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Effect of A23187 ionophore treatment on human blastocyst development—a sibling oocyte study

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Abstract

Purpose To investigate whether treatment with commercially available ready-to-use A23187 ionophore (GM508-CultActive) improves embryo development outcome in patients with a history of embryo developmental problems.

Methods This is a uni-center prospective study in which sibling oocytes of patients with embryos of poor quality on day 5 in the previous cycle were treated or not with CultActive.

Results Two hundred forty-seven metaphase II (MII) oocytes from 19 cycles performed between 2016 and 2019 were included in the study. After ICSI, the sibling oocytes were assigned to the treatment group or to the control group, following an electronically generated randomization list. A number of 122 MII were treated with CultActive and 125 MII had no treatment and were assigned to the control group. No difference in fertilization rate ($p=0.255$) or in the capacity of embryos to reach good quality on day 5 ($p=0.197$) was observed between the two groups. The utilization rates defined as the number of embryos transferred or cryopreserved per mature oocyte ($p=0.438$) or per fertilized oocytes ($p=0.299$) were not significantly different between the treated group and the control group.

Conclusion The results of the current study do not support the use of CultActive in cases with embryo developmental problems.

Keywords Assisted oocyte activation · A23187 ionophore · CultActive · Blastocyst development

Introduction

The development of the preimplantation human embryo begins with fertilization of the oocyte followed by successive cell divisions and cell differentiation events that lead to the formation of the blastocyst able to implant in the endometrial tissue.

For fertilization to take place, the sperm should provide factors necessary to induce calcium oscillations in the ooplasm and consequently to activate the oocyte. This sperm-derived oocyte-activating factor, identified as phospholipase C zeta [1, 2], catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) leading to the generation of diacylglycerol (DAG) and inositol triphosphate (IP3) [3]. The IP3 binds to its receptors on the membrane of the endoplasmic reticulum and induces calcium release into the cytoplasm that will generate Ca^{2+} oscillations [4] necessary for oocyte fertilization and embryo development [5]. These oscillations will last for several hours and modulate essential processes leading to oocyte activation: cortical granule exocytosis, block to polyspermy, cell cycle resumption, and recruitment of maternal mRNA [6, 7].

The absence of Ca^{2+} oscillations leads to failed or poor oocyte fertilization, as in the cases of globozoospermia or spermatozoa of poor quality where the lack of the sperm oocyte activation factor is considered responsible [8, 9]. The use of Ca^{2+} ionophores, such as ionomycin or calcimycin (A23187) during the ICSI procedure artificially increases the level of intracytoplasmic Ca^{2+}, allowing the events of fertilization to take place (assisted oocyte activation, AOA). As a consequence, ionomycin is being used in assisted reproductive technologies (ART) for cases of previously low or total absence of fertilization after insemination by ICSI.

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An alternative use of Ca^{2+} ionophores in ART has also been reported for cases with embryo developmental problems [10, 11]. Experiments on mice oocytes showed that each of the processes leading to oocyte activation has different requirements in terms of the number of Ca^{2+} oscillations necessary for both their initiation and completion [12]. Artificial modulation of the amplitude, frequency, and duration of Ca^{2+} oscillations was shown to impact embryo development and have a considerable effect on the post-implantation period [13–15]. In addition, it was reported that mouse oocyte activation and embryonic development might be mediated by the total summation of Ca^{2+} peaks during the oscillation period [16–18]. As a consequence, A23187 ionophore (in a ready-to-use, commercially available form GM508-CultActive) was used as a strategy to improve embryonic development in cases experiencing pronuclear [10] or embryonic arrest [11].

In the latter study, however, one major limitation was the choice of the control group represented by the previous cycle of the patient. To overcome this shortcoming, we designed a prospective study in which sibling oocytes of patients with embryos of poor quality on day 5 in the previous cycle were treated or not with GM508-CultActive. This study would allow a more accurate investigation of the actual influence of the A23187 ionophore on the development of embryos derived from the same oocyte cohort as the untreated controls.

