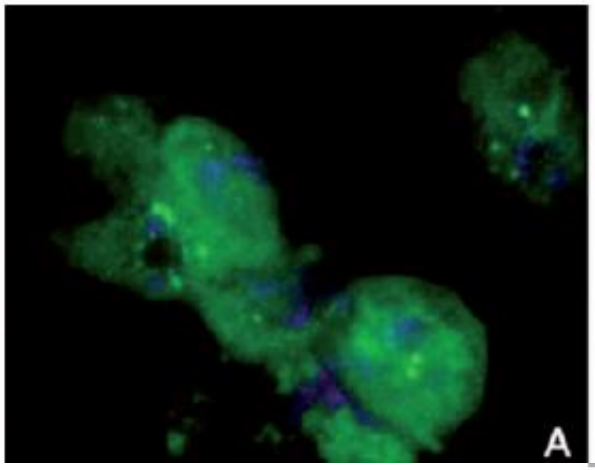
- spontaneous diploidisation

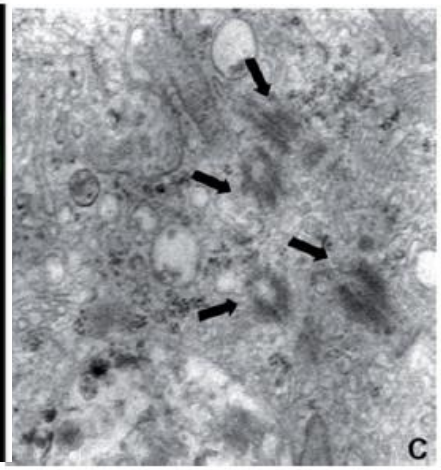
2n PESCs

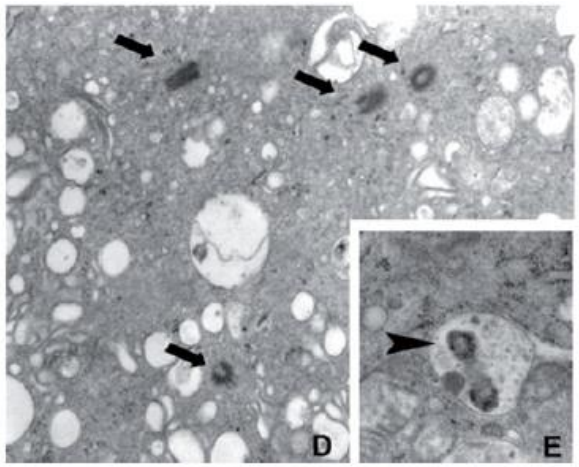
- trophoblast underdeveloped
- little extraembryonic tissue

- abnormal genetic imprinting lost during culture
- study of imprinting and inheritance mechanisms

hPESCs




*Brevini et al 2009*

➡ Supernumerary centrioles ➤ Autophagy vacuoles

# Parthenogenotes and partheno-embryo chimeras



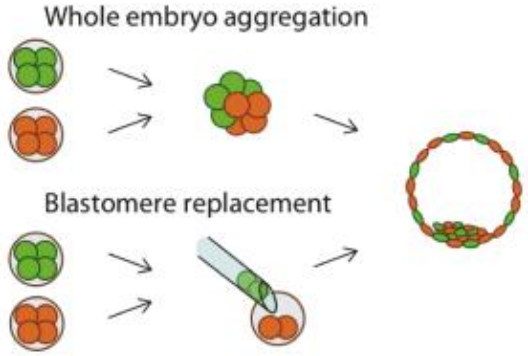

## ❖ Parthenotes

- **developmental arrest**
- physiologically unable to develop to term
- reproductively and in vitro aged eggs prone to spontaneous activation and short-term partnogenetic development in ART !

