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پژوهشگاه رویان



پژوهشگاه رویان  
مرکز تحقیقات پزشکی  
تولیدمثل

پژوهشکده علوم تولیدمثل

نماد ایمان، دانش و بالندگی



سه شنبه ۱۵ اردیبهشت ۱۴۰۵

فاکتورهای اسپرمی فعال سازی تخمک :  
نقش  $PLC\zeta$ ، PAWP، ACTL7A در ART

**Sperm-Borne Oocyte Activation Factors: The role of  
 $PLC\zeta$ , PAWP, and ACTL7A in ART Role**

# Global infertility prevalence estimates

2022 global infertility prevalence estimates are:

Approximately **one in six** people have experienced infertility at some stage in their lives, globally.



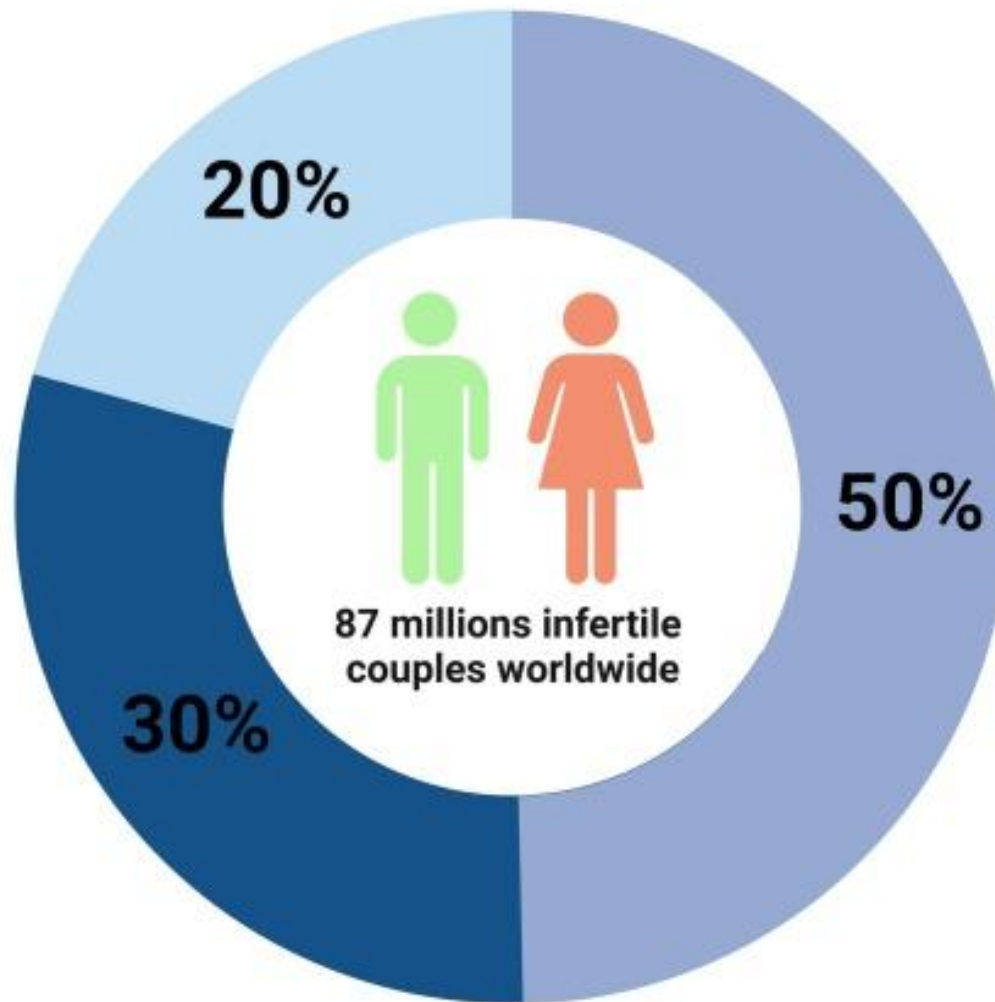
- Female factor
- Male factor
- Combined factor

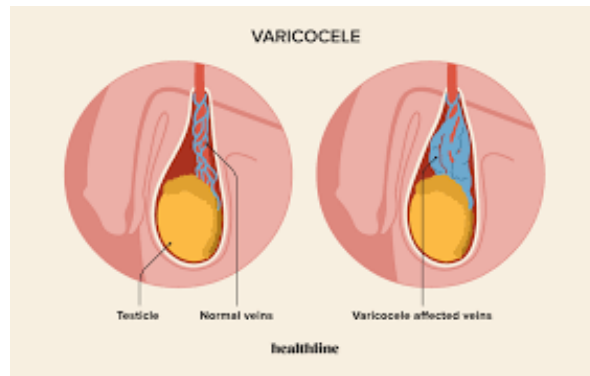
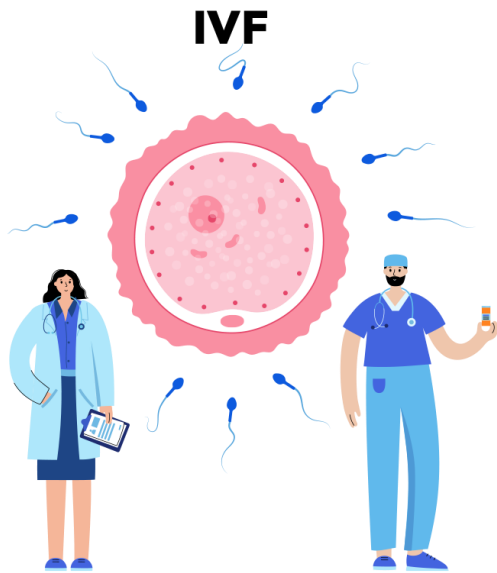
Female infertility causes

- Low AMH
- Polychistic ovaries syndrome
- Endometriosis
- Cervical cancer
- Salpingitis
- Infections

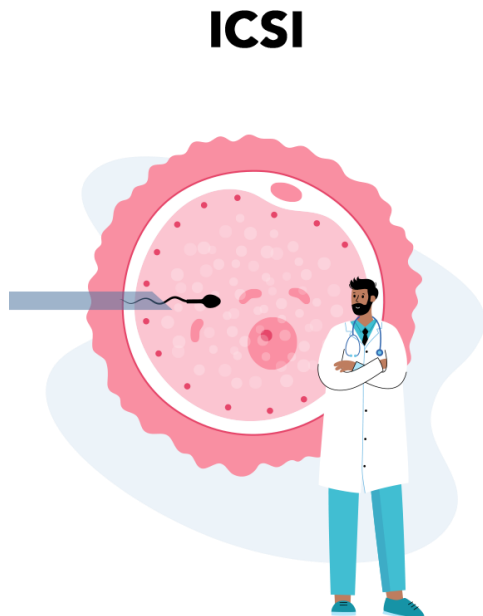
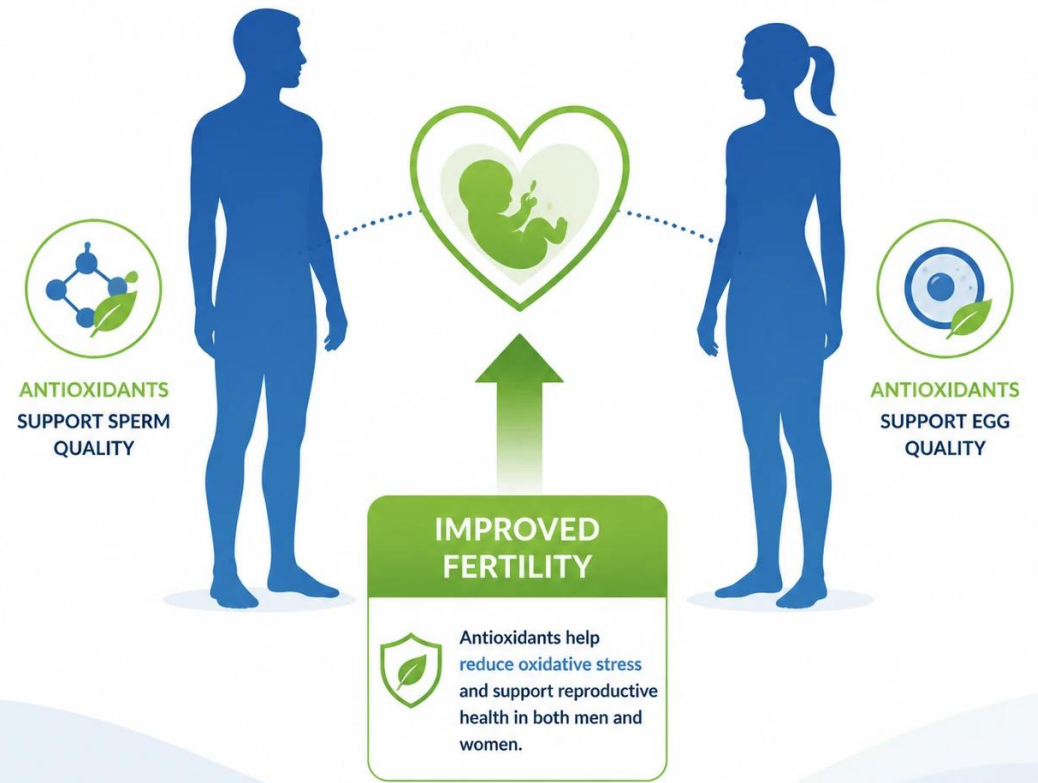
Male infertility causes

- Hormonal dysfunction
- Cryptochidism
- Sertoli-cell-only syndrome
- Varicocele
- Antisperm antibodies
- Klinefelter syndrome



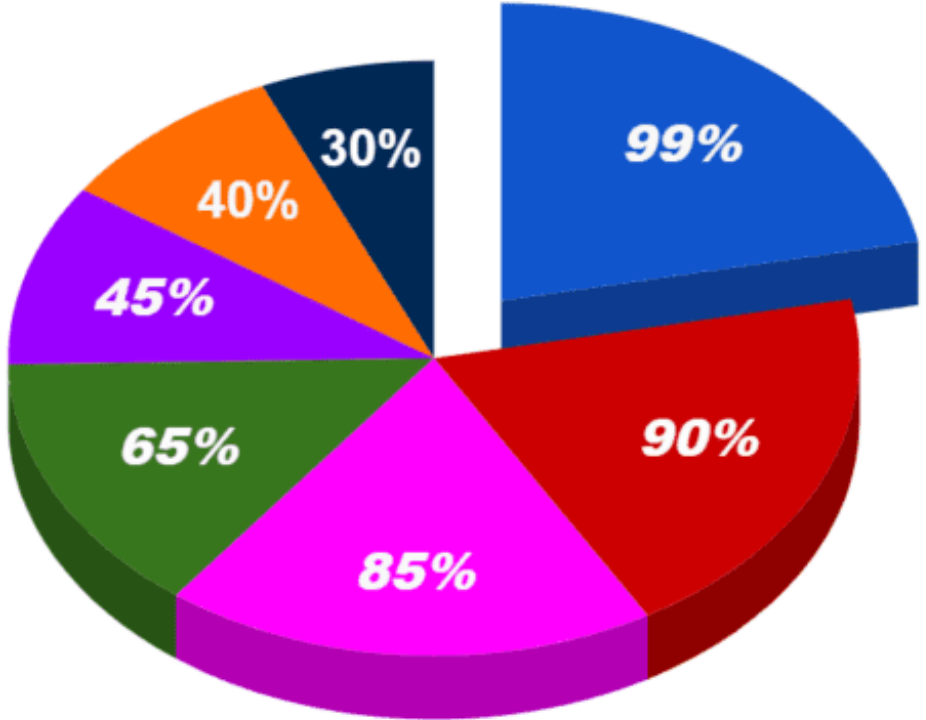


## ANTIOXIDANT THERAPY for BETTER FERTILITY



# PERCENTAGE vs. IVF SUCCESS RATE

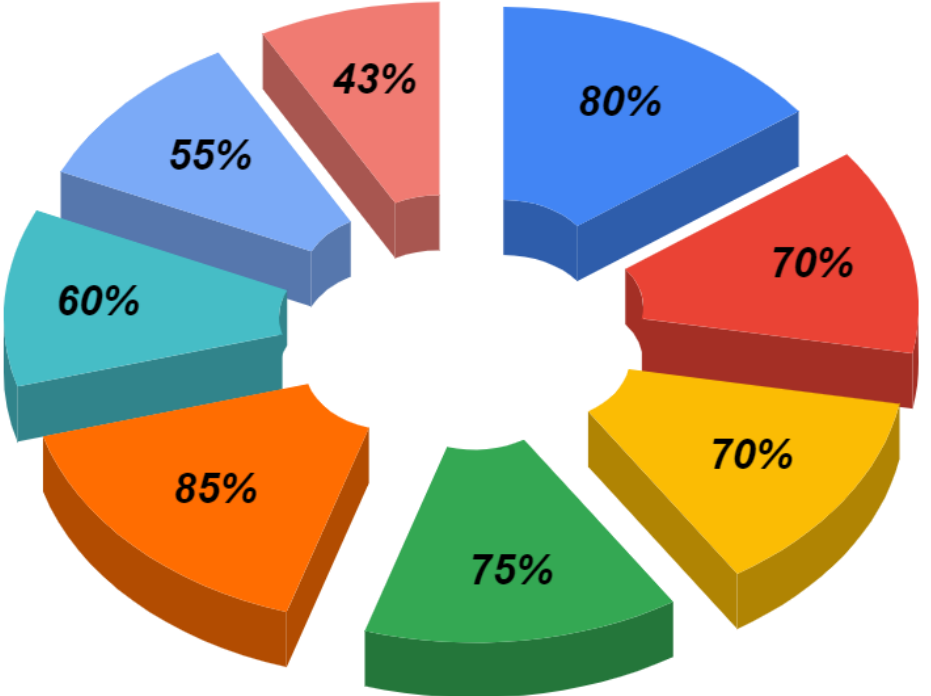
## At Fertility World



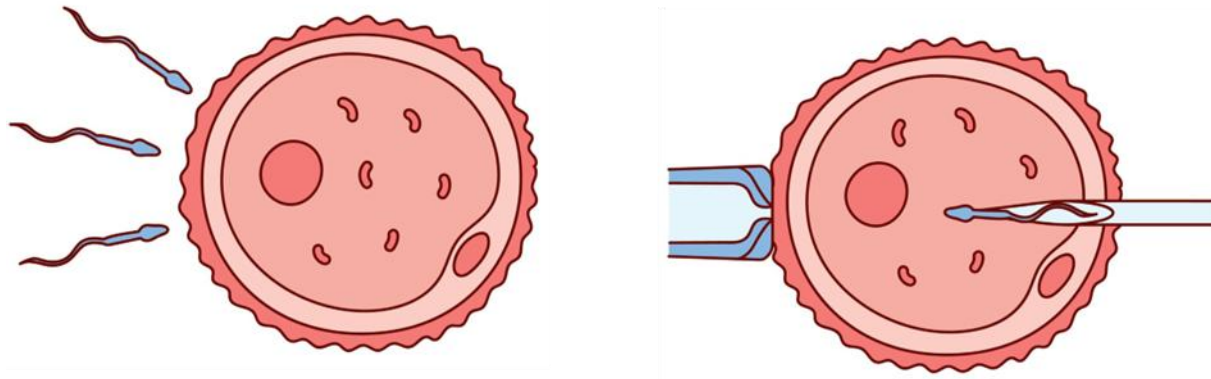
### IVF success rate Age

Fertilityworld IVF clinic

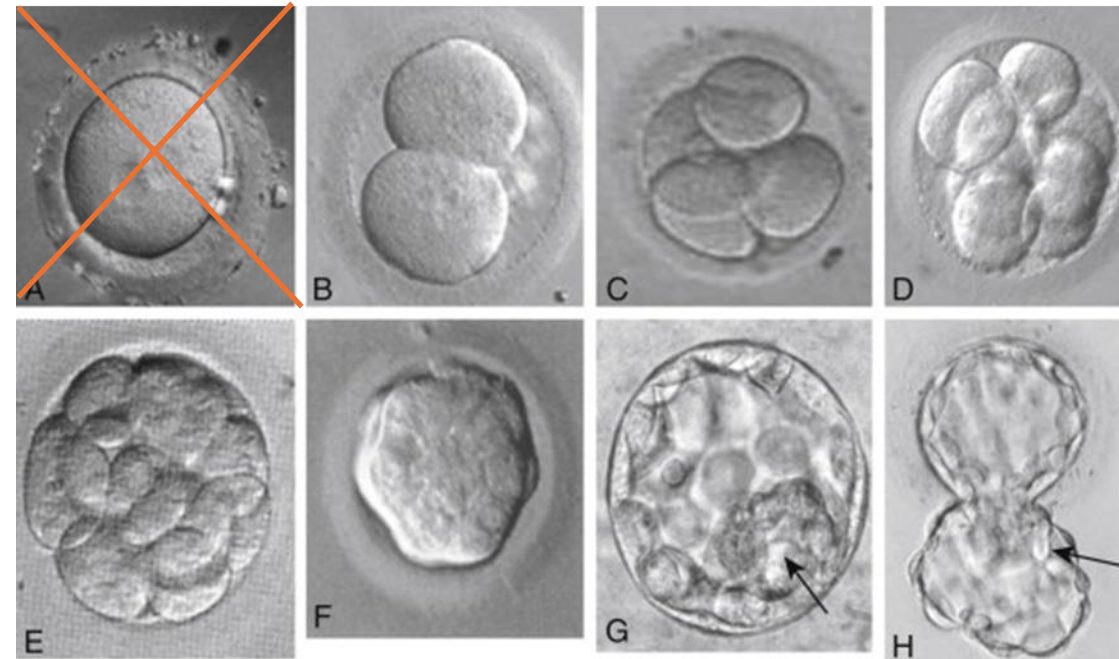
- Women between 21-25 Years of Age is 99%
- Women between 26-30 Years of Age is 90%
- Women between 31-35 Years of Age is 85%
- Women between 36-40 Years of Age is 65%
- Women between 41-45 Years of Age is 45%
- Women between 46-50 Years of Age is 40%
- Women between 50 years and above is 30%



- IVF With Donor Eggs
- IVF With Self Eggs
- IVF With Donor Sperms
- IVF With Self Sperms
- IVF With ICSI
- IVF With FET
- IVF with PESA
- IVF with PGD



- Conventional IVF: about 5–10% of cycles experience total fertilization failure (TFF).
- ICSI (intracytoplasmic sperm injection): about 1–3% TFF.