Materials and methods

Study population

ICSI cycles performed in our center between January 2016 and 2019 were included in the study. Patients were eligible for inclusion in the study if they had undergone at least one previous ICSI cycle in our center with either day 5 transfer of embryos of moderate or poor quality or no transfer on day 5 due to insufficient embryo quality. Therefore, all patients presented in the previous cycle only embryos of moderate and/or poor quality on day 5. Every patient was included only once. Patients with poor fertilization (< 30%) or failed fertilization in the previous cycle were excluded from the study. Cycles with surgically retrieved spermatozoa, egg recipient cycles, in vitro maturation cycles, and preimplantation genetic testing cycles were also excluded. The study has the approval of the Ethical Committee in UZ Brussel (nr B.U.N. 143,201,628,846).

Ovarian stimulation and oocyte retrieval

The ovarian stimulation was performed using either highly purified urinary menotropin (Menopur, Ferring Pharmaceuticals A/S, Denmark) or recombinant FSH (Puregon, Elonva, Pergoveris, Merck Sharp & Dohme, USA; Gonal F, Merck, Germany) in combination with either a GnRH antagonist (Orgalutran, Merck Sharp & Dohme, USA; Cetrotide®, Merck, Germany) or agonist (Suprefact, Aventis Pharma, Germany; Gonapeptyl, Ferring, Switzerland; Decapeptyl, Ipsen Pharma Biotech, France) for pituitary downregulation. Final oocyte maturation and ovulation were triggered as soon as at least 3 follicles ≥ 17 mm were visible on transvaginal pelvic ultrasound. Oocyte retrieval (OR) was carried out by vaginal ultrasound-guided puncture of the ovarian follicles approximately 36 h after ovulation triggering.

Oocyte retrieval and ICSI

Following oocyte retrieval, the cumulus-oocyte-complexes (COCs) were placed in Origio®Fert™ medium (Cooper Surgical, USA) at 6% CO_2/5% O_2 and 37 °C until ICSI procedure. The COC denudation was performed in group in a 100 µl *ICSI*Cumulase® drop (Cooper Surgical, USA) covered with Ovoil (Vitrolife, Sweden). The mature oocytes (Metaphase II) were injected in 5 µl Quinn's AdvantageTM Medium with Hepes (In-Vitro Fertilization, Inc., Cooper Surgical, USA).

Treatment intervention

For the ionophore treatment, the GM508-CultActive bicarbonate-buffered reagent (CultActive) containing an undisclosed concentration of A23187 ionophore (ready-to-use A23187 ionophore; Gynemed, Germany, CE labeled) was used. For this, 60-cm Petri dishes (353,652, Falcon) were prepared as follows: one drop of 30 µl CultActive and 2 drops of 40 µl Origio®Sequential Cleav™ (Cooper Surgical, USA) for each mature oocyte, for a maximum of three mature oocytes treated in one dish. The dishes were covered with Ovoil (Vitrolife, Sweden) and placed in the incubator for at least 4 h at 6% CO_2/5% O_2 and 37 °C use to allow media equilibration prior to use.

Immediately after ICSI, the sibling oocytes were assigned to the treatment group or to the control group, based on an electronically generated randomization list. The oocytes in the control group were placed directly in Origio®Sequential Cleav™ medium (see below).

In the treatment group, the inseminated oocytes were incubated for 15 min in pre-equilibrated CultActive drops, followed by two consecutive washing steps in the 40 µl drops of Origio®Sequential Cleav™ medium. The treated oocytes were further cultured in similar conditions as the ones from the control group (no CultActive treatment after ICSI).

Embryo culture and evaluation

Following ICSI, the inseminated oocytes (both from treated and control groups) were cultured in vitro at 37 °C in an atmosphere of 6% CO_2, 5% O_2, and 89% N_2 in individual 25 µl droplets of culture media (Origio®Sequential Cleav™ until day 3 and Origio®Sequential Blast™ from day 3 until day 5/6, Cooper Surgical, USA) covered with oil (Ovoil, Vitrolife).