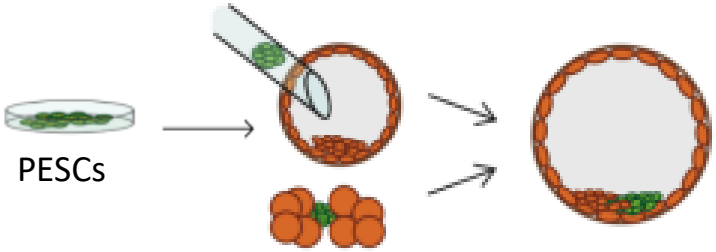
## ❖ Partheno-embryo chimeras

- Generated by

(a) combination of embryo and parthenote blastomeres




(b) injection of PESCs to morula/ blastocyst



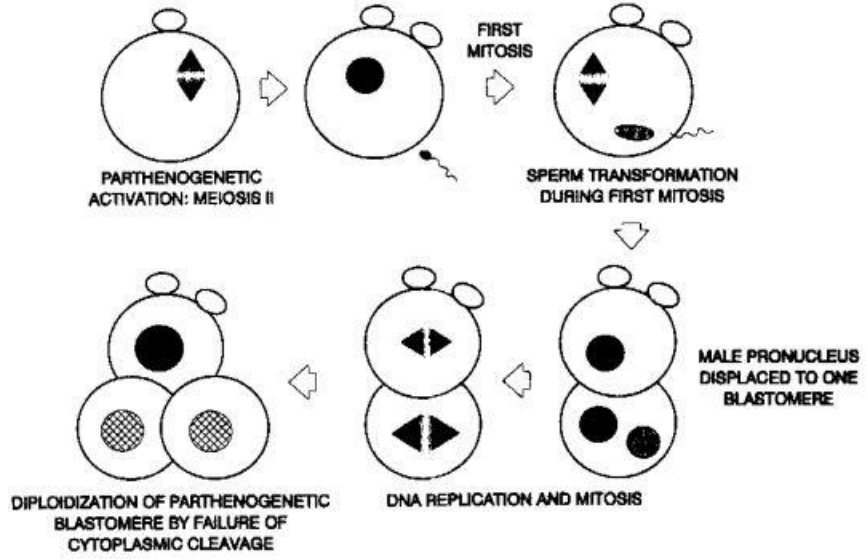

- can develop to term but delayed development
- parthenote cells fail to contribute to tissues of mesoderm and endodermal origin

# Parthenogenesis in humans

- rare event but not impossible

- phenotypic male, blood cells XX karyotype
- 46,XY/46,XX mosaic
- complete maternal isodisomy in blood cells and fibroblasts

- Sex reversal, facial asymmetry, mild festures
- Proposed speculative mechanism: diploidisation and spontaneous activation before syngamy






*article*

## A human parthenogenetic chimaera

Lisa Strain, Jon P. Warner, Thomas Johnston & David T. Bonthron

In mice, parthenogenetic embryos die at the early postimplantation stage as a result of developmental requirements for paternally imprinted genes, particularly for formation of extraembryonic tissues. Chimaeric parthenogenetic↔normal mice are viable, however, due to non-random differences in distribution of their two cell types. Species differences in imprinting patterns in embryo and extra-embryonic tissues mean that there are uncertainties in extrapolating these experimental studies to humans. Here, however, we demonstrate that parthenogenetic chimaerism can indeed result in viable human offspring, and suggest possible mechanisms of origin for this presumably rare event.

*University of Edinburgh, Human Genetics Unit, Department of Medicine, Western General Hospital, Edinburgh EH4 2XU, UK*