# Intracytoplasmic sperm injection (ICSI) for non-male factor indications: a committee opinion

Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology

American Society for Reproductive Medicine and Society for Assisted Reproductive Technology, Birmingham, Alabama

## ASRM-2020:

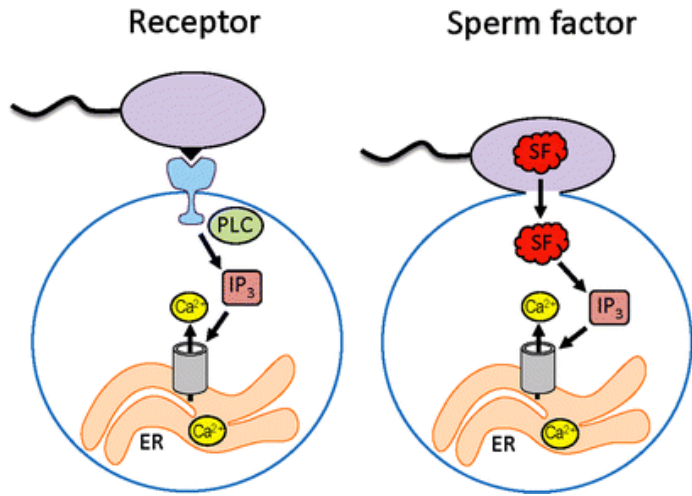
**Two** studies in patients with unexplained infertility compared IVF with ICSI using sibling oocytes.

**The fertilization rates** after ICSI, were higher than IVF group:

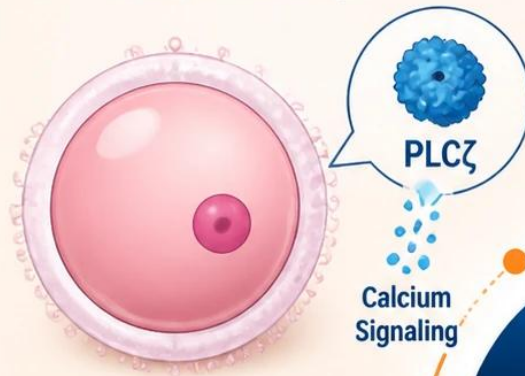
65.3% versus 48.1% ( $P < .001$ ) and 61.0% versus 51.6% ( $P < .001$ ).

**The fertilization failure** occurred more commonly in the IVF groups than in the ICSI groups ( $P < 0.05$ ).

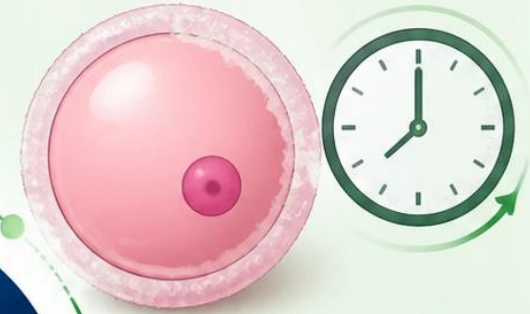
However, these studies used sibling oocytes, and the embryos transferred were a mixture from the IVF and ICSI groups, so no information about the effect of IVF or ICSI on clinical outcomes such as implantation, pregnancy, or livebirth rates could be ascertained.



**1 OOCYTE ACTIVATION DEFECT**  
(PLCζ deficiency)

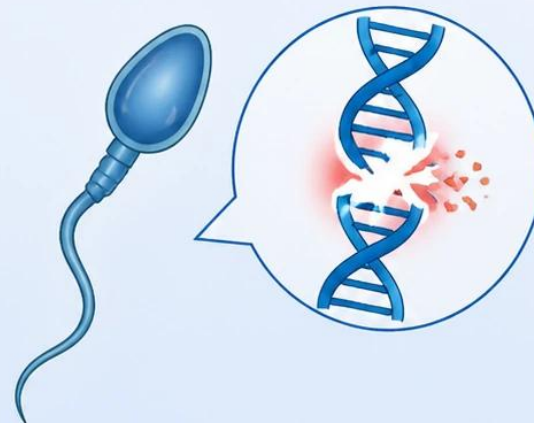


**2 POOR OOCYTE QUALITY**  
(Advanced maternal age)

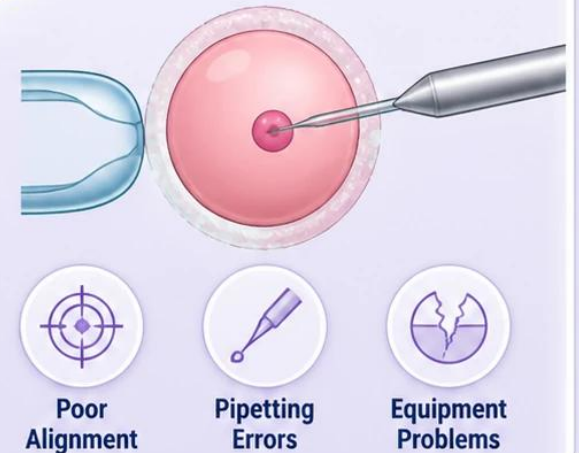


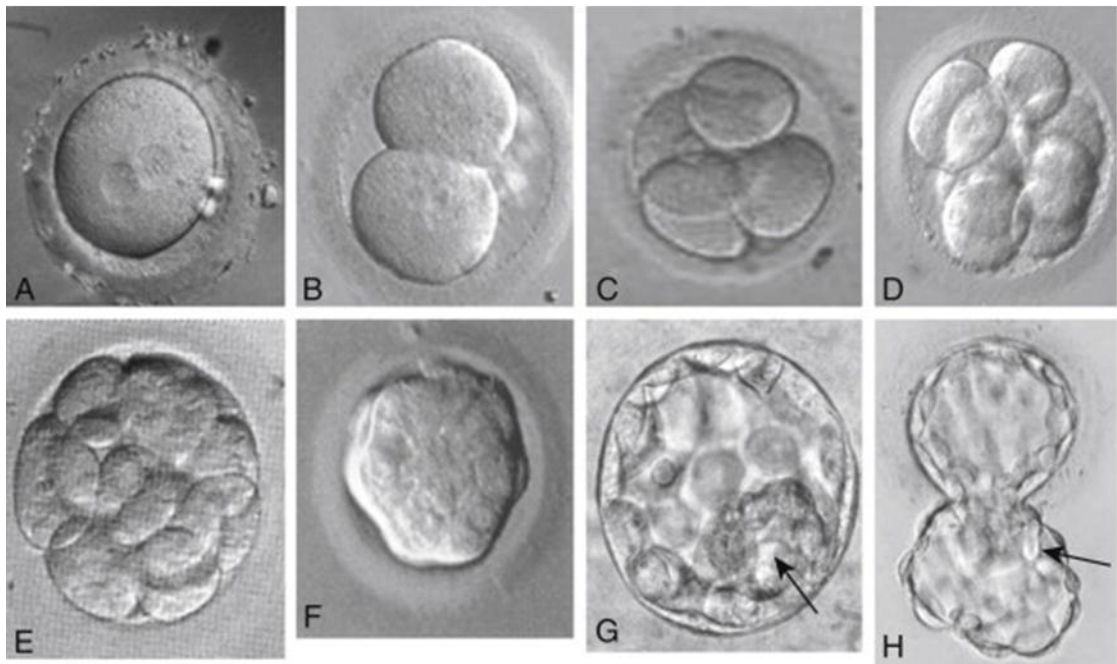
**TOTAL FERTILIZATION FAILURE (TFF)**

**3 SEVERE SPERM DNA DAMAGE**

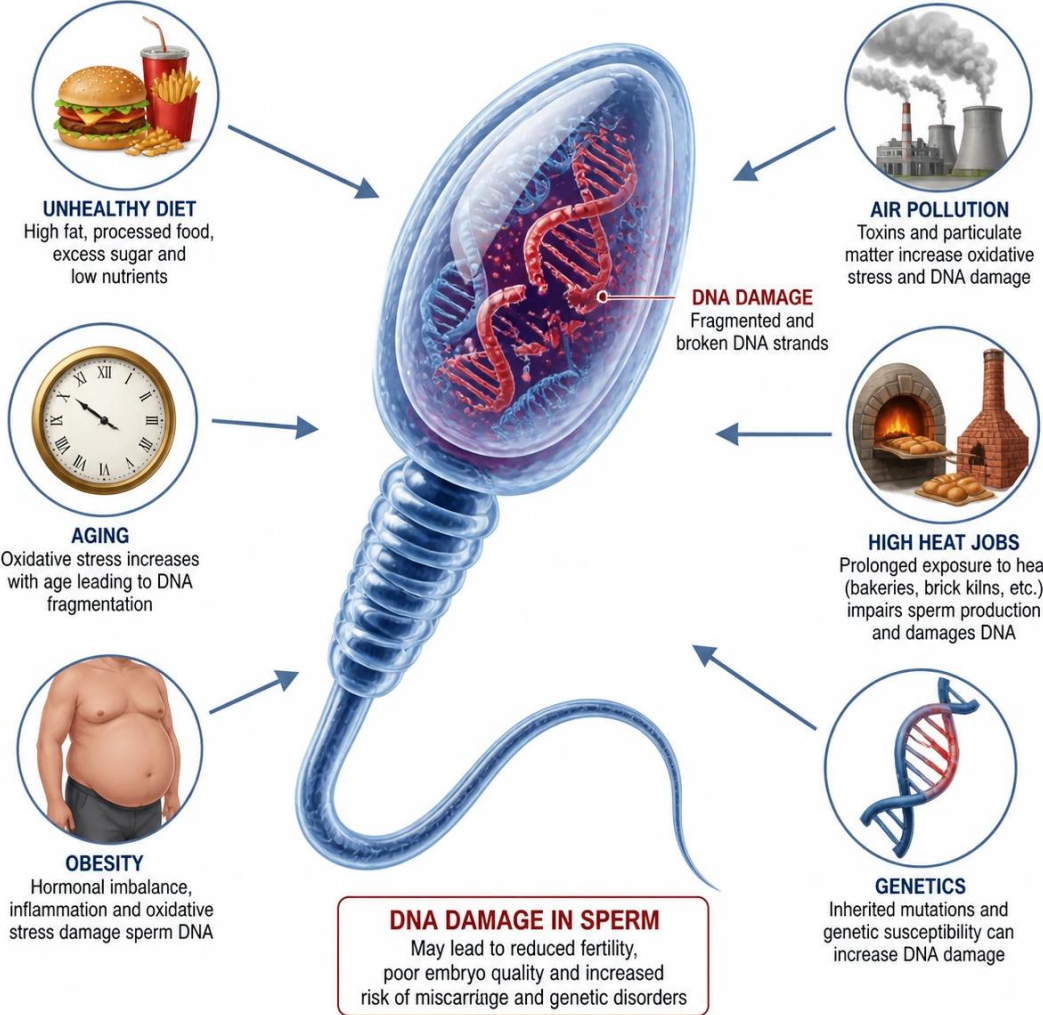


**4 TECHNICAL ISSUES IN ART/ICSI**

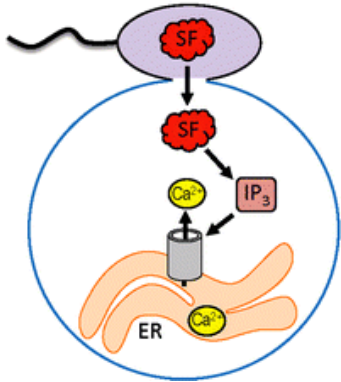




### FACTORS CAUSING DNA DAMAGE IN SPERM



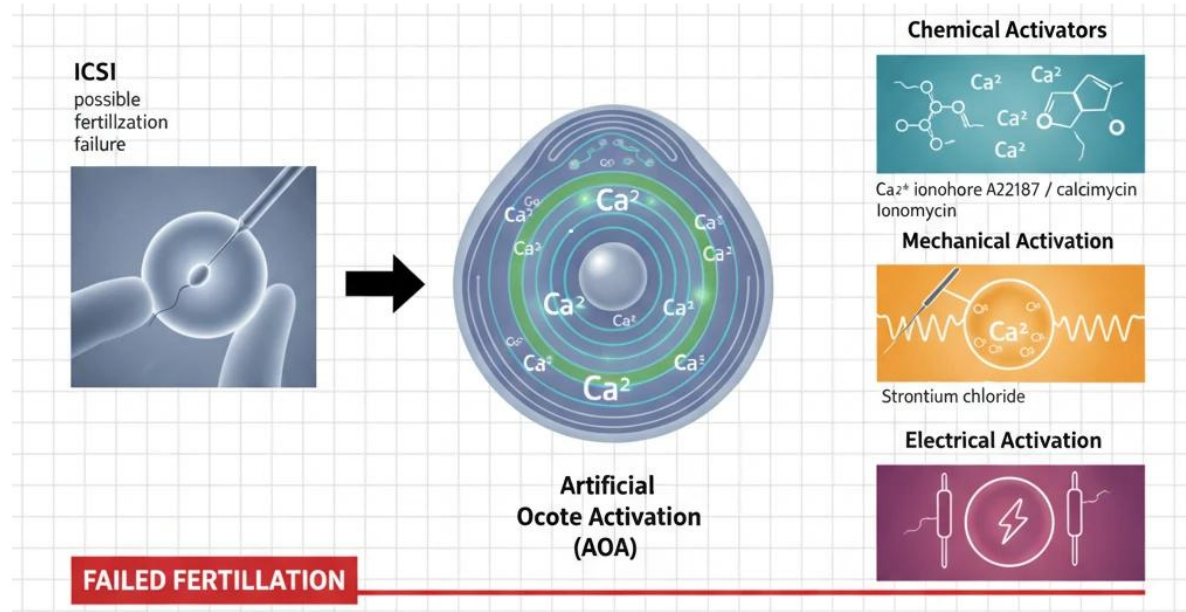
Sperm factor



Failed fertilization after ICSI is commonly reported for globozoospermic couples and can be overcome by artificial oocyte activation (AOA).