Fertilization was assessed 16–18 h after ICSI by the presence of two pronuclei (PN). On day 3, the embryos were evaluated for the number and symmetry of blastomeres, percentage of fragmentation, vacuolization, granulation, and multinucleation. On day 5, the blastocysts were scored according to the grading system by Gardner and Schoolcraft (1999) [19]. Based on all these parameters, an embryo quality score (EQ) was assigned to each embryo based on a predefined algorithm, defining four categories: excellent, good, moderate, or poor as previously described [20].

Regarding day 3 embryos: excellent embryos had at least seven blastomeres with cell stage-specific blastomere size symmetry and had ≤ 10% fragmentation. Good embryos had at least six blastomeres and/or ≤ 20% fragmentation and/or blastomeres with stage-specific cell size symmetry. Moderate embryos had at least four cells and/or 20–50% fragmentation (with or without a combination of vacuoles or granulation) and/or multinucleation in ≤ 50% of the blastomeres, and/or large differences in cell size. Embryos considered to be of poor quality had less than four cells and/or > 50% fragmentation and/or multinucleation in > 50% of the blastomeres (with or without a combination of vacuoles) [20].

Regarding day 5 embryos, the following parameters were used to define the blastocyst quality score: (i) the expansion stage, (ii) the number of cells joining the blastulation, and (iii) the appearance of the trophectoderm (TE) and inner cell mass (ICM). Excellent blastocysts were expanded with TE type A and ICM type A or B. Good blastocysts were full or expanded with a TE type B and ICM type A or B. Moderate quality was obtained in either early blastocysts or full/expanded blastocysts with a type C TE or ICM. Poor-quality embryos were scored as such if they were arrested, compacted, fragmented, degenerated, or lacked a TE or ICM [20].

Embryo transfer and cryopreservation

Fresh embryos were considered eligible for transfer if they reached at least moderate quality and eligible for freezing if they reached at least good quality. The embryo with the best quality from the cohort was chosen for embryo transfer (ET), irrespective of the group they belong to. Each cycle had either a fresh ET or a "freeze-all" policy when, due to medical reasons (risk of OHSS, progesterone rise at the day of ovulation triggering, thin endometrium), the embryos were cryopreserved for later use.

Outcome parameters

The main outcome of the study was the utilization rate defined as the total number of embryos transferred and cryopreserved per fertilized oocyte. Additional embryological parameters such as fertilization rate and embryo quality, as well as clinical outcome, were also reported.

Statistical analysis

Group sample sizes of at least 121 MII in the control group and at least 121 MII in the treatment group to detect a difference in embryo utilization rate between the groups from 15 to 30%, with 80% power. The outcome measures were compared using logistic generalized estimating equations (GEE) regression analysis in order to account for the fact that multiple oocytes were derived from the same treatment cycle. The statistical analysis was performed in Stata version 13.1 and *p*-values below 0.05 were considered statistically significant.

Results

The patient characteristics and the distribution of the baseline cycle characteristics are presented in Table 1. Couples included in the study presented male factor indication ($n = 7$), female factor indication ($n = 6$; age indication (3), PCOS ($n = 1$), tubal ($n = 1$), and endometriosis ($n = 1$)), and unexplained infertility ($n = 6$). Mean female age was 35.3 ± 5.5 years (range of 26–43 years).

The embryological data are presented in Table 2. In 19 cycles, a total number of 301 COCs were retrieved (mean 13.05 ± 10.5), of which 247 (82%) were mature (MII). After ICSI, 122 MII were treated with CultActive and 125 MII had no treatment and were assigned to the control group. Fertilization rate did not differ significantly between the two groups (85.2% vs. 79.2%, respectively; $p = 0.255$). The capacity of fertilized oocytes to develop into good quality embryos (excellent and good) on day 3 was not significantly different between the treatment and the control group (65.4% vs. 70.7%, respectively; $p = 0.281$). Similarly, no significant difference was observed in the blastulation rate (40.4% vs. 38.3%; $p = 0.758$) or in the capacity of embryos to reach good quality (excellent and good) on day 5 (11.5% vs. 18.2%; $p = 0.197$) between the two groups.