*Correspondence should be addressed to D.T.B.*

Normal mammalian development requires functionally distinct genetic contributions from male and female gametes. Though recent studies of differential gene expression from paternal and maternal genomes provide molecular evidence for the phenomenon of genomic imprinting, its existence was originally inferred from embryological studies of the abnormal fate of spontaneously or experimentally induced parthenogenetic or androgenetic embryos, particularly in the mouse. Some mouse strains show high rates of spontaneous parthenogenetic oocyte activation, and in humans, such naturally occurring parthenogenetic development is also well documented, through the study of benign ovarian teratomas. In at least some cases, these originate by development of gametogenic cells which have completed the first meiotic division[1]. However, parthenogenetic or gynogenetic development has not previously been reported in viable human pregnancies. The inability of parthenogenetic mouse embryos to develop beyond the early postimplantation stage results in large part from the need for a paternally imprinted genome for correct formation of extraembryonic tissues[2,3]. In chimaeric normal↔parthenogenetic mice, however, parthenogenetic cells can contribute to many mature tissues, and can even be transmitted through the germline[4-7]. In these chimaeras, there is often selection against the parthenogenetic cells during embryogenesis. This is a non-random phenomenon which occurs particularly in certain tissues (for example virtually eliminating parthenogenetic cells from skeletal muscle), and may reflect the need for tissue-specific imprinted differentiation genes[8-11]. These mouse studies raise the

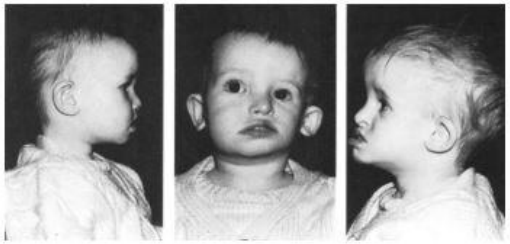


Fig. 1 FD aged 1.2 years. The facial profile from the right is essentially normal, with all the visible abnormalities confined to the left.



*Strain et al 1995*

# Parthenogenesis in humans

- **Implicated in infertility in recurrent miscarriage and pregnancy failure ?**
  - reports of ART cases in which cytogenetic analyses of conceptus biopsy samples found match to maternal side exclusively

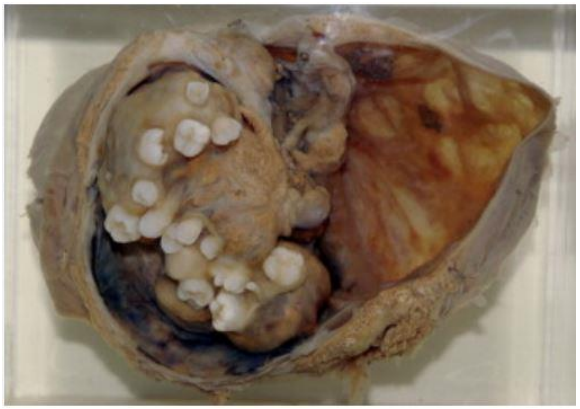
**Table I.** Reported cases with PG in the previous literature

| Authors, Yr (Ref) | Country | Age (yr) | Gynecologic/Obstetric history | Outcome |
|---|---|---|---|---|
| Oliveira *et al.*, 2004 (6) | Brazil | 29 | Left oophorectomy due to ovarian teratoma | Pregnancy |
| Combelles *et al.*, 2011 (13) | USA | 32 | Recurrent miscarriages | No pregnancy |
| Socolov *et al.*, 2015 (14) | African ethnicity | 38 | Salpingectomy | No pregnancy |
| Ye *et al.*, 2020 (15) | China | 38 | Primary infertility with bilateral tubal obstruction | No pregnancy |
| Jiang *et al.*, 2022 (12) | China | 33 | Ectopic pregnancy; salpingectomy | Pregnancy |

*Hegazy et al 2023*

- **Role in occurrence of idiopathic ovarian teratoma ?**

- Slow-growing benign germ cell tumour but can be coverted to malignant
- Composed of derivatives of all 3 germ layers
- Perinatal teratomas believed to originate from mislocalized PGCs but cystic ovarian teratoma mostly found in women of reproductive age

