The present status of artificial oocyte activation in assisted reproductive technology

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Intracytoplasmic sperm injection (ICSI) is the most effective treatment for achieving fertilization in assisted reproductive technology (ART). However, fertilization failure occurs. The incidence of fertilization failure after ICSI is 1–5%. Approximately 50% of fertilization failure cases could be attributed to the abnormality of sperm factor. As the fertilization fails after ICSI using mature sperm, round spermatids and globozoospermia, artificial oocyte activation may provide a means of improving fertilization rates in such cases. The oocyte activation treatments used in clinical research include calcium (Ca) ionophore treatment, electrostimulation and strontium treatment. In terms of the efficiency of oocyte activation, electrostimulation and Ca ionophore gave better outcomes than strontium treatment. Strontium treatment causes Ca^{2+} oscillations in mice, so it has been viewed favorably. However, in human oocytes calcium oscillation has not been observed. The fertilization rate after ICSI was low in the case of globozoospermia and wiht round spermatids. Some cases of pregnancy were achieved by ICSI alone and oocyte activation methods were not essential in these cases. Among the various oocyte activation methods currently used, it should be noted that issues of genetic safety have not been addressed for the combined use of these oocyte activation methods. (Reprod Med Biol 2008; **7**: 133–142)

Key words: calcium ionophore, electrostimulation, fertilization failure, intracytoplasmic sperm injection, oocyte activation.

INTRODUCTION

OOCYTE ACTIVATION IS the process by which oocytes arrested in metaphase II of meiosis are stimulated to resume meiosis.[1] This process is marked by pore formation and secretion in the cortical granules, and release of the second polar body. It is necessary for the initiation of fertilization and occurs when the sperm adheres to the cell membrane of the oocyte. However, it can also be triggered by chemical or physical stimulation in a process known as artificial oocyte activation.

Artificial oocyte activation has been used not only to study the mechanism of oocyte activation,[2] but also in studies of parthenogenesis[3] and nuclear transplantation.[4] Activation of oocytes is precipitated by various types of stimulation, including exposure to ultraviolet light,[5] changes in osmotic pressure,[6] treatment with calcium (Ca) ionophores,[3] electrostimulation,[7] and treatment with strontium,[8] puromycin[9] or cycloheximide.[9]

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Interestingly, the fertilization rate resulting from intracytoplasmic sperm injection (ICSI) is increased when the oocytes undergo artificial oocyte activation in hamsters[10,11] and humans.[12] This raises the possibility that artificial oocyte activation might be used to enhance fertilization rates alongside other techniques. As the fertilization fails after ICSI using mature sperm, immature male germ cells and round head sperm, artificial oocyte activation might provide a means of improving the fertilization rates in such cases.

FERTILIZATION BY INTRACYTOPLASMIC SPERM INJECTION

OOCYTE ACTIVATION OCCURS early in the process of fertilization and involves the resumption of meiosis of oocytes arrested at the metaphase stage of meiosis II. The details of the mechanism are not clear, but a transient increase in calcium ions ($[Ca^{2+}]_i$) inside the oocyte is known to play an important role.[13,14] In ICSI-assisted fertilization, the oocyte is activated by a sperm-derived oocyte activation factor (also known as sperm factor) after the injection.[14–16] A decrease or loss in activity of this factor is a possible cause of fertilization failure with ICSI. When this factor is completely

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

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

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

absent, oocyte activation does not occur and the oocyte remains in metaphase II even if ICSI is carried out. We are able to evaluate the ability of sperm to activate oocytes using mouse oocytes.[17,18] Immobilized sperm injected into the oocyte only undergo decondensation if the sperm nucleoprotein and the oocyte reduction mechanism are normal.[19] When the activity level of the sperm factor is reduced, the oocyte activation mechanism is stimulated at a low level, resulting in a reduction in the level of maturation-promoting factor (MPF) in the oocyte and in the release of the second polar body.[20] However, in some cases, MPF is resynthesized and the activation mechanism of the oocyte is halted. In these cases, premature chromatin condensation (PCC) sometimes occurs after the release of the second polar body and decondensation of the sperm injected into the ooplasm (metaphase III).

Although a transient increase in Ca ions in the oocyte is necessary for oocyte activation, subsequent Ca^{2+} oscillations are not absolutely required. In many oocyte activation methods, the oocyte is activated by inducing a single transient increase in the concentration of Ca^{2+}. Although the functional significance of Ca^{2+} oscillations is not clear, its continuation from the postfertilization to pronuclear fusion stages suggests a role in embryonic development.[21] Furthermore, in mice, parthenogenetic oocytes without Ca^{2+} oscillations develop into blastocysts with a smaller inner cell mass.[22] In addition, Ca^{2+} oscillations affect pronucleus formation,[23] the arrest of early embryo development[24] and post-implantation development.[25]

TYPES AND MECHANISMS OF ARTIFICIAL OOCYTE ACTIVATION

OOCYTE ACTIVATION REQUIRES either a transient increase in Ca^{2+} in the oocyte or a decline in MPF,[26] which normally follows such a Ca spike. Methods

of artificial oocyte activation are usually based on one of these mechanisms (Table 1).

Calcium ionophores

Calcium ionophores, including A23187[1] and ionomycin,[31] are commonly used to induce oocyte activation. The first case in which the combined use of A23187 and ICSI resulted in a successful pregnancy and delivery was reported in 1995.[27] This case used the combination treatment to increase the fertilization rate and several subsequent cases have been reported in recent years.[32–36]

Mechanism

Calcium ionophores help activate the oocyte by increasing the Ca^{2+} permeability of the cell membrane, thereby letting extracellular Ca^{2+} flow into the cell.[37] The oocyte activating effect of A23187 is weakened by the presence of protein, including human serum albumin. When a strong effect is desired, a Ca^{2+}/Mg^{+}-free culture medium should be used, and the osmotic pressure should be corrected using polyvinylpyrrolidone. The addition of albumin may be used to stop the ionophore treatment.

