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Review

Understanding fertilization through intracytoplasmic sperm injection (ICSI)☆

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ABSTRACT

Since the establishment of *in vitro* fertilization, it became evident that almost half of the couples failed to achieve fertilization and this phenomenon was attributed to a male gamete dysfunction. The adoption of assisted fertilization techniques particularly ICSI has been able to alleviate male factor infertility by granting the consistent ability of a viable spermatozoon to activate an oocyte. Single sperm injection, by pinpointing the beginning of fertilization, has been an invaluable tool in clarifying the different aspects of early fertilization and syngamy. However, even with ICSI some couples fail to fertilize due to ooplasmic dysmaturity in relation to the achieved nuclear maturation marked by the extrusion of the first polar body. More uncommon are cases where the spermatozoa partially or completely lack the specific oocyte activating factor. In this work, we review the most relevant aspects of fertilization and its failure through assisted reproductive technologies. Attempts at diagnosing and treating clinical fertilization failure are described.

1. Fertilization

Fertilization is defined as the process resulting from the fusion of the two parental gametes, the oocyte and the spermatozoon. When mammalian oocytes and spermatozoa meet in the oviduct, a series of steps are set in motion that lead to fertilization and ultimately to the development of a new individual. Fertilization induces a cascade of critical events that result in the development of the zygote. Capacitated, free-swimming spermatozoa must initially recognize and bind following cumulus penetration to the zona pellucida of the ovulated oocyte. The sperm cell must complete the acrosome reaction that enables it to penetrate through the thickness of this extracellular coat, then bind to and fuse with the oocyte plasma membrane to activate the ootid. These multiple steps have been postulated to involve receptor–ligand interactions, ion-channel modulations, membrane fusions, and proteolysis [1–4].

During fertilization, the mature oocyte is activated to engage in embryo development. Oocyte activation involves a multitude of molecular changes depending upon the species. Generally, it is triggered by the binding of the male gamete to the oolemma resulting in intracellular Ca^{2+} $[Ca^{2+}]_i$ release within the ooplasm during fertilization [5]. The initial surge of free cytoplasmic Ca^{2+} starts from the site of sperm penetration and expands as a wave through the oocyte [6,7]. While one Ca^{2+} transient is registered in echinoderm, fish, and frog oocytes [7], repetitive calcium oscillations that last up to several hours are observed in mammals [8,9]. In mammals the fertilization-dependent Ca^{2+} oscillations were considered to be due to the release of a soluble cytosolic factor carried by the sperm following fusion with oolemma [10,11]. A putative phospholipase carried within the sperm head catalyzes the hydrolysis of PIP2 (phosphatidylinositol 4,5-bisphosphate) in the plasma membrane releasing IP_3 (inositol 1,4,5-triphosphate) and DAG (diacylglycerol). IP_3 binds to its receptor present on the endoplasmic reticulum membrane, and elicits the flux of Ca^{2+} into the cytoplasm [12] needed to activate the oocyte [13].

2. Sperm-borne oocyte activating factor

To achieve fertilization, spermatozoa must trigger the needed rise in ooplamic Ca^{2+} [14,15]. One of the most important components of the male gamete is the soluble oocyte activating factor that has been recognized in rabbit, hamster, boar, and human spermatozoa [16–18]. One form of this protein once termed oscillin localized in the perinuclear theca of the sperm head that by the mobilization of Ca^{2+} is capable of evoking oscillation patterns similar to that seen

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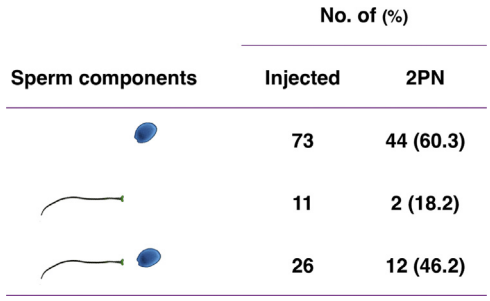