The utilization rates per mature oocyte (13.9% vs. 17.6%; $p = 0.438$) or per fertilized oocytes (16.3% vs. 22.2%; $p = 0.299$) were not significantly different between the CultActive group and the control group.

Table 1 General patient and cycle characteristics

	Patient's age	Infertility	Suppression type	Sperm concentration (mil/ml)	% progressive and non-progressive motility	Nr COC	CultActive-treated group				Control group			
							Nr MII	Nr 2PN	Nr ET	Nr EC	Nr MII	Nr 2PN	Nr ET	Nr EC
1	37	OAT	Antagonist	28.6	61	16	7	5	1	0	8	7	0	0
2	29	Idiopathic	Antagonist	58.5	42	4	2	1	0	0	2	2	1	0
3	26	OAT	Antagonist	13.5	58	12	6	4	0	0	6	6	0	2
4	40	OAT	Antagonist	38.7	30	5	2	0			3	2	0	1
5	36	Idiopathic	Antagonist	80.5	86	17	8	7	0	1	9	8	0	3
6	28	Idiopathic	Antagonist	57.5	60	15	8	6	0	1	7	1	0	1
7	30	PCOS	Antagonist	120.5	16	48	24	20	0	3	24	19	0	4
8	37	OAT	Agonist	0.24	17	6	3	3	1	0	3	3	1	0
9	32	Idiopathic	Antagonist	76.0	48	9	4	4	0	0	5	3	0	0
10	33	Endometriosis	Antagonist	13.5	34	12	6	5	1	0	6	5	0	0
11	35	Tubal	Agonist	114.0	77	10	5	4	0	0	5	3	0	0
12	33	Idiopathic	Antagonist	57.35	32	7	4	4	1	0	3	3	0	1
13	43	Age	Antagonist	38.4	48	6	3	3	1	0	3	1	1	0
14	41	Age	Antagonist	10.1	23	10	5	4	0	1	5	4	0	1
15	43	OAT	Antagonist	0.66	74	5	3	3	1	1	2	2	0	0
16	28	OAT	Antagonist	0.34	27	12	6	6	1	1	6	6	0	2
17	42	Andrologic	Agonist	32.7	71	13	6	6	0	1	7	6	1	1
18	37	Idiopathic	Antagonist	21.8	37	31	15	14	0	1	16	14	0	2
19	41	Age	Agonist	32.6	12	10	5	5	0	0	5	4	0	0

OAT, oligoasthenotheratospermia; *PCOS*, polycystic ovary syndrome; *COC*, cumulus-oocyte-complex. Semen parameters represent values before capacitation; MII, metaphase II oocyte; 2PN, fertilized oocytes; ET, embryos transferred; EC, embryos cryopreserved

Table 2 Comparison of embryological data between the CultActive-treated group and the control group

	CultActive-treated group	Control group	*P* value
No of MII	122	125	
Fertilization/MII (%)			
2PN	104/122 (85.2)	99/125 (79.2)	0.255
1PN	6/122 (4.9)	5 (4.0)	
3PN	2/122 (1.6)	5 (4.0)	
Degenerated	5/122 (4.1)	4 (3.2)	
Not fertilized	5/122 (4.1)	12/125 (9.6)	
Embryo quality day 3/2PN (%)			
Excellent + good	68/104 (65.4)	70/99 (70.7)	0.281
Moderate + poor	36/104 (34.6)	29/99 (29.3)	
Day 3 cell number			
< 6	27	19	
6–7	27	24	
= 8	24	27	
> 8	22	23	
= 8 + > 8	46	50	
Compacting	4	4	
Degenerated	0	2	
Day 5 evaluations			
Blastocyst formation			
Bl1	18	11	
Bl2	8	11	
Bl3	8	10	
Bl4	8	6	
Total blastocyst formation/2PN (%)	42/104 (40.4)	38/99 (38.3)	0.758
Embryo quality day 5/2PN (%)			
Excellent + good	12/104 (11.5)	18/99 (18.2)	0.197
Moderate + poor	92/104 (88.5)	81/99 (81.8)	
Nr of embryos transferred	7	4	
Nr of embryos cryopreserved on day 5	6	9	
Nr of embryos cryopreserved on day 6	4	9	
Utilized embryos (nr of embryos transferred and cryopreserved)	17	22	
Utilization rate per nr of MII (%)	17/122 (13.9)	22/125 (17.6)	0.438
Utilization rate per nr of 2PN (%)	17/104 (16.3)	22/99 (22.2)	0.299