Chemical, mechanical and electrical activators

- Ca<sup>2+</sup> ionophore A23187 (calcimycin);
  - Ionomycin
  - Strontium chloride
- AND electrical



# Application of ICSI-AOA in clinics

- Repeated TFF or LFF after IVF-ICSI
- Severe teratozoospermia (abnormal morphology >98%)  
Globozoospermia
- Testicular sperm extraction (TESE)

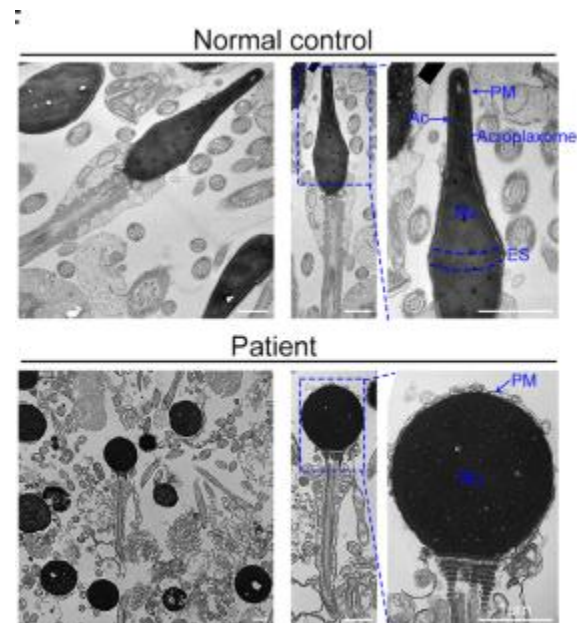
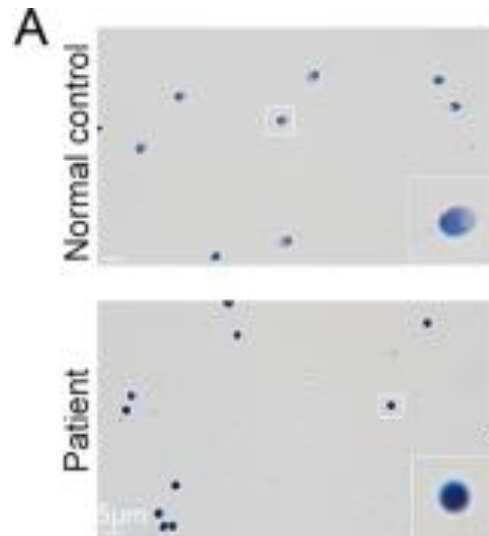
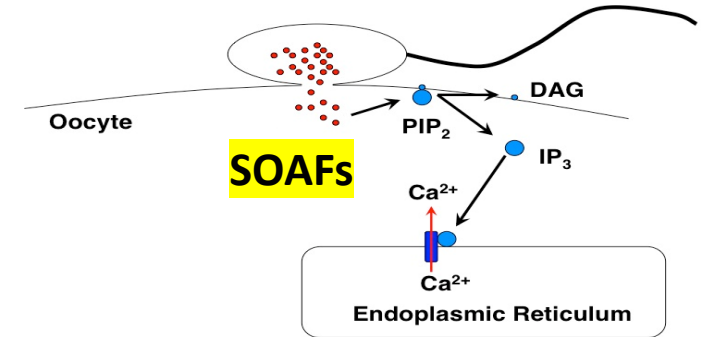
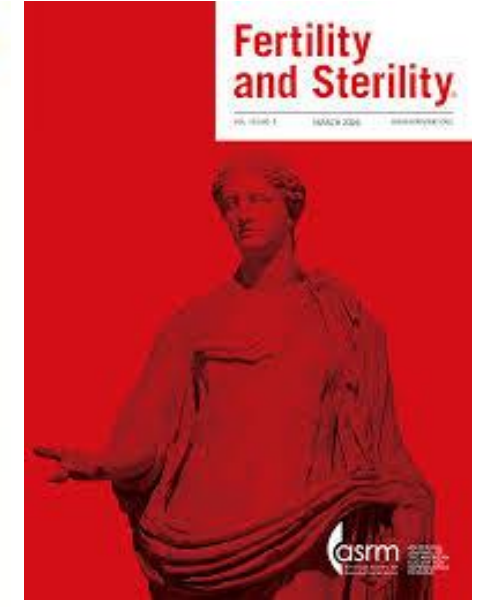
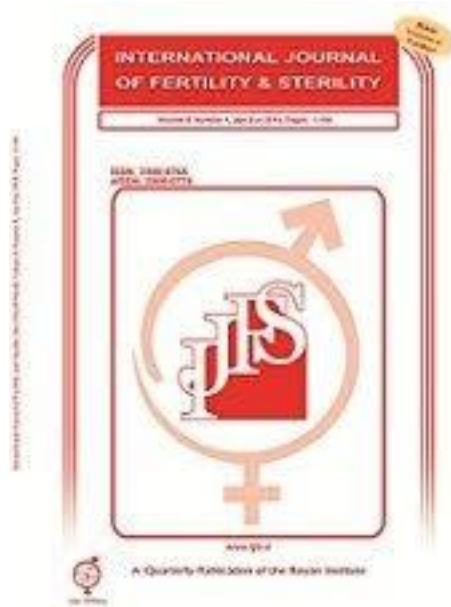


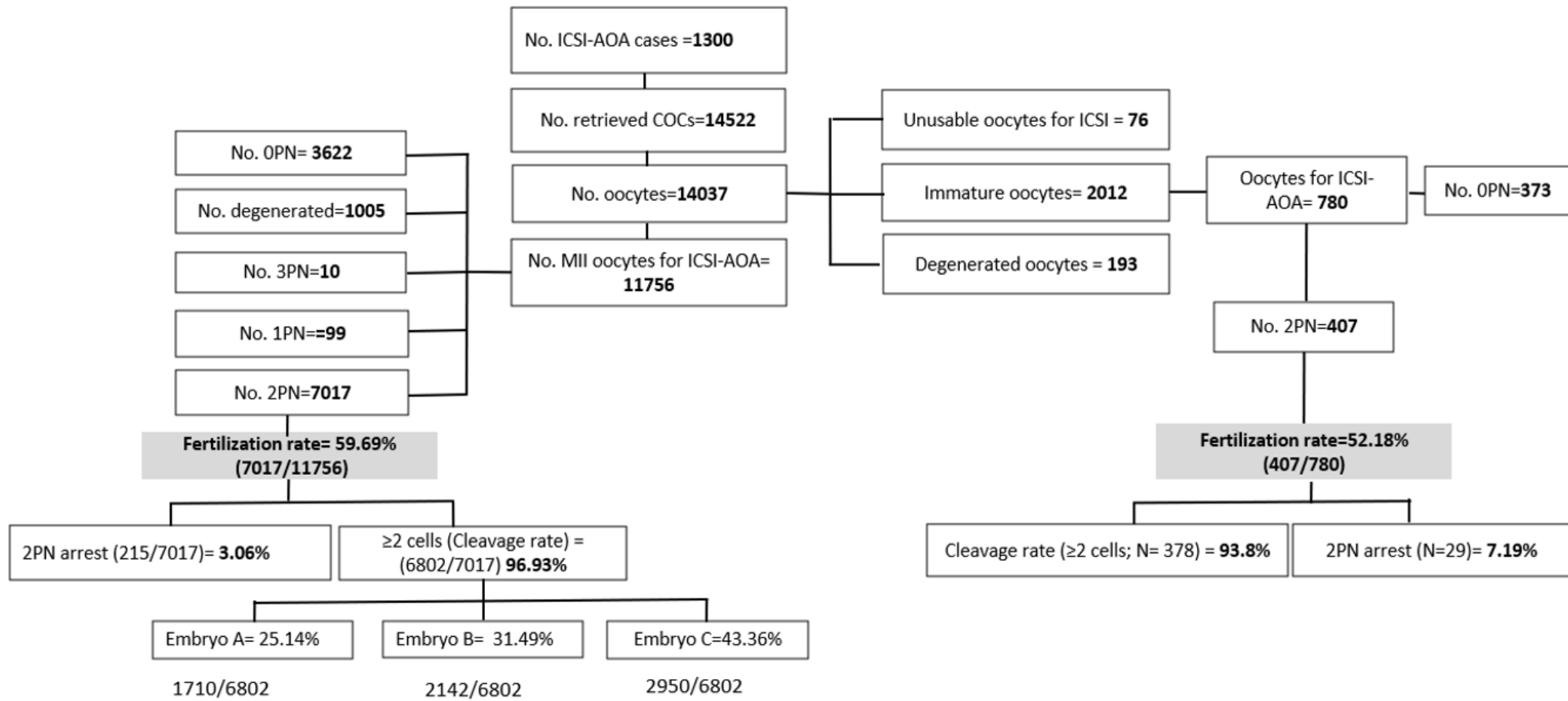
Table 3 Characteristics of each globozoospermic cases and their ICSI-AOA clinical outcomes

	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						

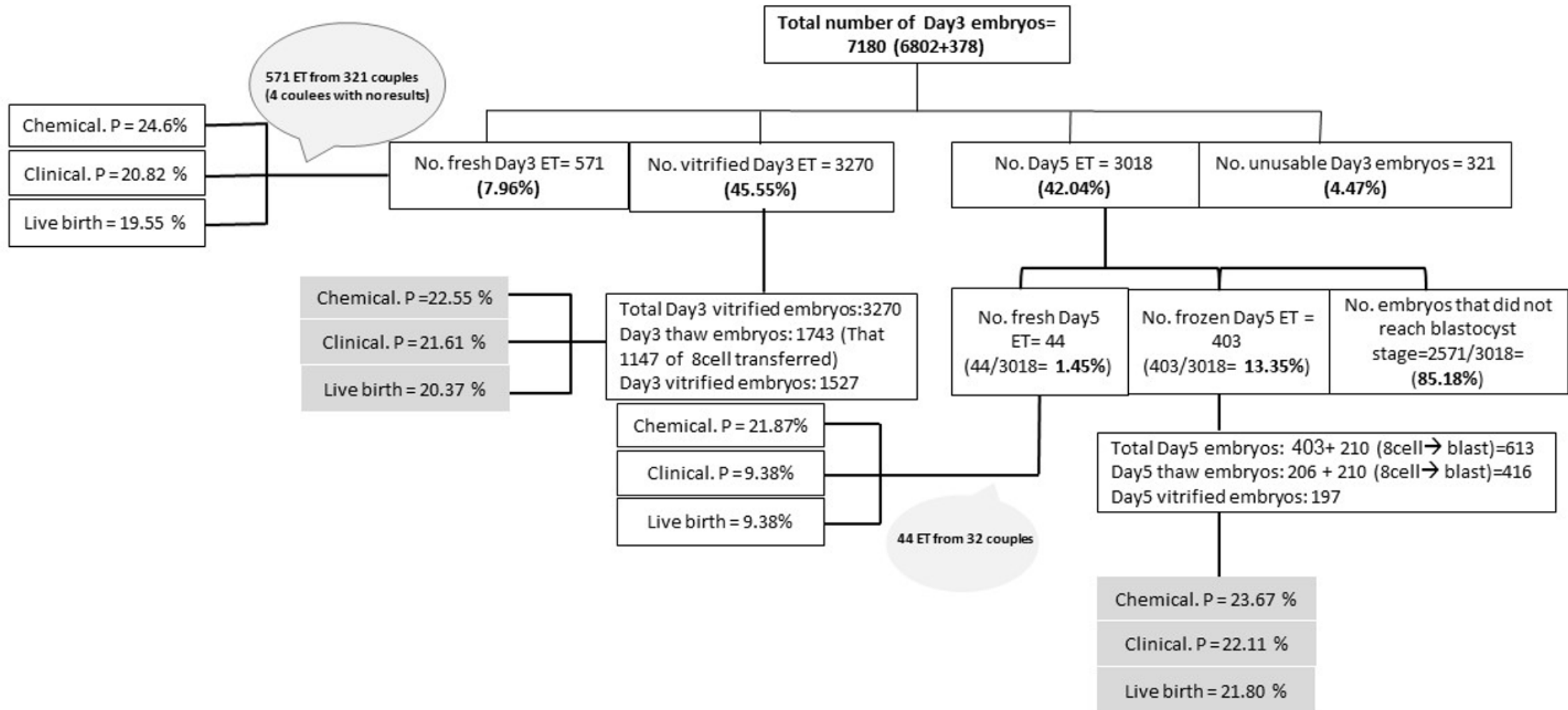
M, Male; FM, Female; G, globozoospermia.

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.





	% of 2PN	% of arrested 2PN	% Cleavage rate
Result of <b>MII</b> Oocytes for ICSI-AOA	59.69%	3.06%	96.93%
Result of <b>Immature</b> oocytes for ICSI-AOA	52.18%	7.19%	93.8%



## Evaluating the Effectiveness of Assisted Oocyte Activation in ICSI: Pairwise Meta-Analyses and Systematic Evidence Evaluation

Mohamed Fawzy ✉, Mohamad AlaaEldein Elsuity, Yasmin Magdi, Mosab Mahmud Rashwan, Mostafa Ali Gad, Nehal Adel, Mai Emad, Dina Ibrahim, Sara El-Gezeiry ... See all authors ▾

First published: 30 January 2025 | <https://doi.org/10.1111/1471-0528.18085>

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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

There is insufficient evidence from the currently available RCTs to make a decision on the efficacy or safety of ICSI-AOA on key reproductive outcomes in couples with previous fertilization failure.



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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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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.**



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



### BIOGRAPHY

Mingrong Lv received her PhD in cell biology from the University of Science and Technology of China in 2015. She has since joined the Reproductive Medicine Center, Department of Obstetrics and Gynecology, at The First Affiliated Hospital of Anhui Medical University. Her research has focused on molecular mechanisms of gamete abnormality.

Mingrong Lv<sup>1,2,3,1</sup>, Dan Zhang<sup>4,1</sup>, Xiaojin He<sup>1,2,3,1</sup>, Beili Chen<sup>1,2,3,1</sup>, Qiang Li<sup>1,5,6</sup>, Ding Ding<sup>1,5,6</sup>, Yan Hao<sup>1,5,6</sup>, Rufeng Xue<sup>1,5,6</sup>, Dongmei Ji<sup>1,5,6</sup>, Weiwei Zou<sup>1,5,6</sup>, Huijuan Zou<sup>1,5,6</sup>, Yajing Liu<sup>1,5,6</sup>, Jianye Wang<sup>1,5,6</sup>, Zhaolian Wei<sup>1,5,6</sup>, Ping Zhou<sup>1,5,6</sup>, Yunxia Cao<sup>1,5,6</sup>, Zhiguo Zhang<sup>1,2,3,\*</sup>

### KEY MESSAGE

Application of A23187 led to increased rates of fertilization, blastulation and high-quality blastocyst formation in certain types of infertile couples and to implantation, clinical pregnancy and live birth rates in most types.

### ABSTRACT

**Research question:** Does calcium ionophore treatment of oocytes improve fertilization rate, embryo development and outcomes in specific groups of infertile couples?

**Design:** This retrospective cohort study involved 796 couples undergoing oocyte activation with calcium ionophore (A23187) after intracytoplasmic sperm injection (ICSI) between 2016 and 2018. All metaphase II oocytes were exposed to 5  $\mu\text{mol/l}$  ionophore for 15 min immediately after ICSI, cultured *in vitro* to the blastocyst stage, and transferred to the uteri of recipients on day 5 or cryopreserved for transfer in the next cycle. The previous cycles of the same patients formed the control group.

**Results:** Among 1261 ICSI cycles and 796 ICSI-artificial oocyte activation (ICSI-AOA) cycles, implantation, positive beta-HCG, clinical pregnancy and live birth rates were significantly ( $P < 0.05$  to  $P < 0.001$ ) improved for all groups, compared with previous cycles, except live birth rate in women with primary ovarian insufficiency (POI). Compared with previous cycles, rates of blastulation (all  $P < 0.001$ ) and high-quality blastocysts ( $P < 0.05$  to  $P < 0.001$ ) were increased significantly for couples with male factor (oligoasthenoteratozoospermia [OAT]), unexplained infertility and couples with both factors in the ICSI-AOA cycles. High-quality blastocyst rate was increased in couples with polycystic ovary syndrome (PCOS) ( $P = 0.0453$ ). Miscarriage rates were decreased significantly ( $P < 0.05$  to  $P < 0.001$ ) in couples with OAT, PCOS and unexplained infertility in the treatment cycles. No significant differences were found for fertilization rate, embryo development or live birth rate in patients with POI between both groups.

**Conclusions:** Artificial oocyte activation was able to 'rescue' the poor reproductive outcomes in certain types of infertile couples with history of failure to achieve pregnancy.

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\*

## Abstract

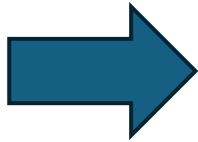
**Background:** Whether artificial oocyte activation (ICSI-AOA) will increase the risk of birth defects remains controversial. Thus, we performed this study to evaluate the risk of birth defects and further compare the incidence of different birth defects types (chromosomal aberrations and non-chromosomal aberrations) in children conceived by ICSI-AOA and conventional intracytoplasmic sperm injection (ICSI) in an enlarged sample size.

**Method:** A comprehensive review of the literatures comparing birth defects in children conceived by ICSI-AOA and conventional ICSI by October 2020 was performed in PubMed, Embase, Cochrane Libraries, Web of Science, and Chinese databases including China National Knowledge Infrastructure, China Biology Medicine disc and Wan Fang. Risk ratios (RR) and 95% confidence intervals (CI) were calculated.

**Results:** 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. 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.

**Conclusion:** Our analysis indicates that ICSI-AOA represents no significant difference in the prevalence of major birth defects or types of birth defects (chromosomal aberrations and non-chromosomal aberrations) comparing with conventional ICSI. This conclusion may provide clinicians evidence-based support in patient counseling and instruction of the application and safety concern about ICSI-AOA.