Fig. 1. Spermatozoa injected into oocytes either as a head only, separated head and tail, and tail only. Regardless of the sperm component injection, fertilization is achieved, however, extremely low in the group that was inseminated with a distinctly separated head and tail.

following sperm extract exposure experiments [16]. The human homologue of this hamster oscillin is glucosamine 6-phosphate isomerase (GPI, GenBank accession number D31766). However, as we observed in our lab, the deployment of antibodies against this compound did not interfere with the sperm extract's ability to activate oocytes, neither the injection of its recombinant form was effective in inducing calcium oscillations [19]. This work as supported by other investigators helped to indicate that this particular molecule was not the specific sperm oocyte activating factor [19,20]. This knowledge helped refocus the quest toward the identification of the actual sperm soluble factor that generates intracellular IP_3, an effort that led to the identification of a phosphoinositide-specific phospholipase C (PLC) [21,22]. Several PLC isoforms, β, γ and δ, present in the spermatozoa have been considered but were found to be absent from chromatographic fractions of sperm extracts that specifically cause Ca^{2+} oscillations [23,24].

The evidence of a novel PLC was first obtained from the examination of short ESTs (expressed sequence tag) from mouse and human testis that enabled the isolation and characterization of a full length cDNA encoding a sperm protein that is now referred to as PLCζ (zeta) [25]. PLCζ triggers Ca^{2+} oscillations in the mouse indistinguishable from those occurring following sperm penetration eliciting oocyte activation and early embryonic development up to the blastocyst stage [26].

Interestingly in another study [27] that assessed the PLCζ as a candidate sperm-borne activating factor detected the active PLCζ isoform in sperm fractions other than the perinuclear theca. It appears that PLCζ was incorporated as part of the acrosome during both mouse and human spermiogenesis to gradually diminish as sperm cells transitioned into elongated spermatids. In addition authors found that PLCζ was expressed and secreted by the epididymal epithelial cells explaining its presence on the sperm head surface. In fact, when IVF was carried out in bulls, PLCζ was no longer detectable following the occurrence of acrosome reaction on the surface of the zona pellucida. This view may appear in contradiction with the role of PLCζ as the activating factor because surgically retrieved testicular spermatozoa in humans consistently are capable of activating an oocyte.

In one of our earlier experiments, we tested the hypothesis that sperm cytosolic factor is a soluble compound deeply sealed within the spermatozoon and may not require spermatozoal integrity. We severed sperm heads from their flagellum and injected the severed parts in several combinations: sperm heads, tails, or head + free tail [28]. Oocytes that survived the injection with isolated sperm heads yielded 66.7% (44/66) fertilization rate, while oocytes injected with only sperm tails, two out 10 formed two pronuclei (possibly from the anextrusion of the second polar body), and those injected with a dissected head along with its corresponding tail in 50.0% (12/24) of the cases gave two pronuclei. It appears that the injection of isolated sperm head can still activate an oocyte (Fig. 1). This is consistent with the hypothesis that PLCζ or the putative activating factor is deeply embedded within the sperm head layers verisimilarly in the perinuclear theca as previously described [29]. This work was in agreement with studies using ICSI have shown that only the sperm head is critical for egg activation and subsequent embryonic development at least in the mouse [30–32]. We closely demonstrated that humans need the integrity of the spermatozoon to ordain the normal chromosomal segregation [28,33,34].

3. Assisted fertilization

Infertility affects approximately 12–15% of couples in their reproductive age [35] and about half of them, 1.2 million infertile couples per year, benefit from assisted reproductive technologies (ART). Since its first human birth in 1978, *in vitro* fertilization (IVF) has been applied almost exclusively to alleviate tubal infertility, but since the very beginning it became clear that the main challenge of inseminating gametes *in vitro* was to achieve a predictable fertilization particularly when men present with suboptimal semen parameters [36].