Fertilization is given as normally fertilized oocytes (2PN), oocytes with one (1PN) or three pronuclei (3PN), degenerated oocytes or not fertilized after ICSI. *P* value < 0.05 is significant

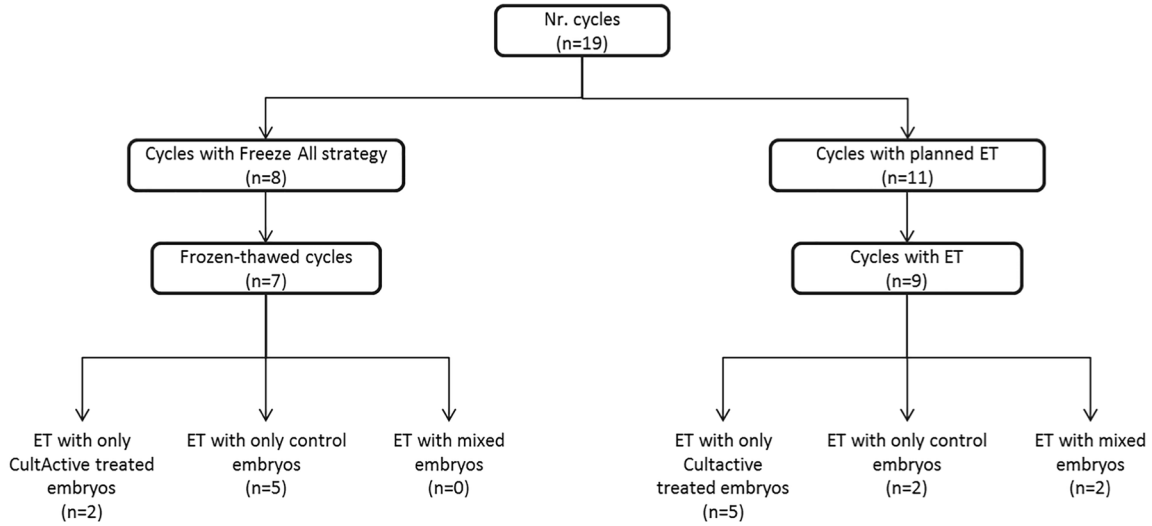
From the 19 cycles, 11 were planned as fresh ET and eight as a freeze-all strategy. From the fresh ET group, two cycles had no ET due to poor embryo quality. Regarding the freeze-all group, in one cycle, no embryos were vitrified due to insufficient quality.

The clinical outcome from the fresh ET cycles and from the first frozen embryotransfer cycle of each patient from the freeze-all group is presented in Table 3: seven single ETs were performed with embryos only from the CultActive-treated group, six single ETs and one double ET with embryos only from the control group, and two ETs performed with mixed embryos. Seven deliveries were reported, with four, two, and one delivery in each category, accordingly. No major malformations at delivery were reported. Data on the weight and height of the seven newborns is presented in Table 3.

Discussion

The aim of the present prospective study was to investigate if Ca^{2+} ionophore treatment with commercially available ready-to-use A23187 ionophore (GM508-CultActive) improves embryo utilization rate in patients with a history of embryo developmental problems. For this purpose, sibling mature oocytes were treated or not with CultActive and the

Table 3 Clinical and neonatal outcome from the fresh ET cycles and from the first frozen-thawed cycle of each patient from the Freeze All group