**Keywords:** Birth defects, AOA, ICSI, Meta-analysis, Chromosomal aberrations





- **Overall, there were 266 live births from 1,317 transfers, with an implantation rate of 14.19% and a live birth rate of 20.20% per cycle, alongside a 1.5% anomaly rate.**
- **Previous research indicates defect rates for children born via natural conception (1.64%), IVF (3.18%), ICSI (3.1%), and ICSI-AOA (3.3%). Our study found a defect rate of 1.5%, which is consistent with these reported ranges.**



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[www.rbmonline.com](http://www.rbmonline.com)



## EDITORIAL

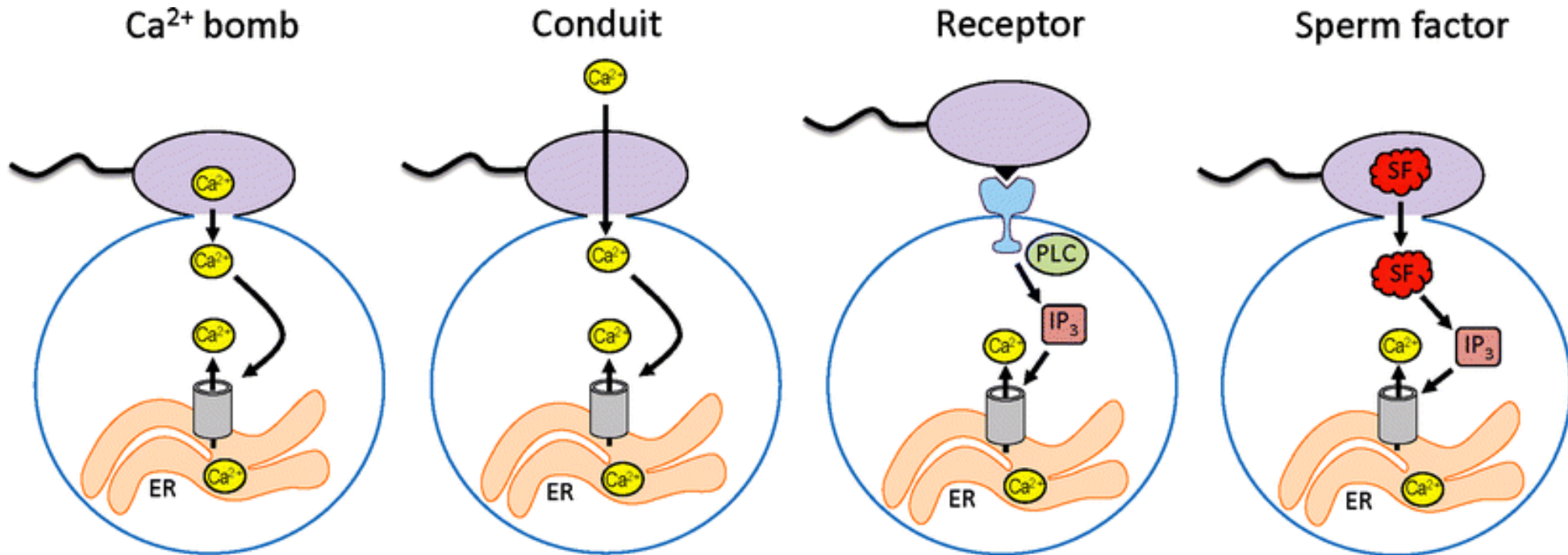
# A plea for caution and more research in the 'experimental' use of ionophores in ICSI

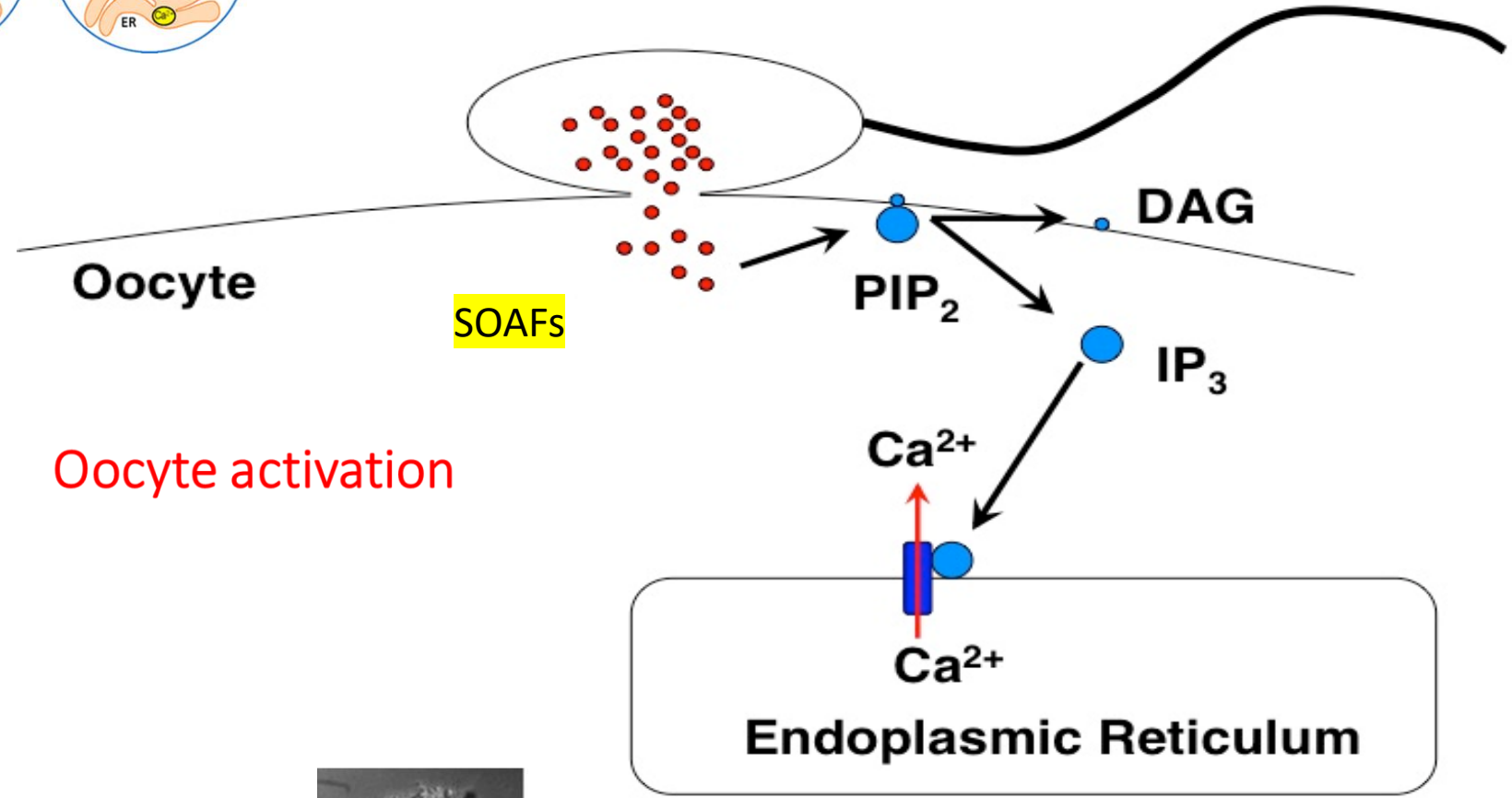
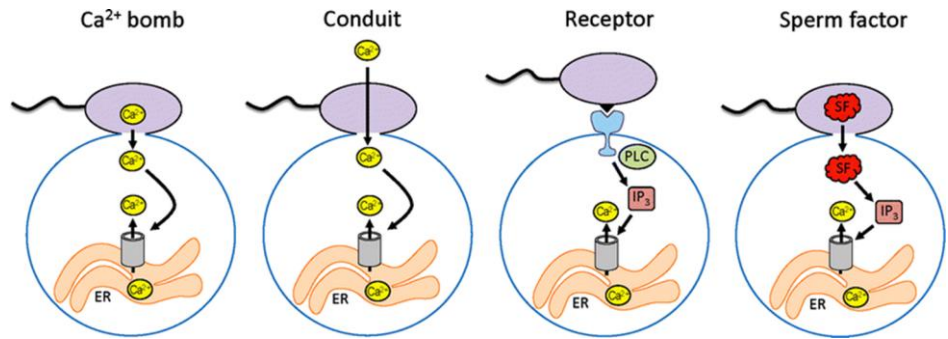


Jonathan van Blerkom, Jacques Cohen, Martin Johnson

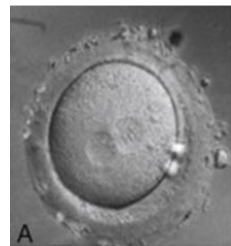


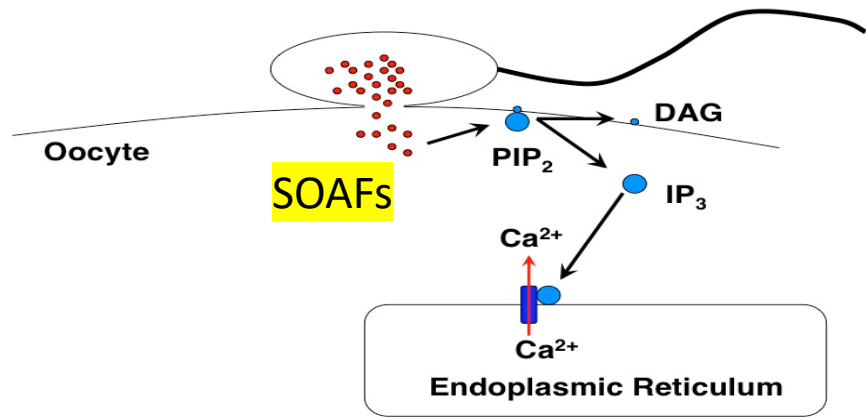
# Oocyte activation



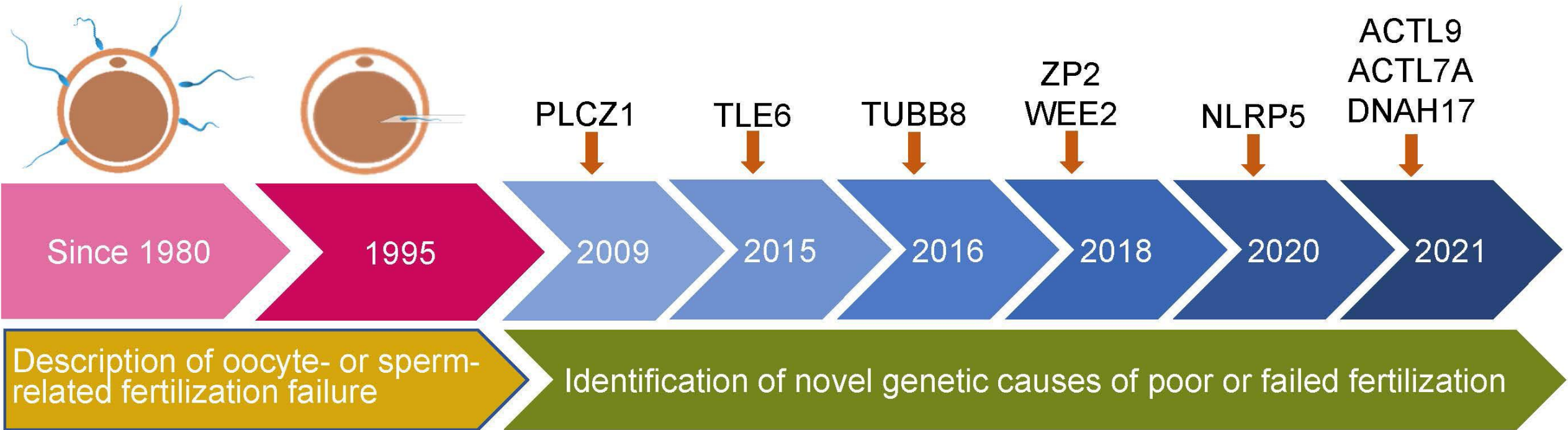


Oocyte activation



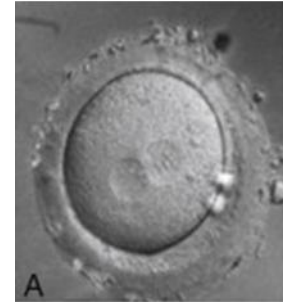


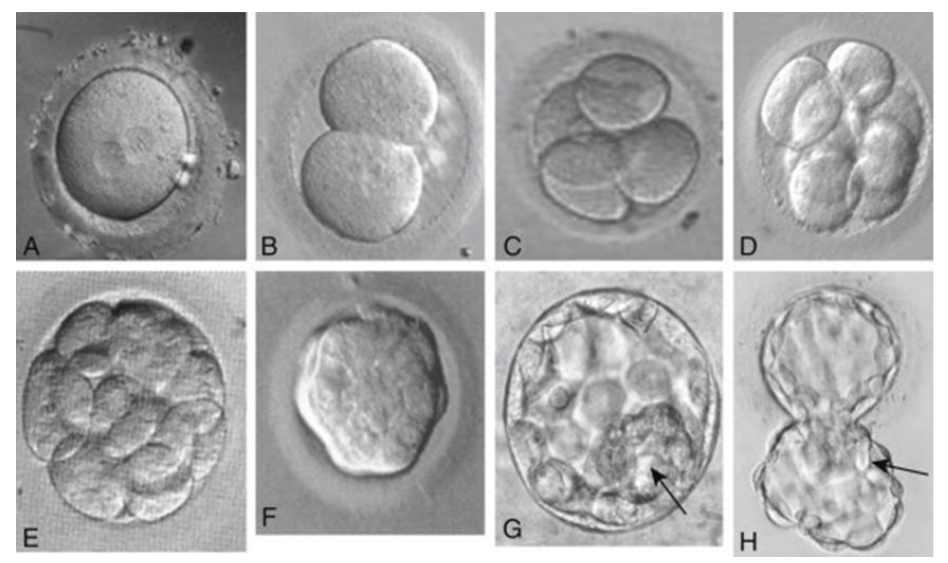
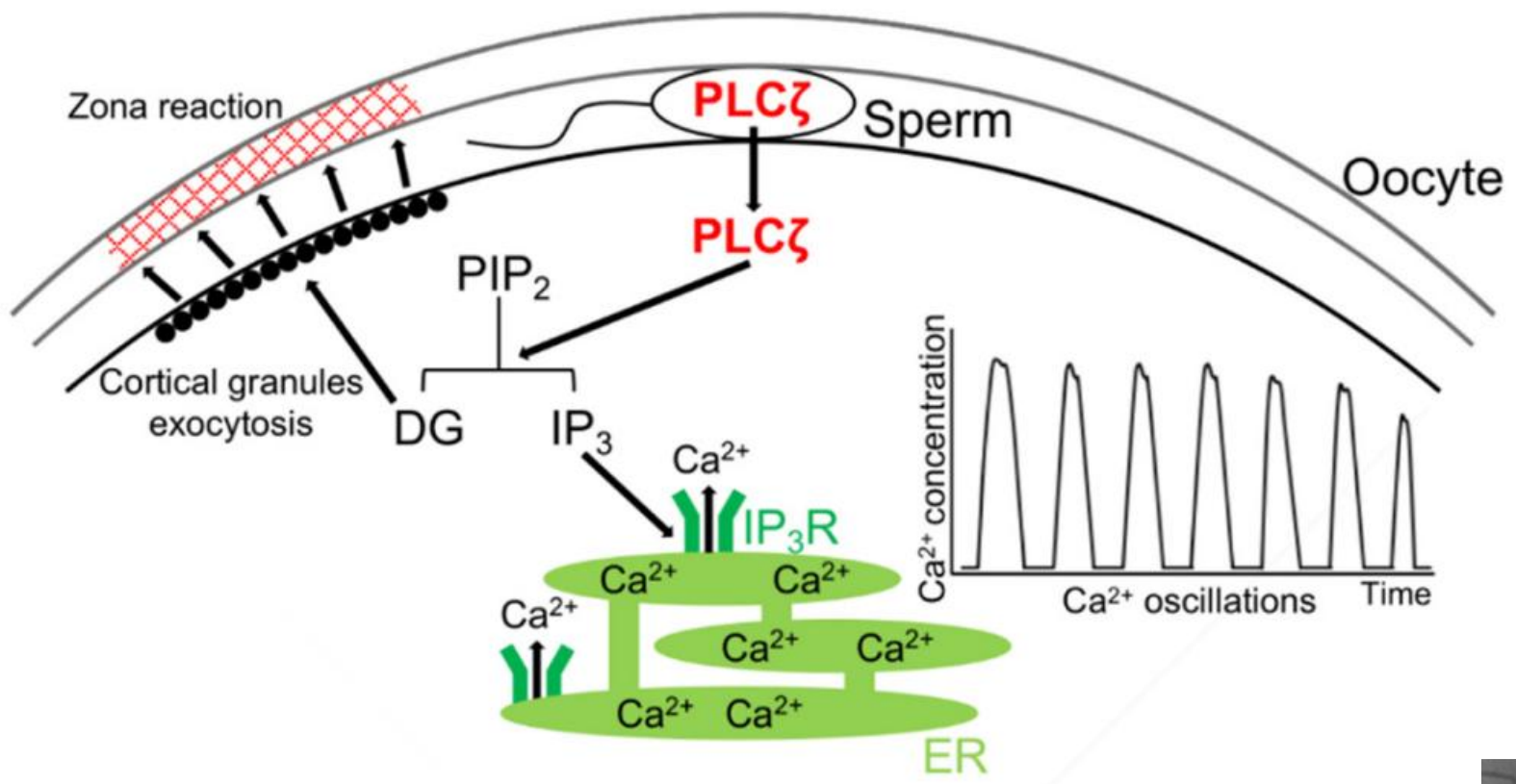
- Oscillin
- TR-KIT
- Nitric Oxide
- Citrate synthase
- PAWP: a sperm-specific WW domain-binding protein
- PLCζ: Phospholipase C zeta
- ACTL7A: Actin Like 7A