In fact, if we exclude infertility indications such as unexplained or concurrent causes, the main reasons for a couples' inability to procreate is almost equally allocated between the female and the male partners. In most instances a man's infertility is attributable to the consistent phenomenon in the inability of spermatozoa to successfully fertilize an oocyte. In the past, the reason was naively attributed to the non-receptive characteristics of the zona pellucida and therefore, a number of procedures were devised to overcome this deficiency. These procedures are generally referred to as techniques of assist fertilization or micromanipulation procedures. The introduction of micromanipulation to handle human gametes has allowed fertilization enhancements for severe oligozoospermia (men with low sperm count), and even asthenozoospermia (poor motility).

When sperm count, motility or morphology were inadequate, various techniques were tested to bypass the zona pellucida. The practical use of micromanipulation started in the mid-80 s with zona drilling (ZD) and partial zona dissection (PZD). Since then, this field has undergone such a rapid evolution that these early approaches have been abandoned in favor of intracytoplasmic sperm injection (ICSI), leaving the use of PZD confined to the 4–8 cell embryo stage (hatching) in an effort to promote implantation. Zona drilling (ZD) first reported by Gordon and Talansky [37] involves the creation of a circumscribed opening in the zona by acid Tyrode's solution applied through a fine glass micropipette. Unfortunately, after insemination, more than one spermatozoon frequently entered such perforated zonas resulting in polyspermic fertilization. Moreover, the use of acidic medium to carry out the drilling had a deleterious effect on the oocyte—an effect not seen in cleavage stage embryos while using the 'hatching' procedure. At the same time as ZD was being tested, mechanical cutting of a hole in the zona was introduced, this was originally devised for nuclear manipulation of fertilized oocytes [38]. Alternative but similar procedures were zona cracking in which the zona was breached mechanically with two fine glass hooks controlled by a micromanipulator [39], and zona softening performed by a brief exposure to trypsin [40] or pronase. Partial zona dissection (PZD) involved cutting of the zona with a glass needle before exposure of the treated oocyte to spermatozoa [41]. For all these techniques, spermatozoa had to be progressively motile and have the potential to undergo acrosome reaction. PZD also carried a distinct risk of injury to the oocytes from the need to produce an opening in the zona of optimal size to allow spermatozoa to penetrate while at the same time limiting this number to prevent polyspermy. Localized laser photoablation was also adopted to introduce a gap of

precise dimensions in the zona, and has resulted in a few healthy offspring [42,43]. However, not only did all these early procedures bring modest improvement in fertilization rate, with PZD being the most useful in that regard, but they were associated with an unsustainable occurrence of polyspermy. Mechanical insertion of spermatozoa directly into the perivitelline space—subzonal sperm injection (SUZI) [44]—was introduced as another way to overcome inadequacies of sperm concentration and motility, and this proved to be more effective than ZD or PZD, particularly following prior induction of the acrosome reaction [45,46]. However, SUZI also remained limited due to its inability to overcome acrosomal abnormalities or dysfunction of the sperm–oolemma fusion process, resulting in mediocre fertilization rates as more severe forms of male infertility were addressed.

The situation changed with the development of a procedure, intracytoplasmic sperm injection (ICSI), that entailed the insertion of a spermatozoon into the ooplasm. This approach emerged as the one capable of providing the most reliable and consistent chances of fertilization [47], because it bypasses all the initial steps of natural fertilization—penetration of the cumulus cells, binding to the zona pellucida, and fusion with the oolemma. The technique was pioneered in animals, initially by Hiramoto [48] in the sea urchin, then by Lin [49] in mammalian (mouse) oocytes. Later, Uehara and Yanagimachi [50] observed relatively high rates of sperm nucleus decondensation after microinjection of human or golden hamster spermatozoa into hamster oocytes, and at the same time ICSI was used to study the time determinants of male pronucleus formation [51,52]. This gamete manipulation approach carried the risk of oocyte injury and lysis [53], and in early studies only about 30% of injected mouse mature oocytes survived the procedure, even when fine micropipettes were used under ideal conditions [54].