	ET with only CultActive treated embryos (n=2)	ET with only control embryos (n=5)	ET with mixed embryos (n=0)	ET with only Cultactive treated embryos (n=5)	ET with only control embryos (n=2)	ET with mixed embryos (n=2)
Positive hCG	2	2*	-	5	1	1
Clinical pregnancy	2	2	-	5	1	1
Clinical abortion	0	1	-	2	0	0
Lost to follow up	0	0	-	1	0	0
Delivery	2	1	-	2	1	1
Birth weight/height (g/cm)	3000/49.5 4300/na	3240/50.0	-	2395/45.0 3760/53.0	2925/48.0	3900/50.5

*1 single ET and 1 double ET (clinical pregnancy with 1 vanishing twin)

embryo development at cleavage and blastocyst stage was evaluated. Our results showed that CultActive treatment is not associated with improved embryo development or utilization rate.

Our data are in contradiction with the previous reports of Ebner et al. [11] and Lv et al. [21]. Both studies published increased blastocyst formation after treatment with A23187 ionophore. In both studies, the oocytes were incubated immediately after ICSI in A23187 solution for 15 min, but is not clear if the same concentration of ionophore was used by the two groups. While Lv et al. used 5 µmol/l A23187, Ebner et al. [11] used (similar to our protocol) CultActive with an undisclosed concentration of ionophore. In addition, both studies use the previous cycle of the patient as a control. By doing this, the possible effect of different stimulation protocols and/or possible differences in quality of culture systems between cycles or intra-individual cycle-to-cycle differences was underestimated. Moreover, Ebner et al. [11] recruited patients from five centers. To overcome these problems, we designed a single-center sibling oocyte study. Only patients with poor embryo quality on day 5 in the previous cycle performed in our center were included. In addition, similar culture conditions were used for both cycles in all patients, but six patients had a different stimulation protocol compared to their previous cycle. Recently, data on embryo morphokinetics following the use of CultActive on sibling oocytes were reported [22]. The study based on a cohort of 78 ICSI patients showed significantly improved fertilization and utilization rates. Our findings contradict these results and may be explained by differences in patient cohorts, embryo transfer protocol, and/or the way data are reported. Shebl et al. [22] included patients with a suspected fertilization problem, which was not the case in our cohort. Contrary to our policy (ET on day 5 only and blastocyst vitrification on day 5/6), Shebl et al. [22] performed blastocyst transfer and vitrification on day 4 or 5. These differences can have an impact on final embryo quality and consequently on utilization rates as different laboratories may have different criteria for transfer and vitrification. No clear description of the criteria to define an embryo's eligibility for transfer or vitrification was mentioned by Shebl et al. [22]. In addition, there is a difference in the way the utilization rate was reported: per number of fertilized oocytes (2PN) in our dataset and per number of embryos in culture to day 5 in the report of Shebl et al. [22]. We consider that, when looking at the effect of a treatment on embryo developmental potential, the best approach is to perform the analysis per normally fertilized oocyte.

The signaling events involved in oocyte activation processes, triggered by the sperm, rely on factors from the

oocyte, like PIP2, DAG, or IP3. This suggests that, besides the sperm, the oocyte also plays a role in oocyte activation. The couples included in this study represent a rather heterogeneous population regarding the cause of infertility. Unfortunately, due to the limited number of patients included, the impact of CultActive treatment on embryological outcomes in different infertility subgroups could not be evaluated.

The present study includes patients with sperm capable of at least initiating oocyte activation as proven by fertilization and early cleavage rates observed in the present and previous cycle. Based on our data, the extra Ca^{2+} present transiently in the cytoplasm due to ionophore exposure has no impact on blastocyst development. Nevertheless, it should be worth mentioning that all transfers of embryos originated following the CultActive led to a clinical pregnancy.

One of the most frustrating aspects of an IVF clinic is the transfer of a blastocyst of lower quality or lack of blastocysts for transfer, in cycles with proven fertilization. Understandably, the use of A23187 ionophore to overcome this situation is tempting. However, little is known about the impact of intracellular Ca^{2+} manipulation in human oocytes. Until now, no adverse neonatal events were reported following Ca^{2+} ionophore treatment [23–25]. From our series, no major malformations were described from the four babies born following transfer with embryos from the CultActive group, results that are in line with our previous report [24].