For human oocytes, serum-containing medium is preferred for its gentler effect on the oocyte. Following treatment with A23187, the Ca^{2+} concentration in the oocyte peaks after approximately 1 min and then gradually decreases.[37] The treatment causes a single transient increase in Ca ion concentration, but no Ca^{2+} oscillation.

Method

1. Dissolve A23187 (C7522; Sigma, St Louis, MO, USA) in dimethyl sulfoxide (DMSO) to make a 1 mmol/L stock solution. The stock solution should be kept frozen at −80°C.
2. Add 10 μL of the stock solution to 990 μL of culture medium (e.g. human tubal fluid [HTF]) and mix to make 10 μmol/L A23187 treatment solution. Use

the treatment solution as soon as possible after preparation and keep it protected from light exposure until use.

3. Add the A23187 treatment solution to the oocyte and place in a 37°C incubator for 5–15 min.
4. After treatment is completed, immediately wash the oocyte three times with standard culture medium and resume incubation.

When oocyte activation with ICSI alone fails, A23187 treatment may be carried out before or after ICSI to improve the fertilization rates. One report describes treating oocytes with A23187 approximately 30 min after performing ICSI.[27]

Electrostimulation

Electrostimulation is used in studies of parthenogenesis or for embryo cloning in the field of animal science. Fundamental research[11,38] and clinical studies[27] of electrostimulation with ICSI in human oocytes have been reported.

Mechanism

The oocyte is placed between two parallel electrode plates. The electric field generated by a direct current voltage causes charged proteins in the lipid bilayer of the cell membrane to move, thereby forming pores in the membrane.[39] Extracellular Ca^{2+} in the culture medium flow into the oocyte through these pores, transiently elevating the interior Ca^{2+} concentration and activating the oocyte. The lower the electrolyte concentration in the culture medium, the more pore formation is stimulated. The pores are thought to take 10–40 min at 37°C to repair, and longer when the temperature is lower.[40] The concentration of Ca^{2+} is elevated immediately after the application of the stimulus, peaks within 1 min, and then gradually decreases and returns to the original level in approximately 5 min, without subsequent oscillation.[11,37] When electrostimulation was applied repeatedly to imitate Ca^{2+} oscillations in unfertilized rabbit oocytes, parthenogenesis occurred and fetuses were obtained.[41]

Method

The actual process of electrostimulation is described as follows:

1. Fill the electrode chamber with Zimmerman solution[39] or Dulbecco's phosphate buffered saline (D-PBS).[11]
2. Place the oocyte (or multiple oocytes) between the electrodes in the chamber.
3. Apply a rectangular wave of electrostimulation using an electroporator (e.g. apply one pulse of 100–150 V for 50–100 μsec when the distance between the electrodes is 1 mm).
4. Incubate the oocyte in standard culture medium.

The conditions for electrostimulation vary according to the electroporator and chamber type (the distance between the electrodes and the material of the electrodes). Conditions must be set in advance using a 1-day-old oocyte. Although D-PBS is used as the pulsing medium in the above method, 0.3 mol/L mannitol solution (containing 100 μmol/L $CaCl_2$ and 100 μmol/L $MgCl_2$) is more typically used in basic research. Because D-PBS contains more electrolytes, the electric current created by electrostimulation would normally be a problem, but the electric resistance between the electrodes is approximately 13 KΩ or higher, and the electric current created is very weak, at approximately 1 mA. Thus, out of concern that changes in the composition of the culture medium might damage the cell, D-PBS is used.

Strontium treatment method

Because strontium treatment has been shown to cause oocyte activation accompanied with Ca^{2+} oscillations in mouse models,[42] this method is preferred when embryonic development is desired. In contrast, only the initial transient increase in cytoplasmic Ca^{2+} concentration is achieved with A23187 treatment and electrostimulation.[37] Another characteristic of strontium treatment is varied efficacy of oocyte activation depending on the species.[29,43–46] It is most effective in mice, but its efficacy has not been sufficiently confirmed in humans. Nonetheless, cases of pregnancy and delivery have been reported in clinical research applications.[29]

Mechanism

Sr^{2+} induced Ca^{2+} transients in an activated oocyte.[47] However, the mechanism by which Sr^{2+} induces Ca^{2+} oscillations in an oocyte remains unclear. Recently, Ca^{2+} oscillations induced by Sr^{2+} were mediated through inositol trisphosphate receptors.[48] Sr^{2+} is thought to move into the oocyte down the concentration gradient, causing Ca to be released from the endoplasmic reticulum.

Method

1. Dissolve $SrCl_2 \cdot 6H_2O$ (Sigma) in distilled water to make 1 mmol/L Sr^{2+} stock solution and store in the deep freezer.
2. Add 20 μL of the stock solution to 1 mL of a Ca^{2+}-free culture medium (such as HTF) to make 20 mmol/L strontium treatment solution.

3. Place the oocyte into the strontium treatment solution and incubate it for 120 min in an incubator.
4. Transfer the oocyte into standard culture medium and incubate.