The first live offspring were obtained in the rabbit following the transfer of sperm-injected oocytes into the oviduct of a pseudopregnant female [55], and soon after that a single ICSI live birth was reported in the bovine [56]. Although applied to human gametes some years earlier [57] as a proof of concept, the first human pregnancies with ICSI did not materialize until 1992 [47].

The ICSI technique is now widely used in assisted reproduction and has provided relevant information about the basic science of fertilization. Mammalian eggs injected with whole mammalian sperm can exhibit normal events of egg activation, including Ca^{2+} release [31,58–60], and can develop to term [30,32,47,61,62]. This suggests that contact of sperm and egg plasma membranes is not a critical step for egg activation. Injecting sperm at various stages of spermiogenesis has demonstrated that elongating spermatids can activate eggs, whereas round spermatids and secondary spermatocytes cannot [63–69]. Egg activation and subsequent development following ICSI are sperm-specific and cannot be substituted by an artifactual introduction of Ca^{2+} into the egg from the outside medium neither by an insertion of a glass pipette with or without the injection of culture medium can sustain an intracellular release of Ca^{2+} to activate the so injected oocytes [58–60,70–72].

More importantly, such micromanipulation approaches have also become a powerful tool for a more comprehensive understanding of the basic elements of oocyte maturation, fertilization, and early development. Micromanipulation techniques now are an important component of the process aimed at the diagnosis and sometimes even the correction of genetic anomalies [73]. Similarly, micromanipulation or microsurgery is employed toward optimization of implantation rates in certain cases [74].

Because of the popularity of ICSI, trends toward the selection of the ideal spermatozoon to provide the most competent gamete capable of providing successful embryo development and implantation have materialized in “motile sperm organellar morphology examination” (MSOME) to assess living male gamete phenotype sperm morphology under high magnification [75]. With this approach, screening is used to select a spermatozoon for ICSI with an optimal shape. The procedure is christened “intracytoplasmic morphologically selected sperm injection” (IMSI), and its use claimed to yield superior clinical outcomes than with conventional ICSI [76]. The promised beneficial impact of IMSI has been described in a series of small studies where the clinical outcome of patients treated by this procedure was compared with that of couples treated by conventional ICSI [77–80].

The high magnification morphological evaluation of viable spermatozoa is carried out using an inverted microscope equipped with interferential contrast Nomarski DIC optics. Maximum optical magnification (100× lens under oil immersion), magnification selector (1.5×), and digital video-coupled magnification (44×) led to a final video monitor magnification of around 6600×. The criteria for evaluating nuclei are smooth, symmetric, oval configuration and homogeneity of the sperm nuclear shape with ‘vacuoles’ not exceeding more than 4% of the nuclear area surface [76]. The role attributed to the sperm nuclear vacuole nor their position within the sperm head is sill unclear. Only transmission electron microscopy would clearly and accurately locate nuclear vacoules [81].

The early ultrastructural studies of human sperm in the 1950s and 60s, revealed that vacuoles in the sperm nucleus have been seen in the large majority of human spermatozoa regardless of the fertility of the donors. Vacuoles in human spermatozoa have in fact been considered as a physiologic finding devoid of consequence on fertility potential [82]. Because of this, the presence of sperm nuclear surface vacuoles portend to sperm DNA defects with consequent impaired embryo developmental competence need to be revisited and analyzed. Another type of sperm head irregularity is a surface “vacuole” or indentation, craters dent, or hollow observed on the sperm coat. In such cases, during sperm morphogenesis, the outer acrosomal membrane misforms and generates what appears to be a vacuole [83]. These vacuole-like structures disappear as the spermatozoon matures in the epididymis or at the time of the acrosome reaction [84]. In other circumstances, however, they seem to increase with temperature (37 °C) and incubation time (≥2 h) [85], most probably due to the plication/vacuolization of the rostral spermiolemma during capacitation. Interestingly, these vacuole-like structures or craters appear in over 90% of spermatozoa from fertile donors with normal semen parameters [86,87].