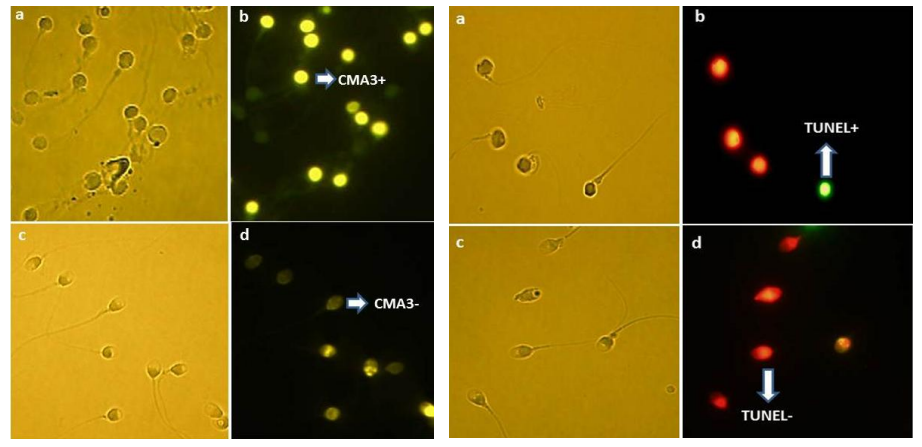
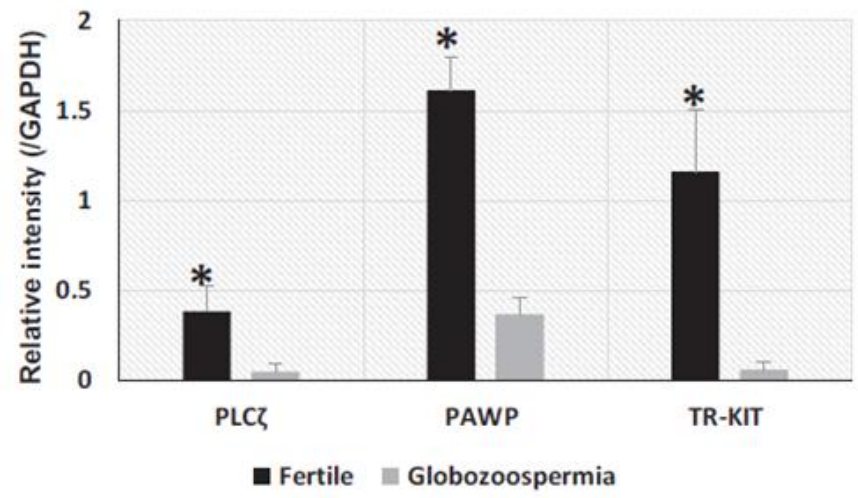
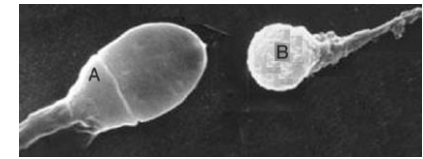


# What is the SOAFs ?

- ✓ Released by spermatozoon upon sperm-oolemma fusion
- ✓ A signaling molecule
- ✓ A male germ cell/testis specific gene product
- ✓ Induces Ca-oscillations
- ✓ Triggers CG exocytosis/PN development/embryo cleavage
- ✓ Present in the acrosome-reacted sperm head





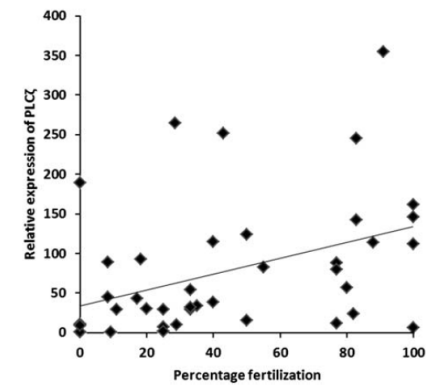
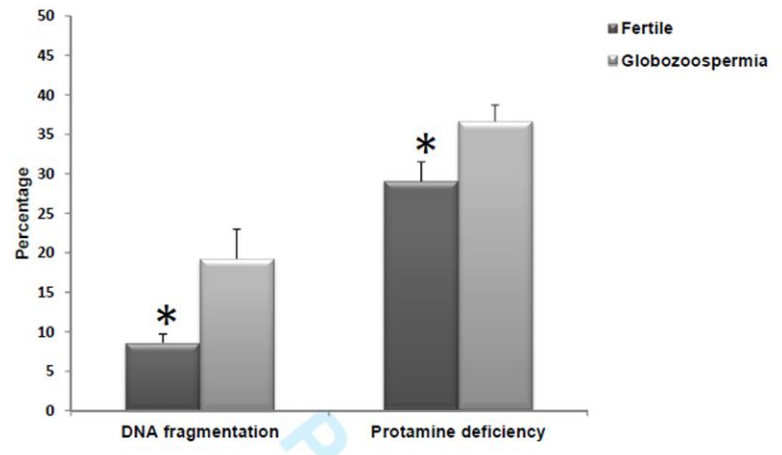
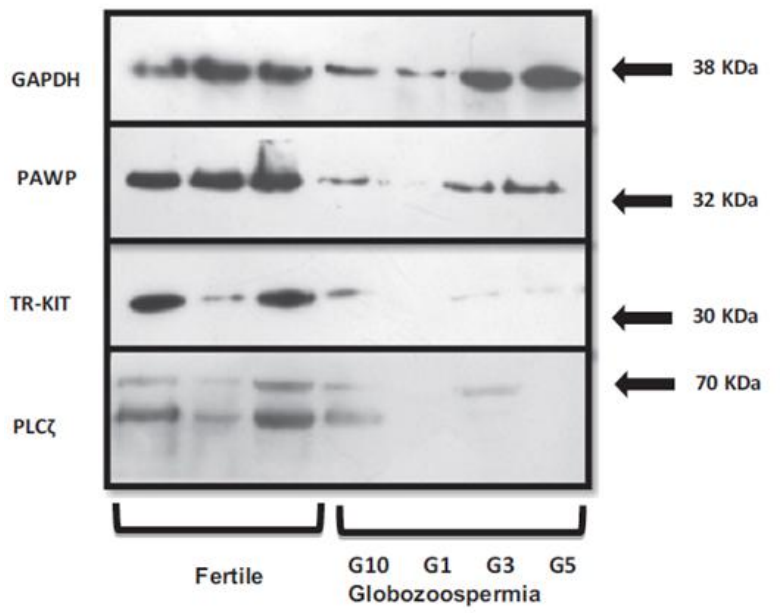


Hum. Reprod. Advance Access published September 6, 2011  
 Human Reproduction, Vol.0, No.0 pp. 1-7, 2011  
 doi:10.1093/humrep/der285

human reproduction ORIGINAL ARTICLE *Andrology*

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

S. Aghajani<sup>1,2</sup>, K. Ghaedj<sup>1,2</sup>, A. Salamian<sup>1</sup>, M.R. Deemeh<sup>1,3</sup>, M. Tavalaei<sup>1</sup>, J. Moshtaghian<sup>2</sup>, J. Parrington<sup>4</sup>, and M.H. Nasr-Esfahani<sup>1,3,5,\*</sup>

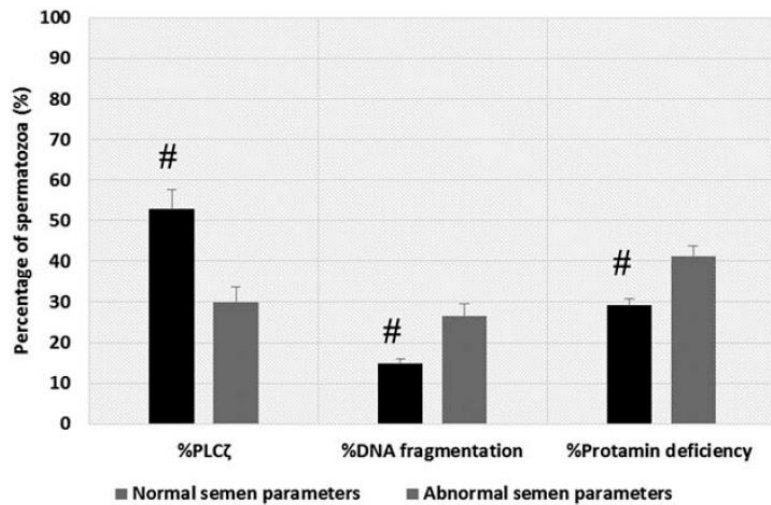


## Relationship between phospholipase C-zeta, semen parameters, and chromatin status

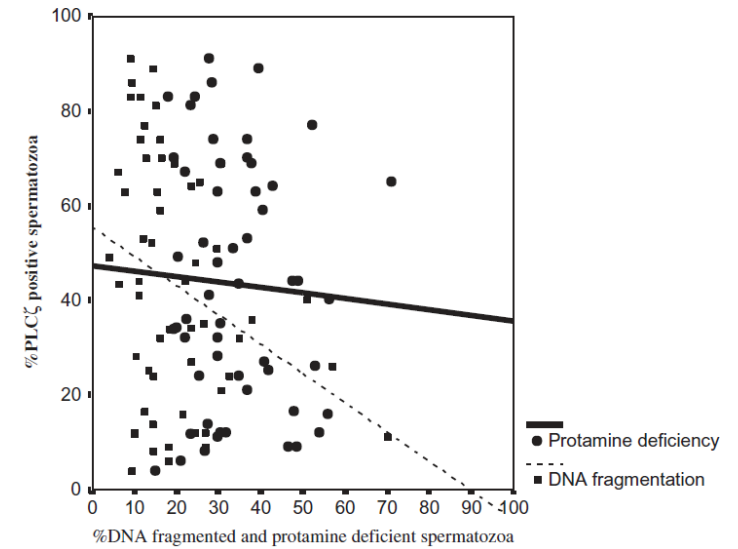
Marziyeh Tavalae, Abbas Kiani-Esfahani & Mohammad H. Nasr-Esfahani

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To link to this article: <http://dx.doi.org/10.1080/19396368.2017.1298006>



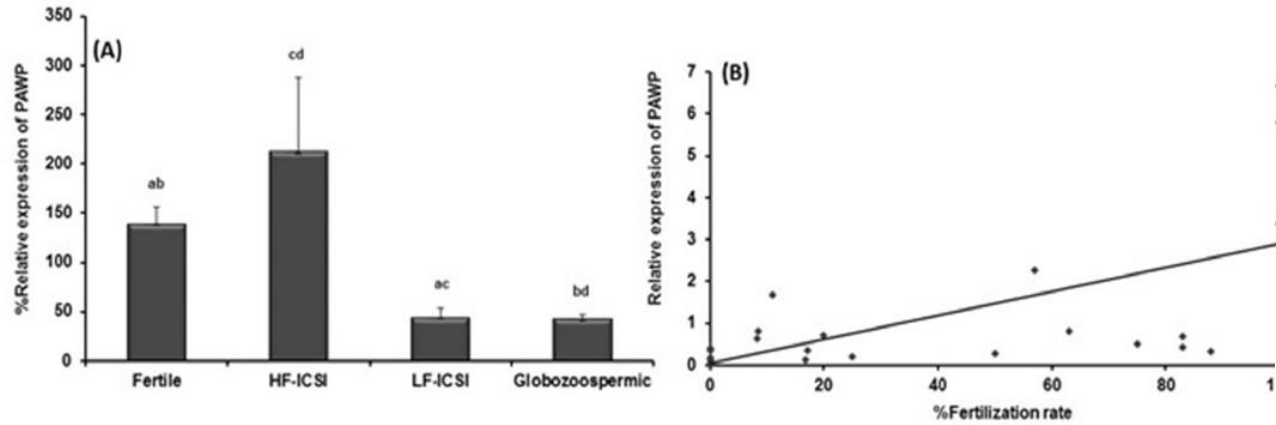
**Figure 1.** Comparison of mean percentage of sperm PLC $\zeta$ , SDF, and protamine deficiency between men with normal and abnormal semen parameters. The mean percentage of PLC $\zeta$  positive spermatozoa was significantly lower, and percentage of SDF and protamine deficient spermatozoa were significantly higher in men with abnormal semen parameters compared to men with normal semen parameters, respectively. SDF: sperm DNA fragmentation; PLC $\zeta$ : phospholipase C-zeta. # shows significant difference between the two groups at  $p < 0.05$ .



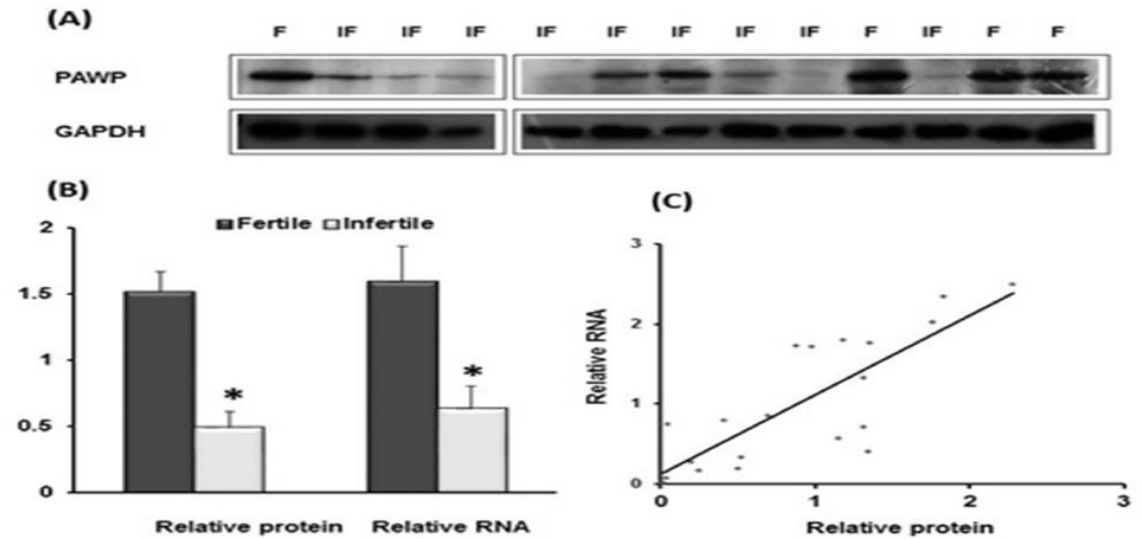
**Figure 3.** Correlation between percentage of PLC $\zeta$  with DNA fragmentation ( $r = -0.3$ ;  $p = 0.026$ ) and protamine deficiency ( $r = -0.05$ ;  $p = 0.702$ ) in total population (32 men with normal semen parameters and 23 men with abnormal semen parameters). PLC $\zeta$ : phospholipase C-zeta.

## Assessment of Sperm PAWP Expression in Infertile Men

Somayeh Tanhaei<sup>1,2</sup>, Solmaz Abdali-Mashhadi<sup>1</sup>, Marziyeh Tavalaei<sup>1</sup>, Soudabeh Javadian-Elyaderani<sup>1</sup>,  
Kamran Ghaedi<sup>2,3</sup>, Seyed Morteza Seifati<sup>4</sup>, Mohammad H. Nasr-Esfahani<sup>1,2,5\*</sup>



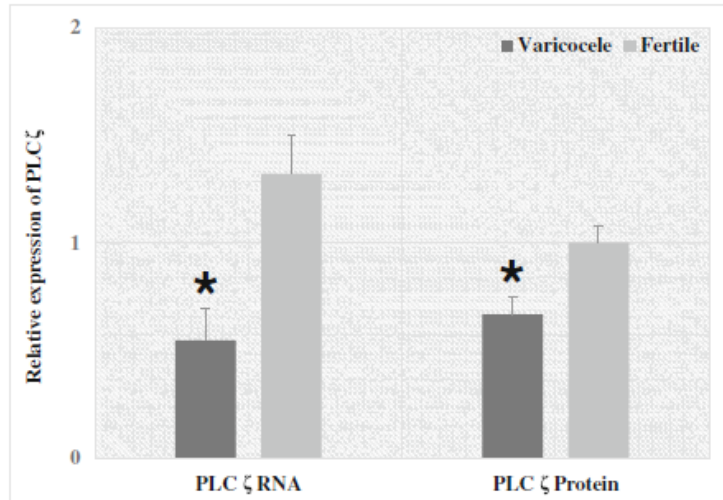
**Figure 1** **A)** Quantitative real-time analysis of PAWP in fertile, high fertilization post-ICSI (HF-ICSI), low fertilization post-ICSI (LF-ICSI), and globozoospermic individuals. (N = 64) **B)** Scatter plot shows significant correlation ( $r = 0.628$ ;  $p = 0.001$ ) between percent fertilization and relative expressions of PAWP (N = 25).



**Figure 2** **A)** Western blot analysis of PAWP in fertile (F) and infertile (IF) individuals. **B)** Comparison of mean relative expression of PAWP RNA and protein level in fertile and infertile individuals and **C)** Correlation analysis between RNA and protein for PAWP ( $r = 0.8$ ;  $p \leq 0.0001$ ). Aster indicates significant difference at  $p < 0.001$  between fertile and infertile individuals (**B**).