The impact of strontium treatment on the chromosomes of gametes has been studied in mice and no effect has been reported.[49]

CLINICAL APPLICATION OF OOCYTE ACTIVATION METHODS

Cases of fertilization failure with ICSI

WHEN ICSI DOES not result in oocyte activation, treatments that artificially activate oocytes are used concomitantly with ICSI to promote fertilization. When ICSI is carried out, the incidence of fertilization failure is 1–5%.[50-54] The number of oocytes used in ICSI is closely associated with the fertilization rate.[52] The incidence of fertilization failure is 13.3% when ICSI is carried out with one oocyte, but decreases to 3.1% with two oocytes and 1% or lower with three or more oocytes. There was no fertilization failure with six or more oocytes.[52] When a small number of oocytes are used in ICSI, the high incidence of failure can be attributed to the poor quality of the collected oocytes or to technical factors (e.g. if a device malfunctioned during the initial ICSI, it would be fixed). Fertilization failure was observed in 5.6% (100/1779) of treatment cycles of ICSI. When fertilization failed in the initial ICSI, the probability of fertilization failure in the second ICSI was 13% and the incidence of such cases is 0.7% in all treatment cycles.[52]

Because the steps between capacitation and sperm–egg fusion are bypassed in ICSI, the problem must lie in later steps of the fertilization process. In particular, abnormalities in oocyte activation, decondensation of the sperm head or pronucleus formation may play a role in fertilization failure. The mechanism of oocyte activation in ICSI is thought to involve the action of phospholipase C zeta (PLCzeta), which is present in the sperm.[55] When the activity of this factor is low or lacking, fertilization fails. In addition, after fertilization, the sperm needs to undergo decondensation, which depends on the condition of the sperm nucleoprotein as well as the reduction system in the oocyte.[19] For example, decondensation can be impaired when the zinc supply from the seminal plasma is decreased because of a disorder, such as prostatitis, or when a large number of disulfide bonds are formed in the nucleoproteins.[56] The sperm aster also plays an important role in the fusion of male and female pronuclei.

Abnormalities in the sperm centrosome that comprise the sperm aster can impair decondensation.[57] When oocytes that were not successfully fertilized following ICSI were observed using chromatin staining, oocyte activation failed to occur in 70% of the oocytes, even though the sperm was successfully inserted into the oocyte.[52] Moreover, when ICSI was carried out using sperm from volunteers whose sperm had been shown to be capable of fertilizing oocytes, fertilization was successful in 70% of the cases. Therefore, for approximately 50% of the oocytes that were not fertilized following ICSI, the failure of fertilization could be attributed to the sperm.[52]

If abnormalities in oocyte activation factor activity are the cause of fertilization failure, the combined use of oocyte activation treatments and ICSI can be considered. However, if the source of the problem lies elsewhere, there is currently no treatment method. The recurrence rate of fertilization failure following ICSI is 13%, which means that in many cases, fertilized oocytes can be obtained from a second round of ICSI.[52] When the risk of fertilization failure is high, however, an oocyte activation treatment combined with ICSI has been used. The oocyte activation treatments used in clinical research include Ca ionophore treatment, electrostimulation and strontium treatment. Table 2 shows the results of clinical research on the combined use of oocyte activation and ICSI in patients with fertilization failure and low fertilization cases following an ICSI attempt, excluding cases using spermatid and those with globozoospermia. We reported the first case of pregnancy and delivery after ICSI with electrostimulation. Since then there have been 10 delivery cases using the same treatment as the fertilization failure case. In terms of the efficiency of oocyte activation, electrostimulation and A23187 gave better outcomes than strontium treatment. Strontium treatment showed variable efficacy. This treatment was developed in studies using mice and has been viewed favorably because it causes Ca^{2+} oscillations and is similar to the physiological stimulus that activates oocytes.[41] However, in human oocytes, Ca^{2+} oscillations have not been observed following treatment and its role in human oocyte activation is not clear.[29] For clinical research, methods with known mechanisms should be selected. Chromosomal analysis of oocytes fertilized using both ICSI and oocyte activation did not show an increased risk from electrostimulation[36] and A23187 + puromycin.[59]

The timing of electrostimulation relative to ICSI is another important factor to consider. When electrostimulation is carried out prior to ICSI, the oocyte tends to

Table 2 Reports on the combined use of oocyte activation methods and intracytoplasmic sperm injection†

Reference	Indication	Types of oocyte activation methods	Cases of pregnancy and delivery
Tesarik *et al.* (1994)[12]	Fresh oocytes	A23187	Research‡
Hoshi *et al.* (1995)[27]	Low fertilization	A23187	Two cases of pregnancy
Yanagida *et al.* (1999)[28]	Fertilization failure	Electrical stimulation	One case of pregnancy and delivery
Yamano *et al.* (2000)[57]	1-day-old unfertilized oocytes	A23187 + puromycin	Research‡
Nakagawa *et al.* (2001)[58]	1-day-old unfertilized oocytes	A23187 + puromycin	Research‡
Tesarik *et al.* (2002)[59]	ICSI patients	Mechanical	One case of pregnancy and delivery
Eldar-Geva *et al.* (2003)[32]	Fertilization failure	A23187	One case of pregnancy and delivery
Murase *et al.* (2004)[30]	Fertilization failure	A23187 + puromycin	One case of pregnancy and delivery
Ebner *et al.* (2004)[60]	Fertilization failure	Mechanical	One case of pregnancy and delivery
Chi *et al.* (2004)[33]	Low fertilization	A23187	One case of pregnancy and delivery
Heindryckx *et al.* (2005)[34]	Fertilization failure	A23187	Three cases of pregnancy
Lu *et al.* (2006)[61]	1-day-old unfertilized oocytes	A23187 + puromycin	Research‡
Manipalviratn *et al.* (2006)[62]	1-day-old unfertilized oocytes	Electrostimulation	Research‡
Ahmady *et al.* (2007)[35]	Immotile testicular sperm	A23187	One case of pregnancy and delivery
Moaz *et al.* (2006)[63]	Low fertilization and fertilization failure	Ionomycin	Research‡
Yanagida *et al.* (2006)[29]	Low fertilization	Strontium	One case of pregnancy and delivery
Nasr-Esfahani *et al.* (2008)[36]	Low fertilization (teratozoospermia)	Ionomycin	Two cases of pregnancy

†Excluding cases using spermatids and globozoospermia. ‡'Research' means that this report was carried out for the purpose of research not a therapeutic purpose. ICSI, intracytoplasmic sperm injection.

degenerate. It is possible that the cell membrane is not sufficiently repaired after electrostimulation[40] and is further damaged by ICSI. Because the cell membrane can also be damaged by Ca ionophore treatment, we treat oocytes with Ca ionophore 30 min after ICSI. In addition, in mice, when the sperm is present during electrostimulation, the incidence of chromosomal aberration (structural abnormality) in the sperm is approximately 50% higher. In such cases, the chromosomes of the oocyte were unaffected. In contrast, when electrostimulation was carried out 30–60 min before ICSI, there were no chromosomal abnormalities, but the oocyte still tends to be damaged.