Higher magnification screening for sperm surface irregularities, however, did not seem to benefit the patients’ clinical outcome in independent investigations [88]. This was true for patients with compromised semen parameters and for those either undergoing first or repeated ART attempts. More detailed morphological observations indicated that in human sperm heads visible irregularities or vacuoles are almost ubiquitous, and appear to be a paraphysiologic finding. Analyses of spermatozoa from different sources, ejaculated or surgically retrieved, also revealed the varying presence and size of vacuoles that develop during the dynamic processes of spermiogenesis and maturation. This surface irregularity did not translate to a higher incidence of DNA fragmentation or aneuploidy, nor to the ability of vacuolated spermatozoa to generate zygotes capable of developing to blastocysts.

4. How to diagnose fertilization failure

Despite the common successful utilization of IVF treatments for infertile couples, cases of total fertilization failure continue to persist. Their occurrence is emotionally devastating for couples, with significant dissipation of logistic resources, and incurred cost. Understanding the etiology of fertilization failure is of critical importance to comfort patients during counseling and devising a successful treatment.

ICSI achieves a fertilization rate between 70 and 80% with ejaculated spermatozoa independently from the sperm's functionality as long as the male gamete is viable [89]. In some ICSI cases, with a frequency ranging from 3 to 5% [90], complete fertilization failure occurs [91–96]. This complete fertilization failure can have various reasons [92–96] but most often occurs because of a nucleus-cytoplasmic maturation asynchrony [96–100]. In a proportion of these cases, however, the inability of the male gamete to generate conceptuses depends upon a defect in the presence or function of the sperm cytosolic factor [101,102].

While ICSI has overcome many gametic defects, it does not completely eliminate fertilization failure. The possible etiologies underlying total fertilization failure are complex [103] and may relate to cycle-specific parameters, oocyte yield and quality, availability of motile spermatozoa and/or severity of sperm defects [90]. Male gamete abnormalities may result in defective sperm DNA decondensation, and/or aberrant pronuclear development, migration and apposition as well as abnormalities at mitosis [104,105]. Some ICSI inseminated oocytes fail to be activated [106] and this activation failure has, under some circumstances, been successfully overcome by exposure to calcium ionophore or electrical pulses to manipulate intracellular calcium influx [107–110].

Several early studies of gamete ultrastructure [94], staining of DNA [111] or whole chromosomes [112,113] have identified abnormal chromatin patterns and/or chromosome numbers, of either paternal or maternal origin, in non-fertilized oocytes. Recent studies on ootid microtubules also revealed abnormal spindle and interphase microtubules, indicating that defects in oocyte cytoplasmic components may also be a cause for failed fertilization [114,115].

One of the simpler approaches to test sperm-oocyte interaction is the hamster ovum penetration test which is an apparently straightforward sperm function assay based on the capacity of human spermatozoa to fuse with zona pellucida-free golden hamster oocytes, leading to subsequent decondensation of the sperm nuclei [116]. This well recognized screening test presumably allows the reproductive specialist to learn more about the ability of the potential of a male gamete to fertilize an oocyte.

Sperm defects, however, are considered the leading cause of activation failure, and given the universally relegated role of PLCζ as the oocyte activating factor; it is highly plausible that defective forms, or abnormal function, of PLCζ may well be the underlying cause of certain types of male-factor infertility and related oocyte activation failure [101,117]. In fact, Heytens and colleagues [117] observed the first genetic link between abnormal PLCζ and male factor infertility by identifying the mutant PLCζ isoform in an infertile non-globozoospermic male. The identification of specific localization patterns of PLCζ in fertile males, and the precise functional relevance of PLCζ subcellular distribution, would provide a key benchmark to which suboptimal spermatozoa may be compared and can be used as a diagnostic tool. Previous work using immunofluorescent techniques has indicated that there appears to be a specific distribution of PLCζ in the sperm head, possibly in the perinuclear theca, that is consistent among fertile spermatozoa [29,117], and an abnormal localization pattern is evident in spermatozoa that consistently failed to fertilize with ICSI [101,117–119]. This argues for a correlation between an abnormal PLCζ activity within the sperm head and its ability to activate the oocyte at fertilization. In a quantitative analysis where real-time PCR was performed, the assessment of the relative expression of PLCζ may provide a useful marker for the ability of the sperm to induce oocyte activation after ICSI more than chromatin maturity, acrosin activity or even the size of the acrosome [120]. In another study that examined the levels of PLCζ protein present in the sperm of men with normal oocyte activation *versus* those that failed to activate found that the oocyte activation deficient group had significantly lower levels of total PLCζ. However, significant amounts of variability was present in the sperm samples of the same man and between the control and oocyte activation deficient group indicating that this assay may not be a clinically viable prognostic indicator of oocyte activation capability [121].