# Same sex parent offsprings?

Cell Stem Cell

CellPress
OPEN ACCESS

Article

## Adult bi-paternal offspring generated through direct modification of imprinted genes in mammals

Zhi-kun Li,[1,3,4,8,*] Li-bin Wang,[1,3,4,8] Le-yun Wang,[1,3,4,8] Xue-han Sun,[1,2,8] Ze-hui Ren,[5,8] Si-nan Ma,[1,6,8] Yu-long Zhao,[1,2,8] Chao Liu,[1,3,4] Gui-hai Feng,[1,3,4] Tao Liu,[7] Tian-shi Pan,[1,6] Qing-tong Shan,[1,6] Kai Xu,[1,3,4] Guan-zheng Luo,[5,*] Qi Zhou,[1,2,3,4,*] and Wei Li[1,2,3,4,9,*]

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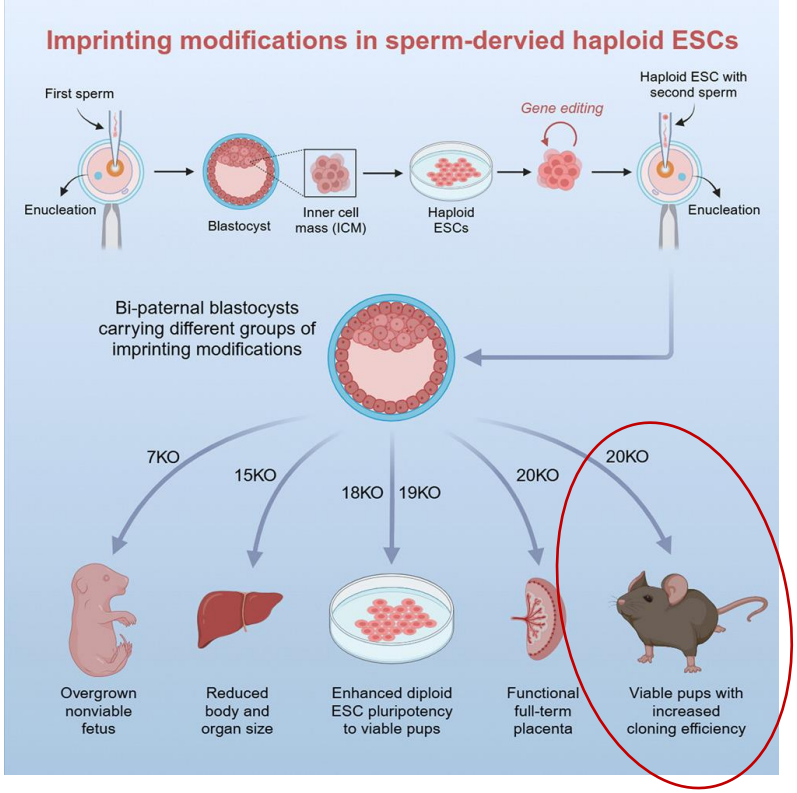
https://doi.org/10.1016/j.stem.2025.01.005



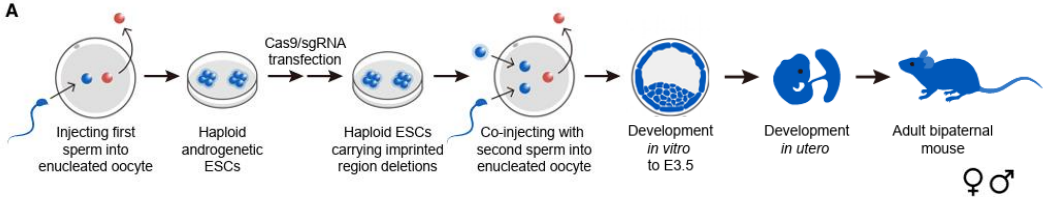

## Rewriting reproduction: With stem cells and CRISPR, scientists breed mice with same-sex parents

### World First As Mouse With 2 Dads Survives To Adulthood

*Imprinting genes have long been a stumbling block for unisexual reproduction in mammals, but scientists have now found a way past it.*


## Mouse With Two Male Parents Survives to Adulthood

Adult 18KO bipaternal (left) and WT mice




- deletion of 20 imprinted regions + haploid hESC generation +SCNT