Special cases of ICSI include cases of round-headed spermatozoa (globozoospermia) and the use of spermatids. Round-headed spermatozoa possess round heads lacking an acrosome.[65] In spermatozoa, the level of sperm factor appears to be low and there are reported cases of fertilization failure associated with it, for which clinical application of ICSI has been reported (Table 3). The reported fertilization rates were 0–42% with ICSI alone and approximately 70% in conjunction with oocyte activation methods. With round-headed spermatozoa, many cases of pregnancy were achieved by ICSI alone[66,68,71–74,77] and oocyte activation methods were

not essential. From clinical research using round spermatids, 14 cases of pregnancy have been reported to date (Table 4).[78,79,81,82,84–86,93,94] Because the activity level of the sperm factor is low in human round spermatids, oocyte activation in conjunction with ICSI has been considered. However, the reported fertilization rates were between 16 and 69% with ICSI alone.[78,79,81–89,92,95,96] Oocyte activation methods were used along with ICSI in a small number of reported cases and the fertilization rate was approximately 40%.[80,81] In our experience, the fertilization rate with round spermatids was 17%.[97] Elongated spermatids seem to have a similar oocyte activation capacity as mature sperm.

ASSISTED ACTIVATION

ASSISTED ACTIVATION IS based on the idea of using oocyte activation methods in conjunction with ICSI in patients without fertilization failure. These patients do not exhibit a low fertilization rate, but this approach is used to further raise the fertilization rate and increase the number of embryos obtained. As this method works on the oocytes that are still unfertilized after ICSI, the number of fertilized oocytes should increase. However, fertilized oocytes would also undergo

Table 3 Reported cases of intracytoplasmic sperm injection for globozoospermia

Reference	Oocyte activation treatment	Fertilization rate (%)	Cases of pregnancy and delivery
Liu *et al.* (1995)[66]	None	–	Two cases of pregnancy
Trokoudes *et al.* (1995)[67]	None	50	One case of pregnancy
Bourne *et al.* (1995)[68]	None	–	None
Battaglia *et al.* (1997)[69]	None	10	None
	A23187	75	None
Rybouchkin *et al.* (1997)[70]	A23187	–	One case of pregnancy
Stone *et al.* (2000)[71]	None	10–42	One case of pregnancy and delivery
Kim *et al.* (2001)[72]	A23187	60	One case of pregnancy and delivery
Zeyneloglu *et al.* (2002)[73]	None	31	One case of pregnancy and delivery
Nardo *et al.* (2002)[74]	None	–	One case of pregnancy and delivery
Kilani *et al.* (2004)[75]	None	38	One case of pregnancy and delivery
Heindryckx *et al.* (2005)[34]	A23187	71–77	Five cases of pregnancy
Khalili *et al.* (2007)[76]	None	0 (four cases)	None
Dirican *et al.* (2007)[77]	None	9, 33 (two cases)	Two cases of pregnancy and delivery

Table 4 Reports on round spermatid injection

Reference	Oocyte activation treatment	Fertilization rate (%)	Cases of pregnancy and delivery
Tesarik *et al.* (1995,96)[78,79]	None	36	Two cases of pregnancy and delivery
Tanaka *et al.* (1996)[80]	Electrostimulation	42	One case of pregnancy
Vanderzwalmen *et al.* (1997)[81]	None	16	None
	A23187	36	One case of pregnancy
Antinori *et al.* (1997)[82]	None	56	Two cases of pregnancy
Yamanaka *et al.* (1997)[83]	None	69	None
Amer *et al.* (1997)[84]	None	25	Four cases of pregnancy
Kahraman *et al.* (1998)[85]	None	26	One case of pregnancy
Barak *et al.* (1998)[86]	None	27	One case of pregnancy and delivery
Al-Hasani *et al.* (1999)[87]	None	18	None
Ghazzawi *et al.* (1999)[88]	None	22	None
Levran *et al.* (2000)[89]	None	44.9	None
Vicdan *et al.* (2001)[90]	None	–	None (0/6)
Khalili *et al.* (2002)[91]	None	–	None (0/7)
Sousa *et al.* (2002)[92]	None	16	None (0/33)
Saremi *et al.* (2002)[93]	None	–	One case of pregnancy and delivery
Amarin *et al.* (2002)[94]	None	–	One case of pregnancy
Ulug *et al.* (2003)[95]	None	42	None
Benkhalifa *et al.* (2004)[96]	None	36	None

treatment. Because the techniques of oocyte activation are still at the clinical research stage, they should not be used for such a purpose. When two or fewer oocytes are used in ICSI, the risk of fertilization failure is higher. When oocyte activation methods were concomitantly used with ICSI in such cases, the fertilization rate, embryonic development rate and pregnancy rate did not show any significant difference and, therefore, these methods were evaluated to be ineffective.[52]

RESCUE ACTIVATION

IN RESCUE ACTIVATION, oocyte activation is used to promote fertilization in oocytes that remain unfertilized following ICSI when the fertilization status is evaluated. The time interval between the collection of the oocyte and the oocyte activation treatment is important. Ontogenesis cannot be expected after aging of the oocyte. With 1-day-old oocytes, the pregnancy rate is extremely

low. When rescue activation was carried out for such oocytes in 52 cases, the fertilization rate was 78% and two cases of pregnancy were obtained, but both resulted in miscarriage.[52] Therefore, this method should not be used clinically for 1-day-old oocytes.[98,99] Rescue ICSI 6 h after insemination has already been shown to be effective in cases of fertilization failure with *in vitro* fertilization.[100] In the same way, rescue activation should be carried out within 6 h after ICSI. When oocyte activation does not occur following ICSI, the head of the injected sperm may undergo decondensation and PCC may occur over time. Premature chromatin condensation is the abnormal condensation of chromatin and can induce damage in the chromosome or DNA. Therefore, an evaluation of fertilization should be done before PCC takes place.[101] Because the incidence of PCC is elevated starting 4 h later when oocyte activation does not occur following ICSI, it is recommended that the fertilization status be evaluated and oocyte activation treatment carried out on unfertilized oocytes within 4 h.[102] If at least one fertilized oocyte is found at the evaluation, oocyte activation treatment is not necessary.