## An association between sperm PLC $\zeta$ levels and varicocele?

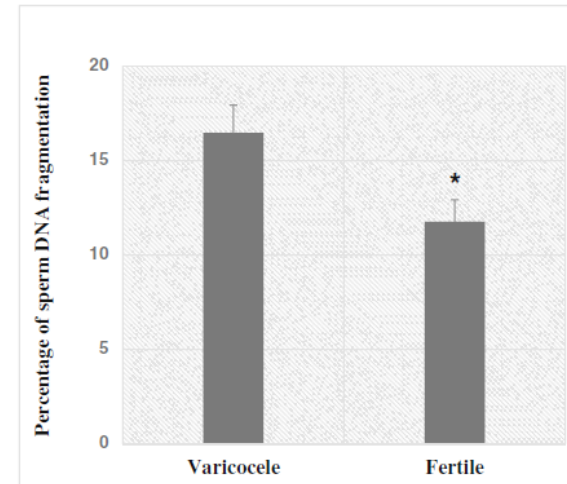
Elham Janghorban-Laricheh<sup>1,2</sup> · Nasim Ghazavi-Khorasgani<sup>1,2</sup> · Marziyeh Tavalaei<sup>1</sup> ·  
Dina Zohrabi<sup>2</sup> · Homayon Abbasi<sup>3</sup> · Mohammad H. Nasr- Esfahani<sup>1,3</sup>



**Fig. 2** Comparison of relative expression of PLC $\zeta$  at both the RNA and protein levels between fertile individuals and men with varicocele. \* $p < 0.05$ , significant differences



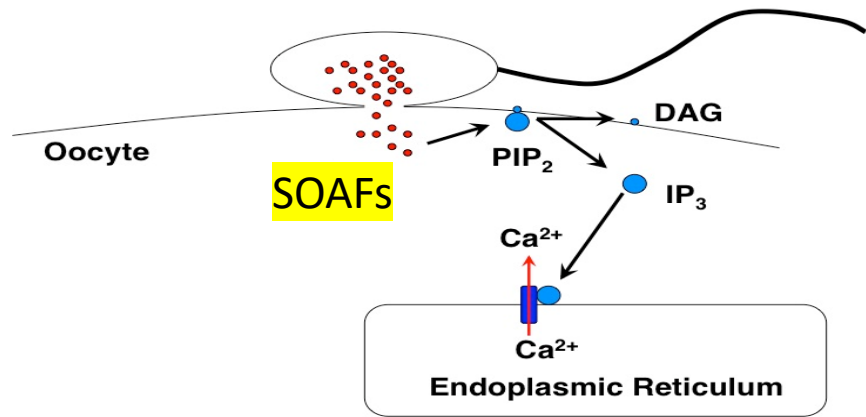
**Fig. 3** Western blot of PLC $\zeta$  and GAPDH in four fertile individuals and five men with varicocele



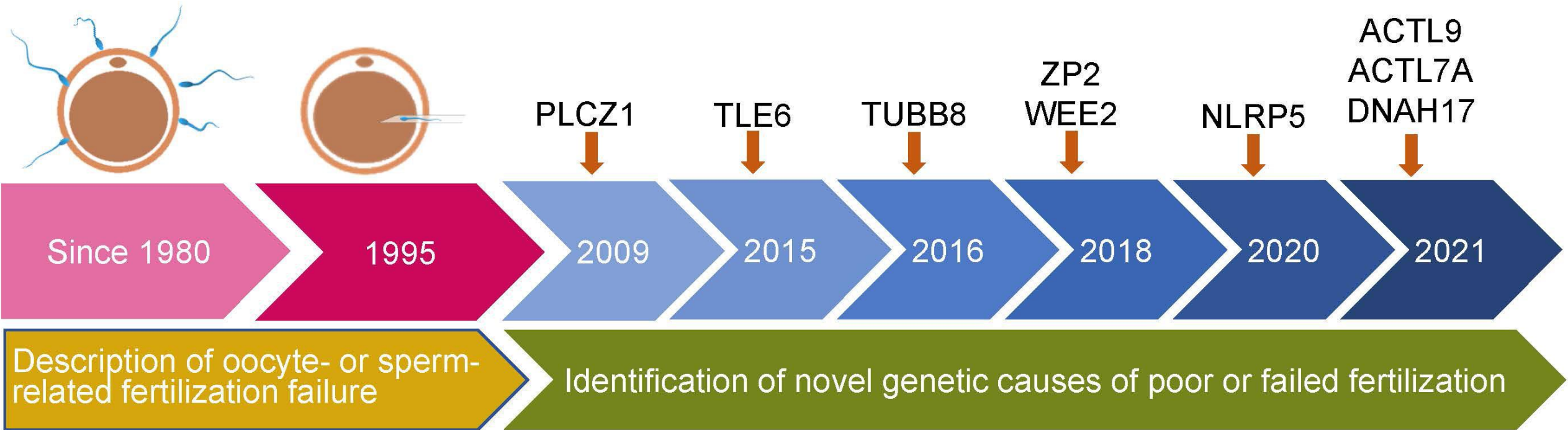
**Fig. 1** Comparison of percentage of sperm DNA fragmentation between fertile individuals and men with varicocele. \* $p < 0.05$ , significant differences

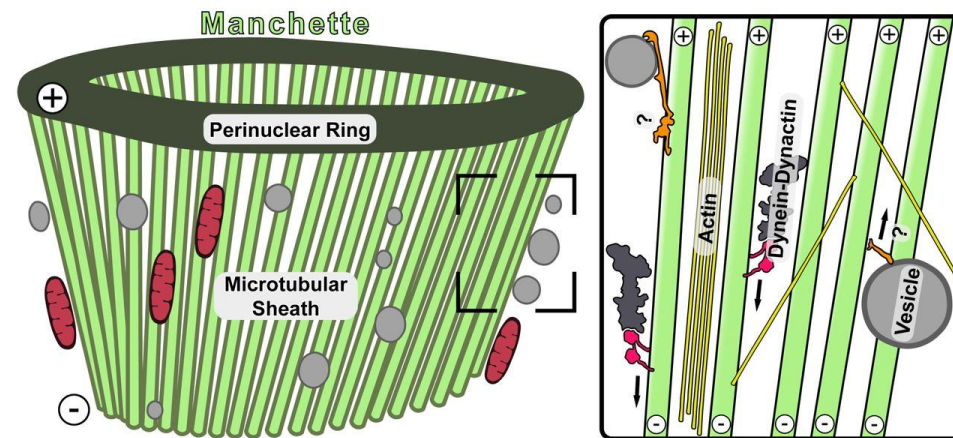
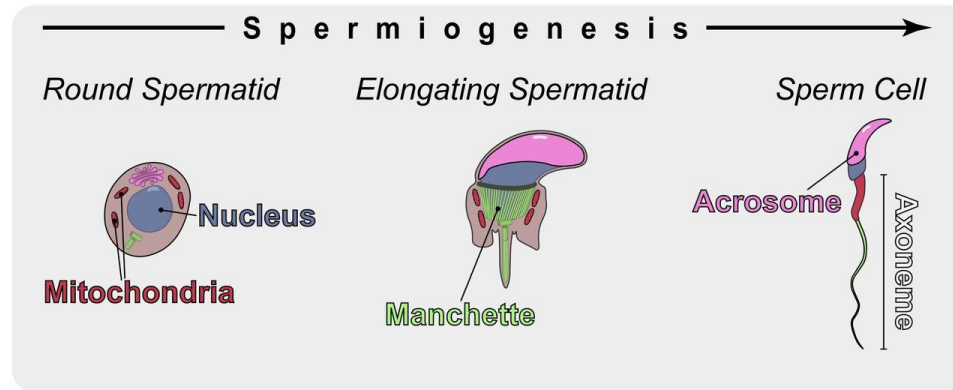
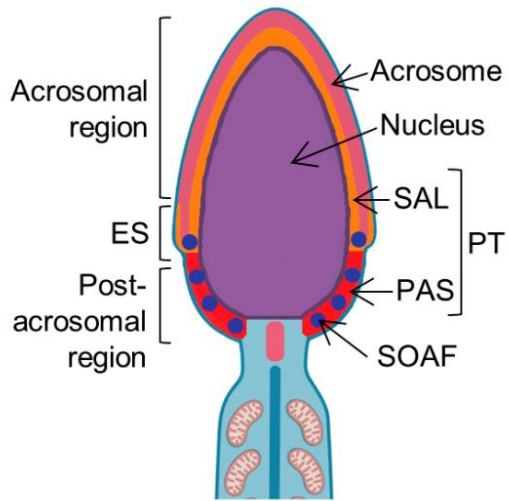
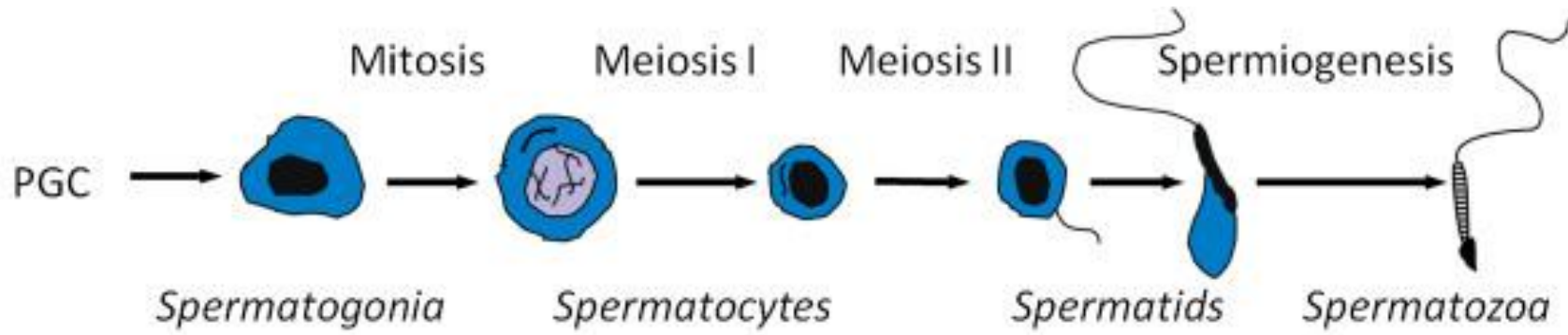
## conclusion

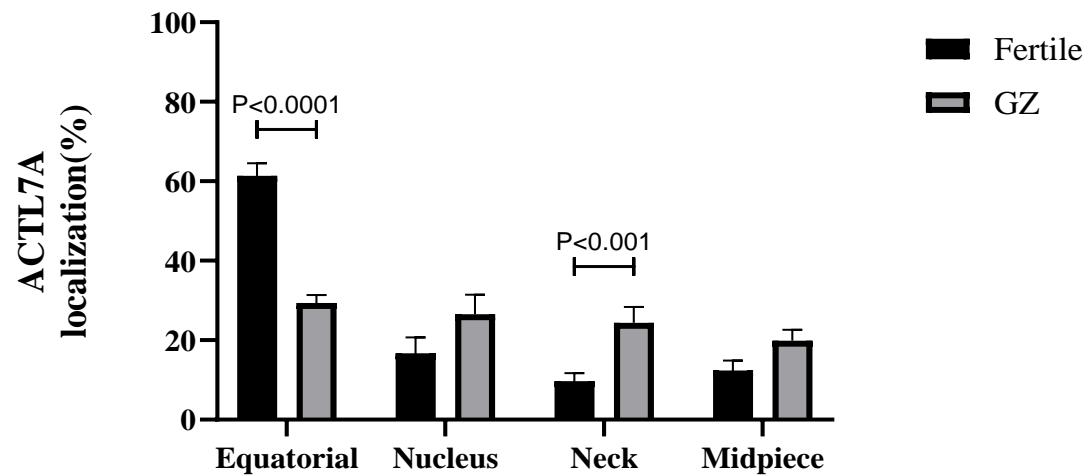
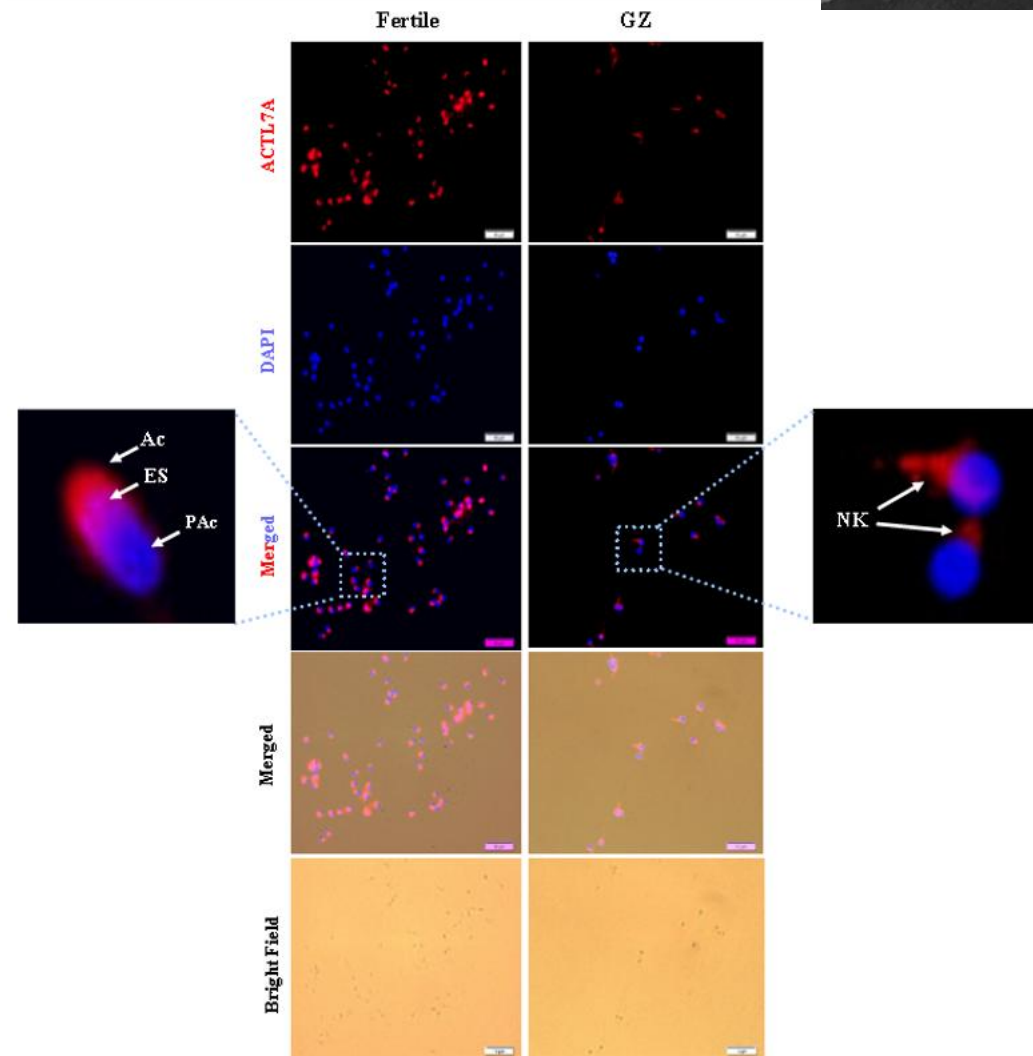
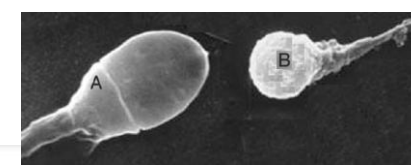
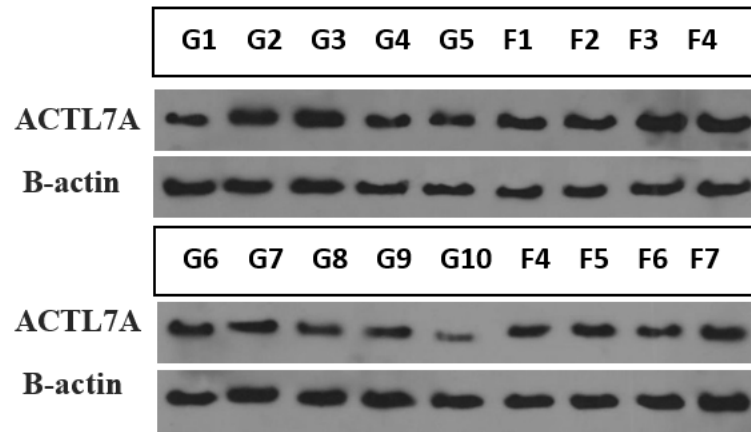
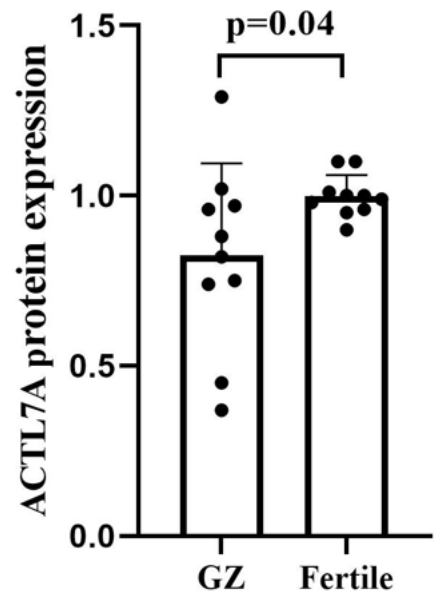
These slides demonstrate that PLC $\zeta$ , together with proteins such as PAWP, is essential for initiating oocyte activation and the fertilization process. A reduction in these proteins is closely associated with structural abnormalities in sperm chromatin. Quantitative assessment of these factors may potentially serve as an accurate diagnostic tool for predicting the success of infertility treatments such as ICSI, as well as identifying the underlying causes of oocyte activation failure.