To better understand the ability of the human spermatozoon to interact with an oocyte without misuse of precious human material, the use of heterologous ICSI insemination of rodent oocyte has been proposed. While hamster oocytes are not suitable for this purpose because they may be easily activated by the injection procedure itself, instead mouse oocytes can be injected with human sperm cells to test their aptitude to activate an oocyte [122]. To test this hypothesis, mouse oocytes were randomized to injection with motile human sperm, sham injection (medium only), and heat-treated or salt-extracted spermatozoa. The motile spermatozoa generated an activation rate of 97% (102/105), while the sham injected and heat-/salt-treated spermatozoa resulted in an activation rate of 14–20% [122]. Once the test oocyte is activated, the human sperm nucleus decondenses forming a pronucleus allowing the chromosome to replicate in a xenogenic environment. Furthermore, a recent study [123] attempted to analyze the ability of a mouse oocyte activation test (MOAT) to provide diagnostic and prognostic information for patients suffering from fertilization failure. The MOAT assay was able to generate three groups of patients depending on the ability of the spermatozoa to activate the mouse oocyte, namely group 1 with $\leq$20%, group 2 21–84%, and group 3 with $\geq$85%. The authors concluded that the patients in group 1 are those that have sperm-related deficiency and the likely cause of previous fertilization failure. However, for group 2, the MOAT test is inconclusive where both sperm and oocyte deficiencies may contribute to their previous fertilization failure, and group 3, sperm-related deficiencies can certainly be refuted.

Among the spermiogenetic anomalies one of the most distinct is represented by globozoospermia (also known as round-headed sperm syndrome) which is a rare type of teratozoospermia accounting for <0.1% of male infertility [124]. Singh [125] described two types of globozoospermia: type I with round-headed spermatozoa lacking the acrosome and acrosomal enzymes and type II with round-headed spermatozoa having remnants of the acrosome. Most often these two variants are represented within the same individuals. The lack of an acrosome, whose production is a postmeiotic event in spermatogenesis, and round sperm heads are its main characteristics. An acrosomeless spermatozoon is unable to penetrate the zona pellucida and therefore unable to reach the oolemma of the oocyte [126,127]. However, even if these spermatozoa are injected in the perivitelline space or within the ooplasm most often fail to fertilize due to the dysruption of the perinuclear theca and, therefore, lack of the sperm cytosolic factor [128]. Even though round-headed spermatozoa can be seldom present in infertile men, only in a few familial cases [129,130], all spermatozoa will have the typical spheric head that require diagnostic confirmation through transmission electron microscopy (TEM). In some rare cases men with a well-defined sperm abnormality such as globozoospermia can be assessed for single gene defects, such as SPATA16 (spermatogenesis associated protein 16) [131] and PICK1 (protein interacting with c kinase 1) [132] which are both presumably involved in the formation of the acrosome. Single gene defects are commonly expected in patients with a specific phenotype. However, the large majority of our patients for male factor infertility suffer from poor semen parameters. For these men, it is difficult to predict whether a single gene defect is exclusively responsible for their inability to procreate or is more likely the result from an interaction of one or more genes that are potentially reflecting environmental influence. In an earlier study on a young couple with known globozoospermia, a multitude of assays was performed to confirm his globozoospermic status