CONCLUSION

ARTIFICIAL OOCYTE ACTIVATION is frequently used in basic research in the field of reproductive technology. Oocyte activation occurs at the initial stage of fertilization and is essential for fertilization. In some cases of fertilization failure with ART, oocyte activation did not occur because of abnormalities in the sperm-derived oocyte activation factor. In 50% of the unfertilized oocytes following ICSI, the failure can be attributed to problems with the sperm.[52] When fertilization failure is predicted with ICSI, oocyte activation methods can be used along with ICSI in a clinical research setting. Oocyte activation methods can also be used as a rescue procedure when fertilization failure is detected within 4 h of ICSI.[101] Among the various oocyte activation methods currently used, Ca ionophore treatment is the easiest to use. However, it should be noted that issues of genetic safety and abnormal imprinting have not been addressed for the combined use of these oocyte activation methods.[102]

ACKNOWLEDGMENTS

REFERENCES

1. Carroll J. The initiation and regulation of Ca^{2+} signalling at fertilization in mammals. *Seminars Cell Dev Biol* 2001; **12**: 37–43.
2. Schuetz AW. Cytoplasmic activation of starfish oocytes by sperm and divalent ionophore A-23187. *J Cell Biol* 1975; **66**: 86–94.
3. Steinhardt RA, Epel D, Carroll EJ Jr, Yanagimachi R. Is calcium ionophore a universal activator for unfertilised eggs? *Nature* 1974; **252**: 41–43.
4. Wakayama T, Perry ACF, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* 1998; **394**: 369–374.
5. Bradshaw J, Jung T, Fulka J Jr, Moor RM. UV irradiation of chromosomal DNA and its effect upon MPF and meiosis in mammalian oocytes. *Mol Reprod Dev* 1995; **41**: 503–512.
6. Mahowald AP, Goralski TJ, Caulton JH. In vitro activation of Drosophila eggs. *Dev Biol* 1983; **98**: 437–445.
7. Prather RS, Eichen PA, Nicks DK, Peters MS. Artificial activation of porcine oocytes matured in vitro. *Mol Reprod Dev* 1991; **28**: 405–409.
8. Whittingham DG, Siracusa G. The involvement of calcium in the activation of mammalian oocytes. *Exp Cell Res* 1978; **113**: 311–317.
9. Siracusa G, Whittingham DG, Molinaro M, Vivarelli E. Parthenogenetic activation of mouse oocytes induced by inhibitors of protein synthesis. *J Embryol Exp Morphol* 1978; **43**: 157–166.
10. Hoshi K, Yanagida K, Sato A. Pretreatment of hamster oocytes with Ca^{2+} ionophore to facilitate fertilization by ooplasmic micro injection. *Hum Reprod* 1992; **7**: 1992.
11. Yanagida K, Katayose H, Hoshi K, Yazawa H, Sato A. Effect of electrical stimulation on oocyte activation after intracytoplasmic sperm injection. *J Mamm Ova Res* 1997; **14**: 132–138.
12. Tesarik J, Testart J. Treatment of sperm-injected human oocytes with Ca^{2+} ionophore supports the development of Ca^{2+} oscillations. *Biol Reprod* 1994; **51**: 385–391.
13. Miyazaki S, Shirakawa H, Nakada H, Honda Y. Essential role of the inositol 1,4,5-triphosphate receptor/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. *Dev Biol* 1993; **158**: 62–78.
14. Swann K. A cytosolic sperm factor stimulates repetitive calcium increases and mimics fertilization in hamster eggs. *Development* 1990; **110**: 1295–1302.
15. Stice SL, Robl JM. Activation of mammalian oocytes by a factor obtained from rabbit sperm. *Mol Reprod Dev* 1990; **25**: 272–280.
16. Tesarik J, Sousa M, Testart J. Human oocyte activation after intracytoplasmic sperm injection. *Hum Reprod* 1994; **9**: 511–518.
17. Rybouchkin A, Dozortsev D, de Sutter P, Qian C, Dhont M. Intracytoplasmic injection of human spermatozoa into

mouse oocytes: a useful model to investigate the oocyte-activating capacity and the karyotype of human spermatozoa. *Hum Reprod* 1995; **10**: 1130–1135.

18 Araki Y, Yoshizawa M, Abe H, Murase Y, Araki Y. Use of mouse oocytes to evaluate the ability of human sperm to activate oocytes after failure of activation by intracytoplasmic sperm injection. *Zygote* 2004; **12**: 111–116.

19 Yanagimachi R. Mammalian fertilization. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction*, 2nd edn. Raven Press, New York, 1994; 189–317.

20 Moor RM. Regulation of the meiotic cycle in oocytes of domestic mammals. *Ann NY Acad Sci* 1988; **541**: 248–258.

21 Jones K, Carroll J, Merriman J, Whittingham D, Kono T. Repetitive sperm-induced Ca^{2+} transients in mouse oocytes are cell cycle dependent. *Development* 1995; **121**: 3259–3266.

22 Bos-Mikich A, Whittingham DG, Jones KT. Meiotic and mitotic Ca^{2+} oscillations affect cell composition in resulting blastocysts. *Dev Biol* 1997; **182**: 172–179.