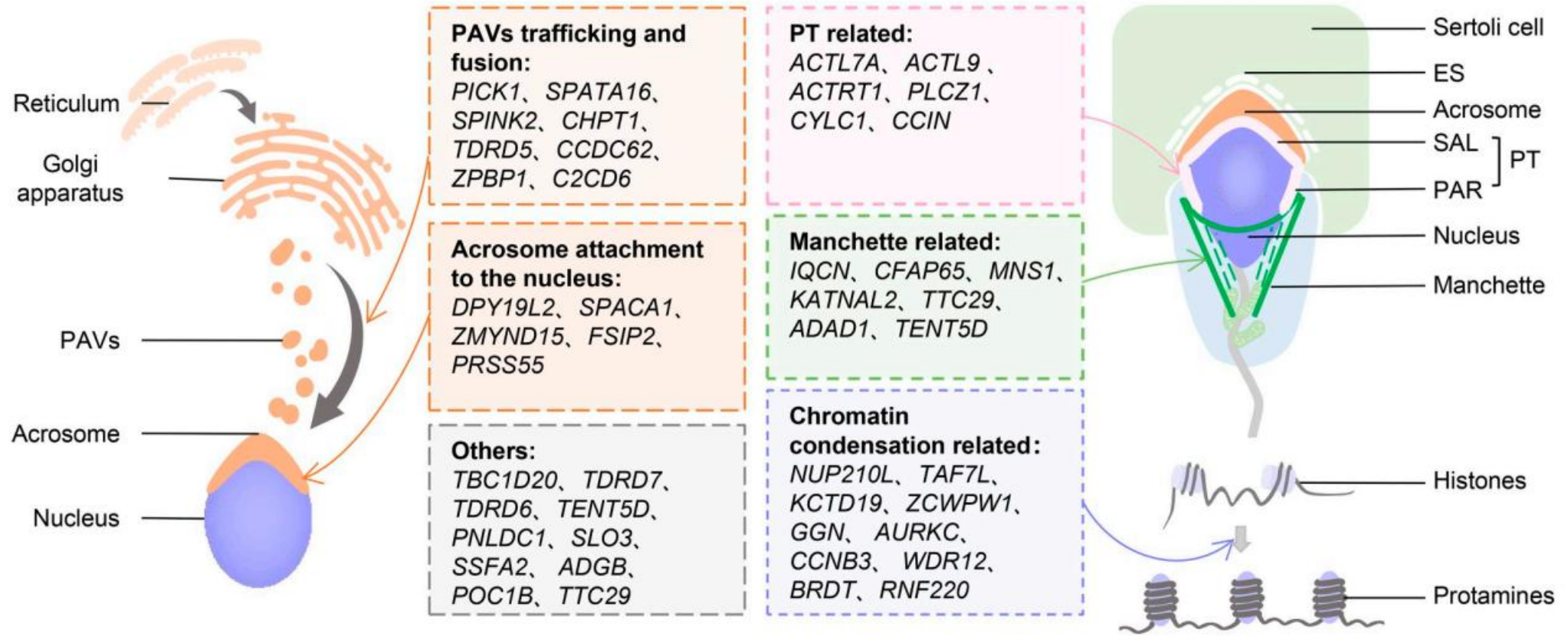
- Oscillin
- TR-KIT
- Nitric Oxide
- Citrate synthase
- PAWP: a sperm-specific WW domain-binding protein
- PLCζ: Phospholipase C zeta
- ACTL7A: Actin Like 7A

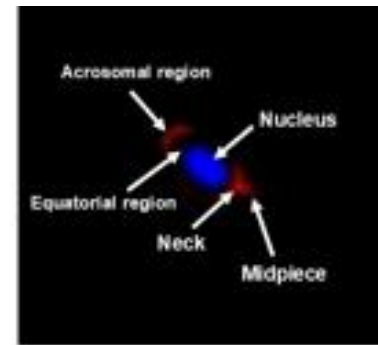
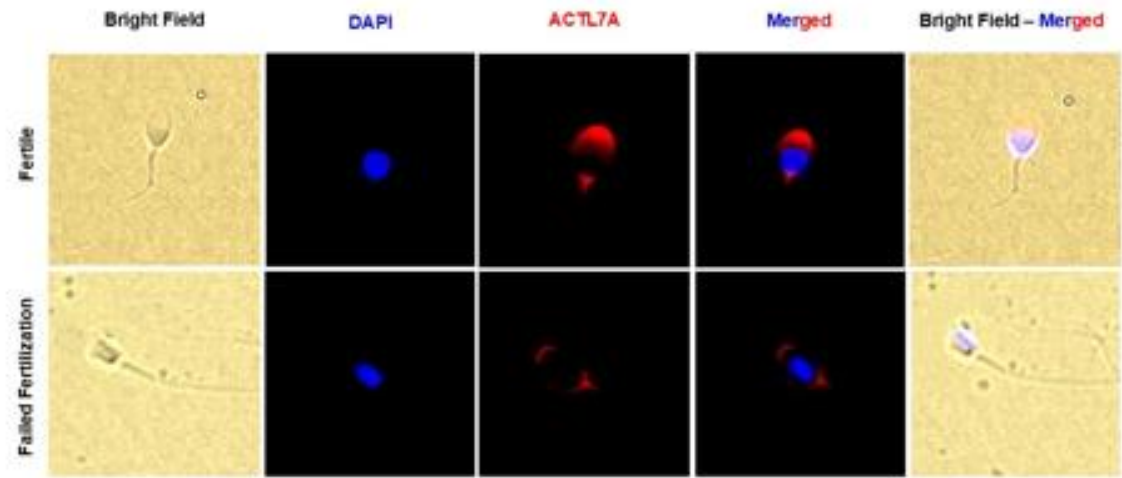
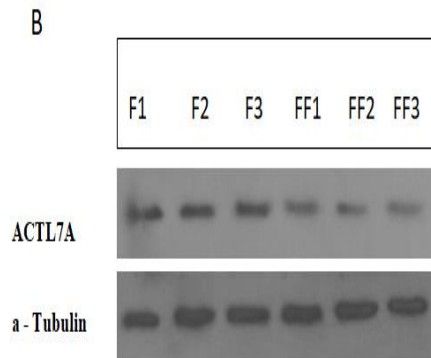
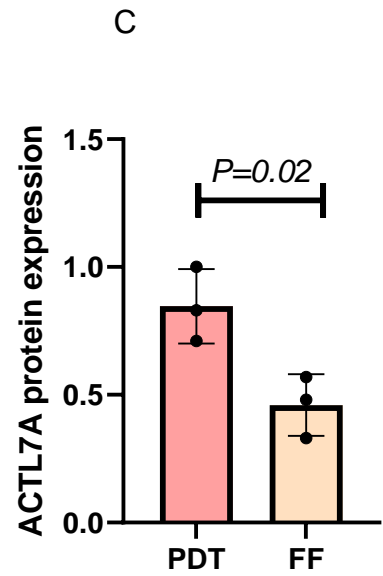
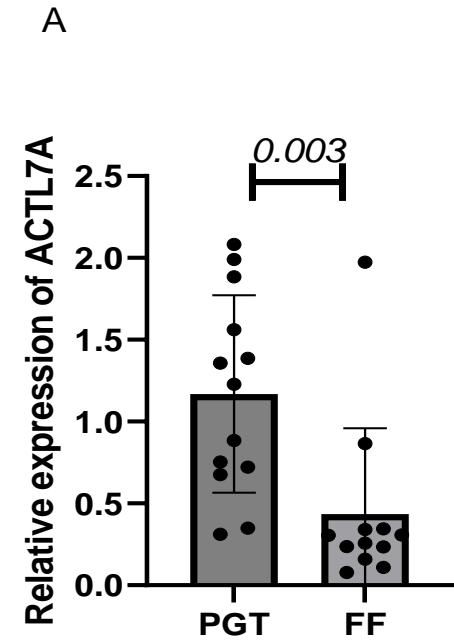




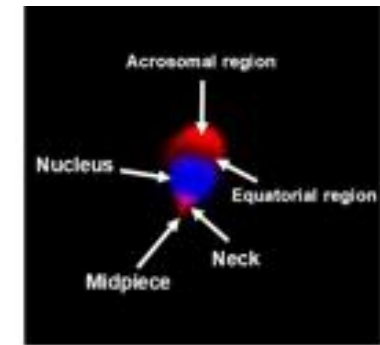


**UNDER REVIEW**





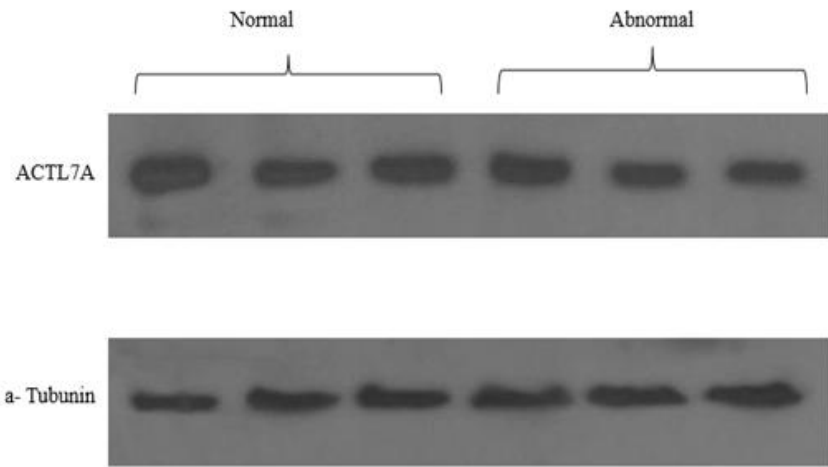
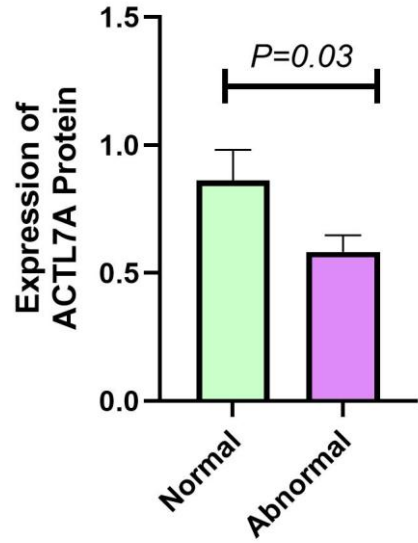
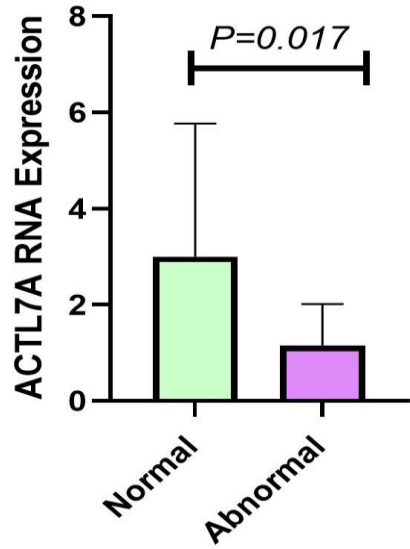
Sperm with abnormal ACTL7A



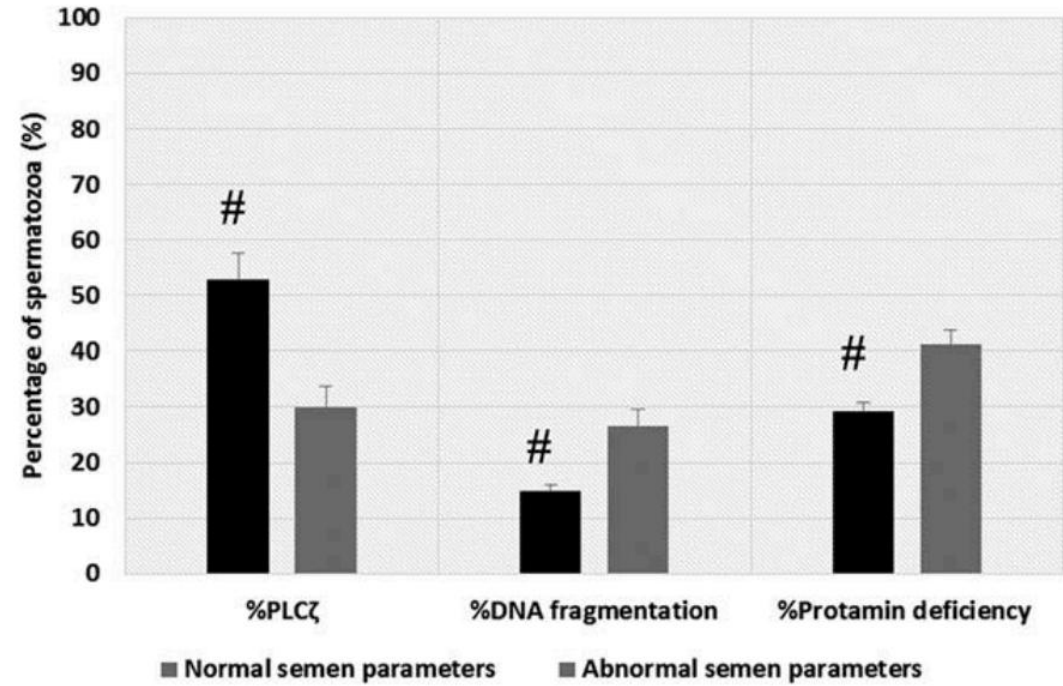
Sperm with normal ACTL7A

**Immunofluorescence localization of ACTL7A protein in human spermatozoa.**

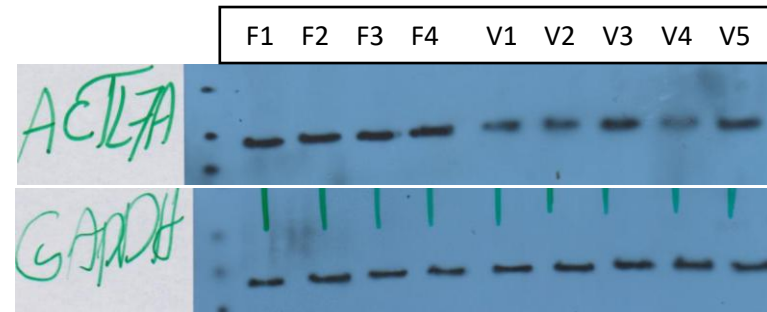
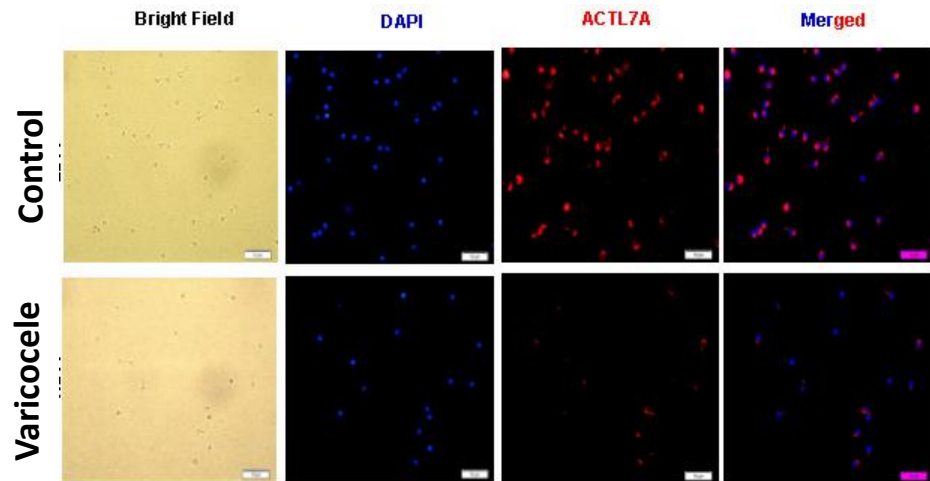
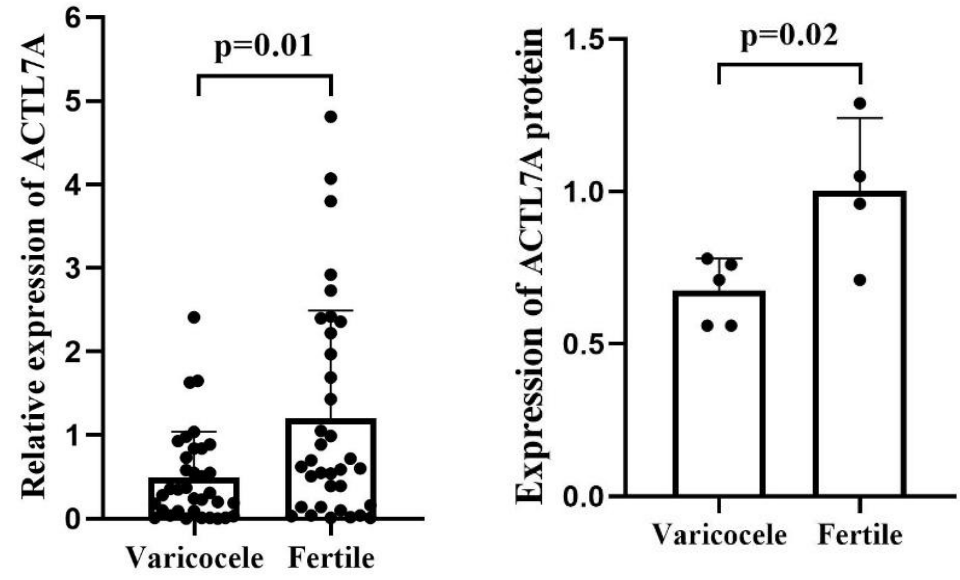
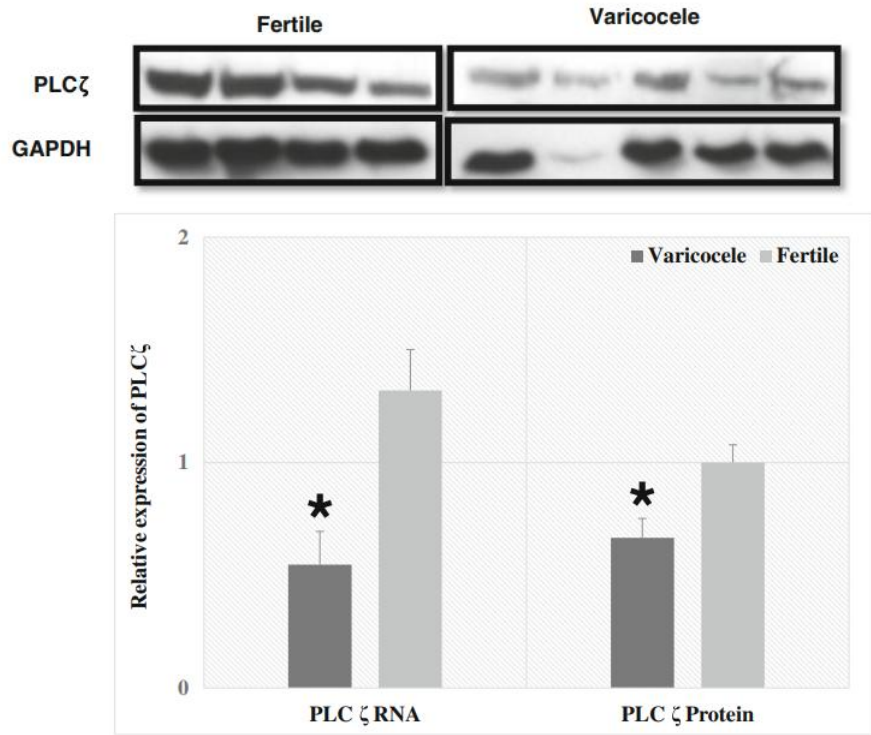
**UNDER REVIEW**