The results reported here should be confirmed in a larger size study. Before more research is performed, the use of Ca^{2+} ionophore in ART should be limited to strict indications, where its benefit has been proven, as in cases of globozoospermia or previously failed or low fertilization [26, 27].

The main strength of our study is its prospective, sibling oocyte nature that allows us to evaluate the impact of CultActive on embryo development within the same treatment cycle, and the fact that all cycles and previous cycles were performed in a single center. The limitation of our study is the small number of patients included.

In conclusion, the results of the current study do not support the use of CultActive in cases with embryo developmental problems.

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Declarations

Conflict of interest The authors declare no competing interests.

References

1. Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney ML, Swann K, Lai FA. PLC zeta: a sperm-specific trigger of Ca(2+) oscillations in eggs and embryo development. Development. 2002;129(15):3533–44.
2. Swann K, Larman MG, Saunders CM, Lai FA. The cytosolic sperm factor that triggers Ca2+ oscillations and egg activation in mammals is a novel phospholipase C: PLCzeta. Reproduction. 2004;127:431–9.
3. Yeste M, Jones C, Amdani SN, Patel S, Coward K. Oocyte activation deficiency: a role for an oocyte contribution? Hum Reprod Update. 2016;22(1):23–47. https://doi.org/10.1093/humupd/dmv040.
4. Whitaker M. Calcium at fertilization and in early development. Physiol Rev. 2006;86(1):25–88. https://doi.org/10.1152/physrev.00023.2005.
5. Jones KT. Intracellular calcium in the fertilization and development of mammalian eggs. Clin Exp Pharmacol Physiol. 2007;34(10):1084–9. https://doi.org/10.1111/j.1440-1681.2007.04726.
6. Schultz RM, Kopf GS. Molecular basis of mammalian egg activation. Curr Top Dev Biol. 1995;30:21–62. https://doi.org/10.1016/s0070-2153(08)60563-3.
7. Amdani SN, Jones C, Coward K. Phospholipase C zeta (PLCζ): oocyte activation and clinical links to male factor infertility. Adv Biol Regul. 2013;53(3):292–308. https://doi.org/10.1016/j.jbior.2013.07.005.
8. Yoon SY, Jellerette T, Salicioni AM, Lee HC, Yoo MS, Coward K, et al. Human sperm devoid of PLC, zeta 1 fail to induce Ca2+ release and are unable to initiate the first step of embryo development. J Clin Invest. 2008;118:3671–81. https://doi.org/10.1172/JCI36942.
9. Heytens E, Parrington J, Coward K, Young C, Lambrecht S, Yoon SY, Fissore RA, Hamer R, et al. Reduced amounts and abnormal forms of phospholipase C zeta in spermatozoa from infertile men. Hum Reprod. 2009;24:2417–28. https://doi.org/10.1093/humrep/dep207.
10. Darwish E, Magdi Y. A preliminary report of successful cleavage after calcium ionophore activation at ICSI in cases with previous arrest at the pronuclear stage. Reprod Biomed Online. 2015;31(6):799–804. https://doi.org/10.1016/j.rbmo.2015.08.012.
11. Ebner T, Oppelt P, Wöber M, Staples P, Mayer RB, Sonnleitner U, Bulfon-Vogl S, Gruber I, Haid AE, Shebl O. Treatment with Ca2+ ionophore improves embryo development and outcome in cases with previous developmental problems: a prospective multicenter study. Hum Reprod. 2015;30(1):97–102. https://doi.org/10.1093/humrep/deu285.
12. Ducibella T, Huneau D, Angelichio E, Xu Z, Schultz RM, Kopf GS, Fissore R, Madoux S, Ozil JP. Egg-to-embryo transition is driven by differential responses to Ca(2+) oscillation number. Dev Biol. 2002;250(2):280–91.
13. Ozil JP, Huneau D. Activation of rabbit oocytes: the impact of the Ca2+ signal regime on development. Development. 2001;128(6):917–28.
14. Malcuit C, Kurokawa M, Fissore RA. Calcium oscillations and mammalian egg activation. J Cell Physiol. 2006;206(3):565–73. https://doi.org/10.1002/jcp.20471.
15. Machaty Z. Signal transduction in mammalian oocytes during fertilization. Cell Tissue Res. 2016;363(1):169–83. https://doi.org/10.1007/s00441-015-2291-8.
16. Ozil JP, Banrezes B, Tóth S, Pan H, Schultz RM. Ca2+ oscillatory pattern in fertilized mouse eggs affects gene expression and development to term. Dev Biol. 2006;300(2):534–44. https://doi.org/10.1016/j.ydbio.2006.08.041.
17. Tóth S, Huneau D, Banrezes B, Ozil JP. Egg activation is the result of calcium signal summation in the mouse. Reproduction. 2006;131(1):27–34. https://doi.org/10.1530/rep.1.00764.
18. Ferrer-Buitrago M, Bonte D, De Sutter P, Leybaert L, Heindryckx B. Single Ca2+ transients vs oscillatory Ca2+ signaling for