23 Lawrence Y, Ozil J, Swann K. The effects of a Ca^{2+} chelator and heavy-metal-ion chelators upon Ca^{2+} oscillations and activation at fertilization in mouse eggs suggest a role for repetitive Ca^{2+} increases. *Biochem J* 1998; **335**: 335–342.

24 Gordo A, Rodrigues P, Kurokawa M *et al*. Intracellular calcium oscillations signal apoptosis rather than activation in in vitro aged mouse eggs. *Biol Reprod* 2002; **66**: 1828–1837.

25 Ozil J, Huneau D. Activation of rabbit oocytes: the impact of the Ca^{2+} signal regime on development. *Development* 2001; **128**: 917–928.

26 Gerhart J, Wu M, Kirschner M. Cell cycle dynamics of an M-phase-specific cytoplasmic factor in Xenopus laevis oocytes and eggs. *J Cell Biol* 1984; **98**: 1247–1255.

27 Hoshi K, Yanagida K, Yazawa H, Katayose H, Sato A. Intracytoplasmic sperm injection using immobilized or motile human spermatozoon. *Fertil Steril* 1995; **63**: 1241–1245.

28 Yanagida K, Katayose H, Yazawa H *et al*. Successful fertilization and pregnancy following ICSI and electrical oocyte activation. *Hum Reprod* 1999; **14**: 1307–1311.

29 Yanagida K, Morozumi K, Katayose H, Sato A. Successful pregnancy after ICSI with strontium oocyte activation in low rates of fertilization. *Reprod Biomed Online* 2006; **13**: 2006.

30 Murase Y, Araki Y, Mizuno S *et al*. Pregnancy following chemical activation of oocytes in a couple with repeated failure of fertilization using ICSI: case report. *Hum Reprod* 2004; **19**: 1604–1607.

31 Navara CS, First NL, Schatten G. Microtubule organization in the cow during fertilization, polyspermy, parthenogenesis, and nuclear transfer: the role of the sperm aster. *Dev Biol* 1994; **162**: 29–40.

32 Eldar-Geva T, Brooks B, Margalioth EJ, Zylber-Haran E, Gal M, Silber SJ. Successful pregnancy and delivery after calcium ionophore oocyte activation in a normozoospermic patient with previous repeated failed fertilization after intracytoplasmic sperm injection. *Fertil Steril Supplement* 2003; **3**: 1656–1658.

33 Chi HJ, Koo JJ, Song SJ, Lee JY, Chang SS. Successful fertilization and pregnancy after intracytoplasmic sperm injection and oocyte activation with calcium ionophore in a normozoospermic patient with extremely low fertilization rates in intracytoplasmic sperm injection cycles. *Fertil Steril* 2004; **82**: 475–477.

34 Heindryckx B, Van der Elst J, De Sutter P, Dhont M. Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI. *Hum Reprod* 2005; **20**: 2237–2241.

35 Ahmady A, Michael E. Successful pregnancy and delivery following intracytoplasmic injection of frozen–thawed nonviable testicular sperm and oocyte activation with calcium ionophore. *J Androl* 2007; **28**: 13–14.

36 Nasr-Esfahani MH, Razavi S, Javdan Z, Tavalaee M. Artificial oocyte activation in severe teratozoospermia undergoing intracytoplasmic sperm injection. *Fertil Steril* 2008; **16** [Epub ahead of print].

37 Swann K, Ozil JP. Dynamics of the calcium signal that triggers mammalian egg activation. *Int Rev Cytol* 1994; **152**: 183–222.

38 Zhang J, Wang CW, Blaszczyk A *et al*. Electrical activation and in vitro development of human oocytes that fail to fertilize after intracytoplasmic sperm injection. *Fertil Steril* 1999; **72**: 509–512.

39 Zimmerman U, Vienken J. Electric field-induced cell-to-cell fusion. *J Membr Biol* 1982; **67**: 165–182.

40 Bates GW, Saunders A, Sowers AE. Electrofusion. In: Sowers AE, ed. *Cell Fusion*. Plenum Press, New York, 1987; 367–395.

41 Ozil JP. The parthenogenetic development of rabbit oocytes after repetitive pulsatile electrical stimulation. *Development* 1990; **109**: 117–127.

42 Cheek TR, McGuinness OM, Vincent C, Moreton RB, Berridge MJ, Johnson MH. Fertilisation and thimerosal stimulate similar calcium spiking patterns in mouse oocytes but by separate mechanisms. *Development* 1993; **119**: 179–189.

43 Kato M, Ishikawa A, Hochi S, Hirabayashi M. Effect of activation regimens for rat oocytes on full-term development after round spermatid injection. *Contemp Top Lab Anim Sci* 2004; **43**: 13–15.

44 Tateno H, Kamiguchi Y. Parthenogenetic activation of Chinese hamster oocytes by chemical stimuli and its cytogenetic evaluation. *Mol Reprod Dev* 1997; **47**: 72–78.

45 Méo SC, Yamazaki W, Leal CL, de Oliveira JA, Garcia JM. Use of strontium for bovine oocyte activation. *Theriogenology* 2005; **63**: 2089–2102.

46 Okada K, Miyano T, Miyake M. Activation of pig oocytes by intracytoplasmic injection of strontium and barium. *Zygote* 2003; **11**: 159–165.

47 Kono T, Jones KT, Mikich AB, Whittingham DG, Carroll J. A cell cycle-associated change in Ca^{2+} releasing activity leads to

the generation of Ca^{2+} transients in mouse embryos during the first mitotic division. *J Cell Biol* 1996; **132**: 915–923.

48 Zhang D, Pan L, Yang LH, He XK, Huang XY, Sun FZ. Strontium promotes calcium oscillations in mouse meiotic oocytes and early embryos through InsP3 receptors, and requires activation of phospholipase and the synergistic action of InsP3. *Hum Reprod* 2005; **20**: 3053–3061.