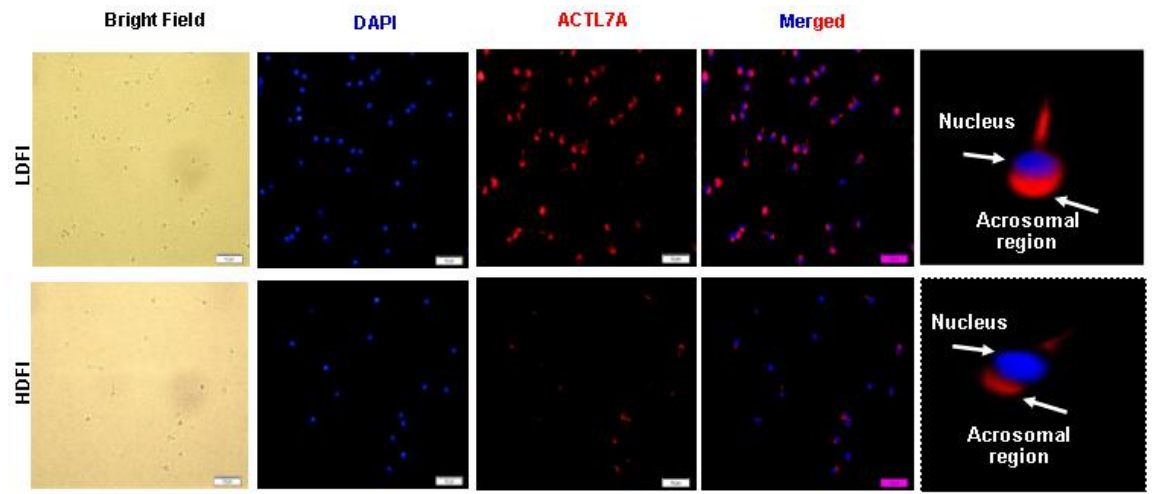
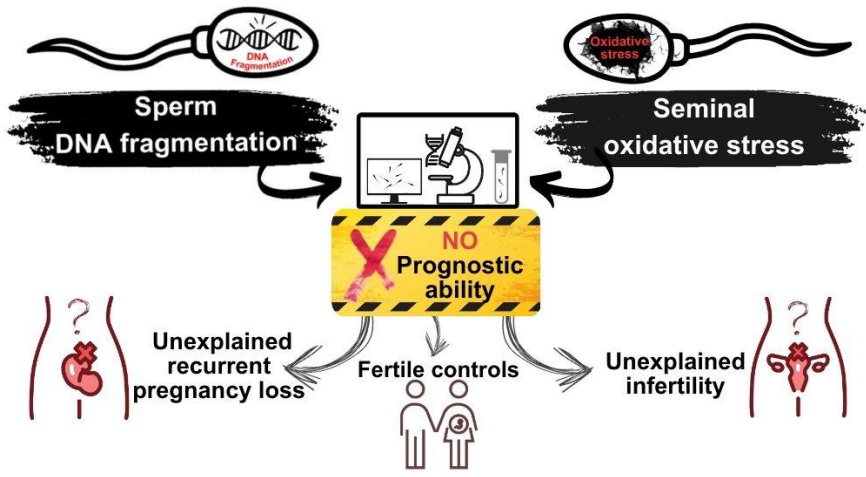


SYSTEMS BIOLOGY IN REPRODUCTIVE MEDICINE

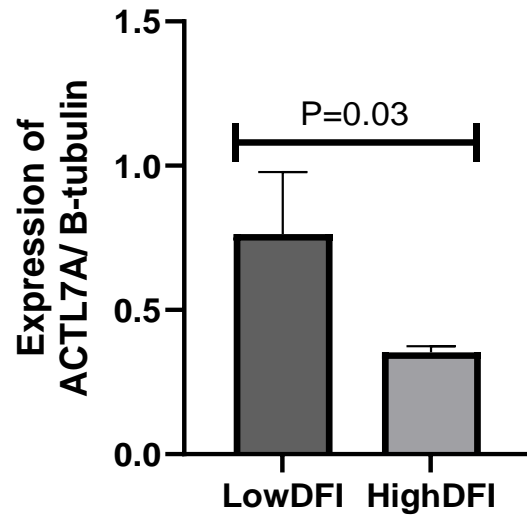
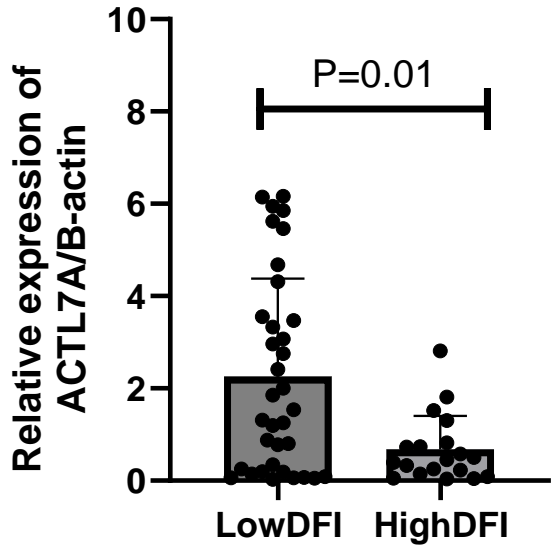
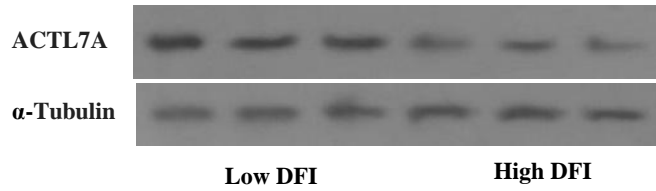


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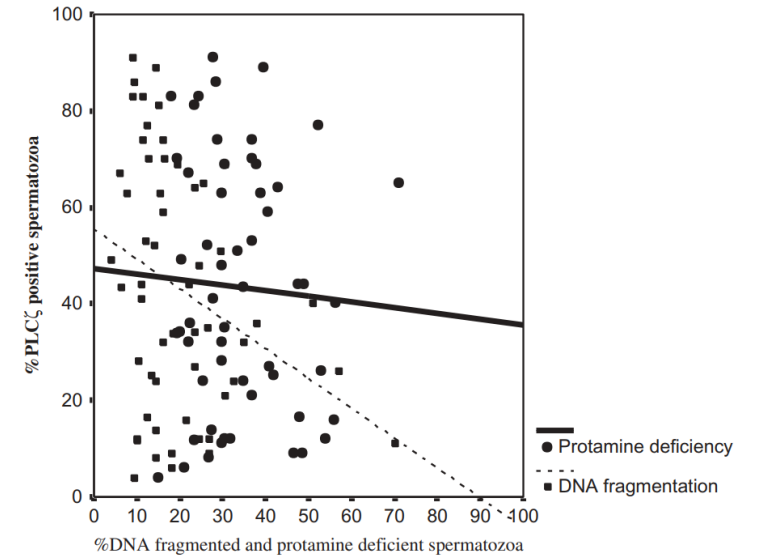


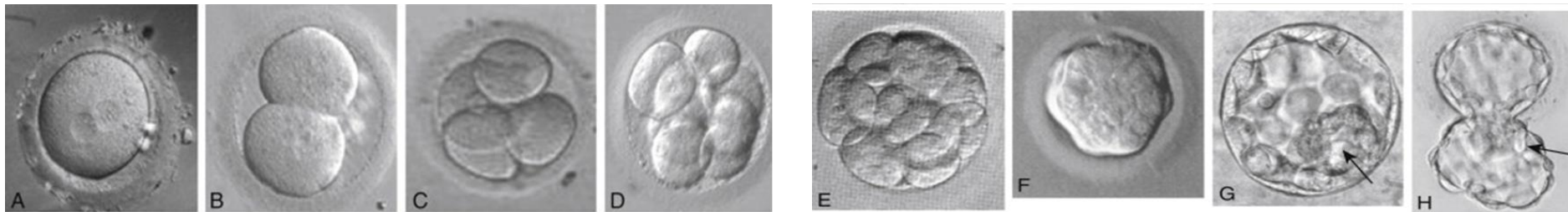


IJFS (In press)



SYSTEMS BIOLOGY IN REPRODUCTIVE MEDICINE

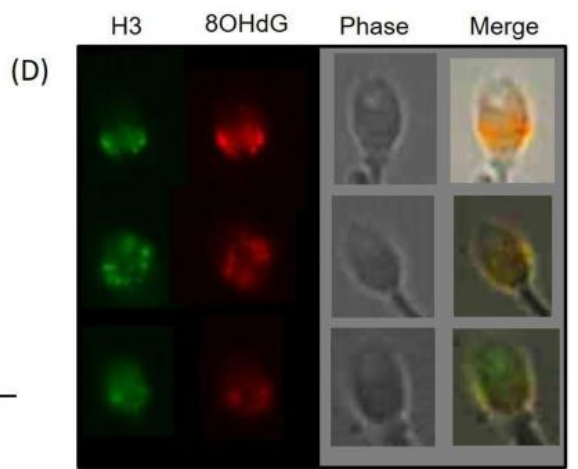
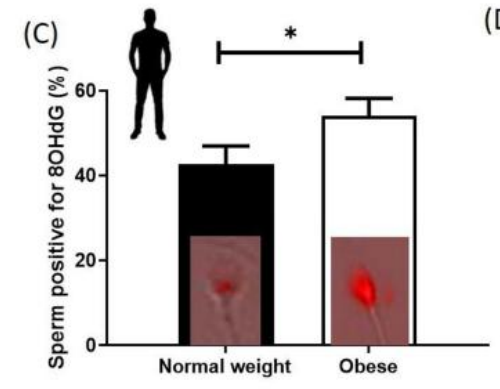
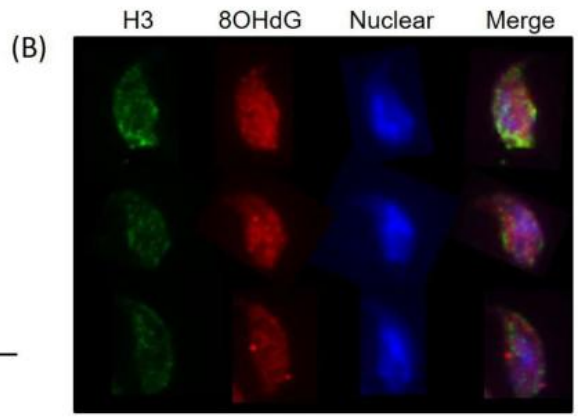
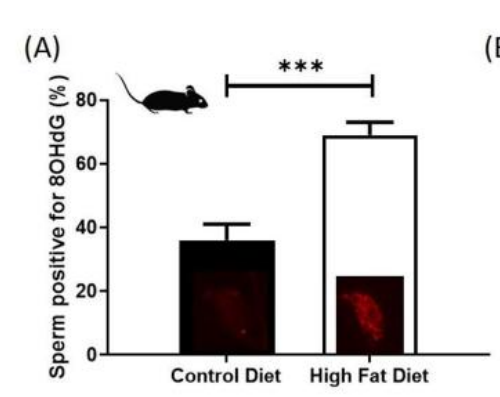
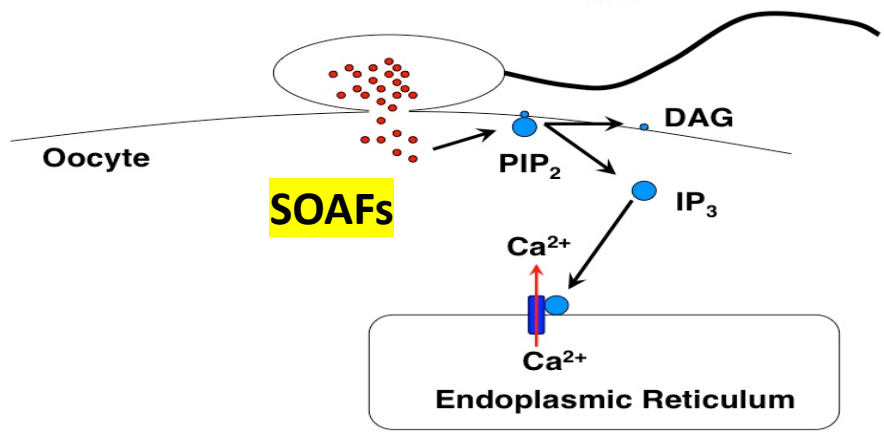




## Sperm DNA Fragmentation



- Predict blastocyst rate**  
Tello Mora et al 2018, Kim et al 2019
- Implantation failure**  
Ribas-Maynou et al 2019
- Miscarriage risk**  
Kamkar et al 2018
- Pregnancy: IVF 65%↓, ICSI 31%↓**  
(>2,000 cycles) Simon et al 2017



## **Conclusion:**

Artificial oocyte activation (AOA) after ICSI — especially when using calcium ionophore — can significantly improve fertilization rates, embryo development, pregnancy, and live birth outcomes, particularly in cases with poor or failed fertilization.

To date, most studies have shown that AOA does not increase the risk of congenital anomalies, miscarriage, or developmental problems, and overall it is considered a relatively safe technique.

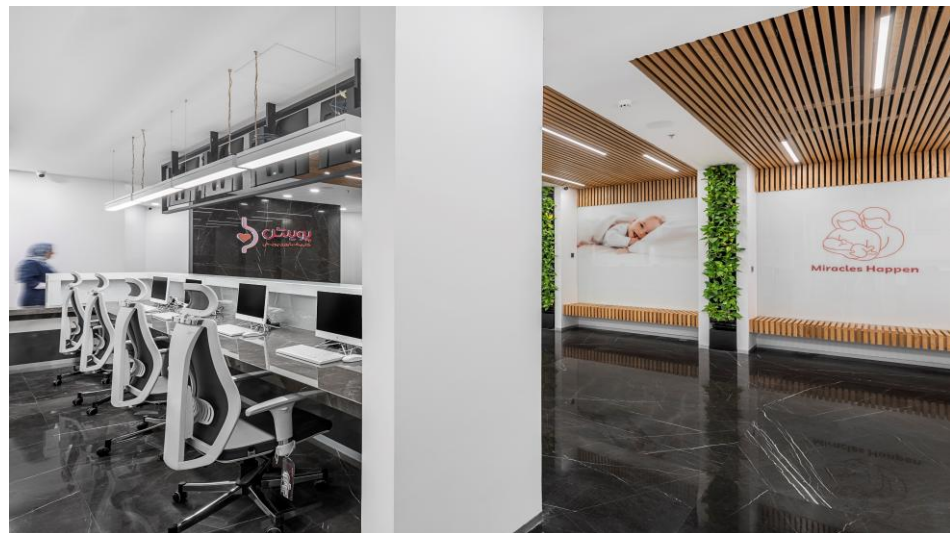
However, because artificial activation cannot perfectly replicate the natural physiological process, and long-term data remain limited, AOA should be applied only in well-defined cases where there is clear clinical evidence of oocyte activation deficiency or repeated fertilization failure, rather than being used routinely for all ICSI patients.



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Attention**