assisted oocyte activation: limitations and benefits. Reproduction. 2018;155(2):105–19. https://doi.org/10.1530/REP-17-0098.
19. Gardner DK, Schoolcraft WB. In-vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. Towards reproductive certainty: fertility and genetics beyond 1999. Carnforth: Parthenon Press; 1999. p. 378–88.
20. De Munck N, Santos-Ribeiro S, Mateizel I, Verheyen G. Reduced blastocyst formation in reduced culture volume. J Assist Reprod Genet. 2015;32(9):1365–70. https://doi.org/10.1007/s10815-015-0541-z.
21. Lv M, Dan Zhang D, He X, Chen B, Li Q, Ding D, Hao Y, Xue R, Ji D, et al. Artificial oocyte activation to improve reproductive outcomes in couples with various causes of infertility: a retrospective cohort study. Reprod Biomed Online. 2020;40(4):501–9. https://doi.org/10.1016/j.rbmo.2020.01.001.
22. Shebl O, Trautner PS, Enengl S, Reiter E, Allerstorfer C, Rechberger T, Oppelt P, Ebner T. Ionophore application for artificial oocyte activation and its potential effect on morphokinetics: a sibling oocyte study. J Assist Reprod Genet. 2021;38(12):3125–33. https://doi.org/10.1007/s10815-021-02338-3.
23. Miller N, Biron-Shental T, Sukenik-Halevy R, Klement AH, Sharony R, Berkovitz A. Oocyte activation by calcium ionophore and congenital birth defects: a retrospective cohort study. Fertil Steril. 2016 1;106(3):590–6. https://doi.org/10.1016/j.fertnstert.2016.04.025
24. Mateizel I, Verheyen G, Van de Velde H, Tournaye H, Belva F. Obstetric and neonatal outcome following ICSI with assisted oocyte activation by calcium ionophore treatment. J Assist Reprod Genet. 2018;35(6):1005–10. https://doi.org/10.1007/s10815-018-1124-6.
25. Li B, Zhou Y, Yan Z, Li M, Xue S, Cai R, Fu Y, Hong et al. Pregnancy and neonatal outcomes of artificial oocyte activation in patients undergoing frozen-thawed embryo transfer: a 6-year population-based retrospective study. Arch Gynecol Obstet. 2019 Oct;300(4):1083–92. https://doi.org/10.1007/s00404-019-05298-3
26. Moaz MN, Khattab S, Foutouh IA, Mohsen EA. Chemical activation of oocytes in different types of sperm abnormalities in cases of low or failed fertilization after ICSI: a prospective pilot study. Reprod Biomed Online. 2006;13(6):791–4. https://doi.org/10.1016/s1472-6483(10)61025-5.
27. Heindryckx B, De Gheselle S, Gerris J, Dhont M, De Sutter P. Efficiency of assisted oocyte activation as a solution for failed intracytoplasmic sperm injection. Reprod Biomed Online. 2008;17(5):662–8. https://doi.org/10.1016/s1472-6483(10)60313-6.