49 Morozumi K, Tateno H, Yanagida K, Katayose H, Kamiguchi Y, Sato. A. Chromosomal analysis of mouse spermatozoa following physical and chemical treatments that are effective in inactivating HIV. *Zygote* 2004; **12**: 339–344.

50 Moomjy M, Sills ES, Rosenwaks Z, Palermo GD. Implications of complete fertilization failure after intracytoplasmic sperm injection for subsequent fertilization and reproductive outcome. *Hum Reprod* 1998; **13**: 2212–2216.

51 Ludwig M, Strik D, Al-Hasani S, Diedrich K. No transfer in a planned ICSI cycle: we cannot overcome some basic rules of human reproduction. *Eur J Obstet Gynecol Reprod Biol* 1999; **87**: 3–11.

52 Yanagida K. Complete fertilization failure in ICSI. *Hum Cell* 2004; **17**: 187–193.

53 Liu J, Nagy Z, Joris H *et al*. Analysis of 76 total fertilization failure cycles out of 2732 intracytoplasmic sperm injection cycles. *Hum Reprod* 1995; **10**: 2630–2636.

54 Esfandiari N, Javed MH, Gotlieb L, Casper RF. Complete failed fertilization after intracytoplasmic sperm injection – analysis of 10 years' data. *Int J Fertil Womens Medical* 2005; **50**: 187–192.

55 Saunders CM, Larman MG, Parrington J *et al*. PLC zeta: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. *Development* 2002; **129**: 3533–3544.

56 Kuvist U. Importance of spermatozoal zinc as temporary inhibitor of sperm nuclear chromatin decondensation ability in man. *Acta Phisiol Scand* 1980; **109**: 79–84.

57 Terada Y, Nakamura S, Simerly C *et al*. Centrosomal function assessment in human sperm using heterologous ICSI with rabbit eggs: a new male factor infertility assay. *Mol Reprod Dev* 2004; **67**: 360–365.

58 Yamano S, Nakagawa K, Nakasaka H, Aono T. Fertilization failure and oocyte activation. *J Med Invest* 2000; **47**: 1–8.

59 Nakagawa K, Yamano S, Moride N, Yamashita M, Yoshizawa M, Aono T. Effect of activation with Ca ionophore A23187 and puromycin on the development of human oocytes that failed to fertilize after intracytoplasmic sperm injection. *Fertil Steril* 2001; **76**: 148–152.

60 Tesarik J, Rienzi L, Ubaldi F, Mendoza C, Greco E. Use of a modified intracytoplasmic sperm injection technique to overcome sperm-borne and oocyte-borne oocyte activation failures. *Fertil Steril* 2002; **78**: 619–624.

61 Ebner T, Moser M, Sommergruber M, Jesacher K, Tews G. Complete oocyte activation failure after ICSI can be overcome by a modified injection technique. *Hum Reprod* 2004; **19**: 1837–1841.

62 Lu Q, Zhao Y, Gao X *et al*. Combination of calcium ionophore A23187 with puromycin salvages human unfertilized oocytes after ICSI. *Eur J Obstet Gynecol Reprod Biol* 2006; **126**: 72–76.

63 Manipalviratn S, Ahnonkitpanit V, Numchaisrika P, Chompurat D, Pansatha J, Suwajanakorn S. Results of direct current electrical activation of failed-to-fertilize oocytes after intracytoplasmic sperm injection. *J Reprod Med* 2006; **51**: 493–499.

64 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**: 791–794.

65 Kullander S, Rausing A. On round-headed human spermatozoa. *Int J Fertil* 1975; **20**: 33–40.

66 Liu J, Nagy Z, Joris H, Tournaye H, Devroey P, Van Steirteghem A. Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia. *Hum Reprod* 1995; **10**: 626–629.

67 Trokoudes KM, Danos N, Kalogirou L *et al*. Pregnancy with spermatozoa from a globozoospermic man after intracytoplasmic sperm injection treatment. *Hum Reprod* 1995; **10**: 880–882.

68 Bourne H, Liu DY, Clarke GN, Baker HW. Normal fertilization and embryo development by intracytoplasmic sperm injection of round-headed acrosomeless sperm. *Fertil Steril* 1995; **63**: 1329–1332.

69 Battaglia DE, Koehler JK, Klein NA, Tucker MJ. Failure of oocyte activation after intracytoplasmic sperm injection using round-headed sperm. *Fertil Steril* 1997; **68**: 118–122.

70 Rybouchkin A, Van Der Elst J, De Sutter P, Dhont M. 'Globe-headed spermatozoa' and ICSI. *Fertil Steril* 1998; **69**: 361–362.

71 Stone S, O'Mahony F, Khalaf Y, Taylor A, Braude P. A normal livebirth after intracytoplasmic sperm injection for globozoospermia without assisted oocyte activation: case report. *Hum Reprod* 2000; **15**: 139–141.

72 Kim ST, Cha YB, Park JM, Gye MC. Successful pregnancy and delivery from frozen–thawed embryos after intracytoplasmic sperm injection using round-headed spermatozoa and assisted oocyte activation in a globozoospermic patient with mosaic Down syndrome. *Fertil Steril* 2001; **75**: 445–447.

73 Zeyneloglu HB, Baltaci V, Duran HE, Erdemli E, Batioglu S. Achievement of pregnancy in globozoospermia with Y chromosome microdeletion after ICSI. *Hum Reprod* 2002; **17**: 1833–1836.

74 Nardo LG, Sinatra F, Bartoloni G, Zafarana S, Nardo F. Ultrastructural features and ICSI treatment of severe teratozoospermia: report of two human cases of globozoospermia. *Eur J Obstet Gynecol Reprod Biol* 2002; **104**: 40–42.

75 Kilani Z, Ismail R, Ghunaim S *et al*. Evaluation and treatment of familial globozoospermia in five brothers. *Fertil Steril* 2004; **82**: 1436–1439.

76 Khalili MA, Kalantar SM, Vahidi S, Ghafour-Zadeh M. Failure of fertilization following intracytoplasmic injection of round-headed sperm. *Ann Saudi Med* 1998; **18**: 408–411.

77 Dirican EK, Isik A, Vicdan K, Sozen E, Suludere Z. Clinical pregnancies and livebirths achieved by intracytoplasmic injection of round headed acrosomeless spermatozoa with and without oocyte activation in familial globozoospermia: case report. *Asian J Androl* 2007: [Epub ahead of print].

78 Tesarik J, Mendoza C, Testart J. Viable embryos from injection of round spermatids into oocytes. *N Engl J Med* 1995; **333**: 525.

79 Tesarik J, Mendoza C. Spermatid injection into human oocytes. I. Laboratory techniques and special features of zygote development. *Hum Reprod* 1996; **11**: 772–779.

80 Tanaka A, Nagayoshi M, Awata S *et al.* Clinical evaluation of round spermatid injection (ROSI) into human oocytes. *Fertil Steril* 1996; **Suppl.**: S99.

81 Vanderzwalmen P, Zech H, Birkenfeld A *et al.* Intracytoplasmic injection of spermatids retrieved from testicular tissue: influence of testicular pathology, type of selected spermatids and oocyte activation. *Hum Reprod* 1997; **12**: 1203–1213.

82 Antinori S, Versaci C, Dani G, Antinori M, Pozza D, Selman HA. Fertilization with human testicular spermatids: four successful pregnancies. *Hum Reprod* 1997; **12**: 286–291.

83 Yamanaka K, Sofikitis NV, Miyagawa I *et al.* Ooplasmic round spermatid nuclear injection procedures as an experimental treatment for nonobstructive azoospermia. *J Assist Reprod Genet* 1997; **14**: 55–62.

84 Amer M, Soliman E, el-Sadek M, Mendoza C, Tesarik J. Is complete spermiogenesis failure a good indication for spermatid conception? *Lancet* 1997; **350**: 116.

85 Kahraman S, Polat G, Samli M *et al.* Multiple pregnancies obtained by testicular spermatid injection in combination with intracytoplasmic sperm injection. *Hum Reprod* 1998; **13**: 104–110.

86 Barak Y, Kogosowski A, Goldman S, Soffer Y, Gonen Y, Tesarik J. Pregnancy and birth after transfer of embryos that developed from single-nucleated zygotes obtained by injection of round spermatids into oocytes. *Fertil Steril* 1998; **70**: 67–70.

87 Al-Hasani S, Ludwig M, Palermo I *et al.* Intracytoplasmic injection of round and elongated spermatids from azoospermic patients: results and review. *Hum Reprod* 1999; **14**: 97–107.

88 Ghazzawi IM, Alhasani S, Taher M, Souso S. Reproductive capacity of round spermatids compared with mature spermatozoa in a population of azoospermic men. *Hum Reprod* 1999; **14**: 736–740.

89 Levran D, Nahum H, Farhi J, Weissman A. Poor outcome with round spermatid injection in azoospermic patients with maturation arrest. *Fertil Steril* 2000; **74**: 443–449.

90 Vicdan K, Isik AZ, Delilbaşi L. Development of blastocyst-stage embryos after round spermatid injection in patients with complete spermiogenesis failure. *J Assist Reprod Genet* 2001; **18**: 78–86.

91 Khalili MA, Aflatoonian A, Zavos PM. Intracytoplasmic injection using spermatids and subsequent pregnancies: round versus elongated spermatids. *J Assist Reprod Genet* 2002; **19**: 84–86.

92 Sousa M, Cremades N, Silva J *et al.* Predictive value of testicular histology in secretory azoospermic subgroups and clinical outcome after microinjection of fresh and frozen–thawed sperm and spermatids. *Hum Reprod* 2002; **17**: 1800–1810.

93 Saremi A, Esfandiari N, Salehi N, Saremi MR. The first successful pregnancy following injection of testicular round spermatid in Iran. *Arch Androl* 2002; **48**: 315–319.

94 Amarin ZO, Jamal HS, Rouzi AA. Successful pregnancy after round spermatid microinjection. *Saudi Med J* 2002; **23**: 113–114.

95 Ulug U, Bener F, Akman MA, Bahceci M. Partners of men with Klinefelter syndrome can benefit from assisted reproductive technologies. *Fertil Steril* 2003; **80**: 903–906.

96 Benkhalifa M, Kahraman S, Biricik A *et al.* Cytogenetic abnormalities and the failure of development after round spermatid injections. *Fertil Steril* 2004; **81**: 1283–1288.

97 Yanagida K, Yazawa H, Katayose H. Oocyte activation induced by spermatids and the spermatozoa. *Int J Androl* 2000; **23**: 63–65.

98 Winston NJ, Braude PR, Johnson MH. Are failed-fertilized human oocytes useful? *Hum Reprod* 1993; **8**: 503–507.

99 Sjögren A, Lundin K, Hamberger L. Intracytoplasmic sperm injection of 1 day old oocytes after fertilization failure. *Hum Reprod* 1995; **10**: 974–975.

100 Chen C, Kattera S. Rescue ICSI of oocytes that failed to extrude the second polar body 6 h post-insemination in conventional IVF. *Hum Reprod* 2003; **18**: 2118–2121.

101 Suganuma R, Walden CM, Butters TD *et al.* Alkylated imino sugars, reversible male infertility-inducing agents, do not affect the genetic integrity of male mouse germ cells during short-term treatment despite induction of sperm deformities. *Biol Reprod* 2005; **72**: 805–813.

102 DeBaun MR, Niemitz EL, Feinberg AP. Association of in vitro fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum Genet* 2003; **72**: 156–160.