[133]. Patient had an unremarkable physical exam, 46XY karyotype, negative Yq microdeletion, DNA damage of his unprocessed semen was remarkably high at 80% and dropped when swim-up was performed, with normal sperm aneuploidy rates. PLCζ was not detected by immunofluoresnce or Western blotting. In this case, ICSI followed by oocyte activation with calcium ionophore resulted in high rates of fertilization and an ongoing pregnancy [133]. In a case report, where the patient was diagnosed with globozoospermia as assessed by MSOME, somatic karyotype, sperm FISH, and genetic screening of SPATA16 and DPY19L2, the use IMSI in this case generated 2 good quality embryos for transfer that resulted in a delivery of a healthy child [123]. Finally, in a case series of three patients with globozoospermia, MSOME identified increased levels of PLCζ relative to sperm from the sample that did not undergo MSOME. In fact, novel patterns of PLCζ in spermatozoa exhibiting an acrosomal bud were observed. Each patient was able to obtain a pregnancy and go to term except for one who had a miscarriage after a fresh and frozen embryo transfer [134]. In addition, a particular mouse strain, the wobbler, is a useful model for globozoospermia and could also be used to study abnormalities in PLCζ expression [135]. The wobbler mouse is characterized by motor neuron disease where the males are infertile and whose sperm cells are round-headed lacking the acrosome. The presence of PLCζ in the spermatozoa was lacking after immunohistochemistry but PLCζ protein expression was found in the testis. The wobbler sperm cells also had reduced fertilizing capacity that was restored only after artificially activating the oocyte [135].

For patients that have experienced failed or low fertilization rates with ICSI, it is important to have an assay to diagnose the underlying reason for the failure. The crucial question is how to distinguish the activation deficiency due to a lack or depressed activity of the sperm factor from the impaired ability of the oocyte to respond to this factor. One way is to perform heterologous ICSI to evaluate the activation capacity of human spermatozoa by injection into mouse oocytes [128,136] as previously mentioned. However, this is time consuming due to the need to stimulate and harvest mouse oocytes and be impractical or unachievable in a hospital or private setting. As we have tested in our practice, an alternative method would be to search for the presence of the PLCζ within the spermatozoa utilizing a polyclonal antibody [29,119]. Cases of failed fertilization where lack of the sperm cytosolic factor have been clearly identified can be offered assisted oocyte activation.

The failure of fertilization due to sperm dysfunction attributable to the PLCζ ineffectiveness can only be encountered in about 1% of the men affected with infertility. The incidence, however, of the oocyte's inability to be activated in our clinic is about 3%. Obviously, independently from the PLCζ the female gamete has a responsibility in the process of oocyte activation. It is clear that an immature ooplasm is incapable of participating to the activation cascade as are germinal vesicle (GV) or metaphase I (MI) stages. Since the shift toward the routine use of recombinant medication associated with hypothalamic suppression with the GnRH antagonist, complete failed fertilization has become more recurrent in patients with history of successful fertilization in stimulated cycles even in presence of adequate number of oocytes and apparently normal semen specimen [99].

The developmental competence that is acquired by the oocyte as a result of the completion of meiosis to metaphase II (MII) plays a critical role during fertilization and subsequent stages of preimplantation embryonic development [137]. Several factors may be related to an inability of the oocyte to activate. First, the timing of maturational events appears to be tightly regulated [137–140] and the competence to undergo nuclear and cytoplasmic maturation is acquired independently during folliculogenesis [141]. Oocytes are retrieved from varying follicle sizes may carry an immature cytoplasm despite the premature extrusion of the first polar body. It has been postulated that such an asynchrony between nuclear and cytoplasmic maturation may be responsible for the inability of the ooplasm to support decondensation of the sperm nucleus [141,142]. In support of this idea, meiotic spindle visualization reveals that these apparently mature oocytes (first polar body extruded) may still be at telophase I or prometaphase II [143–145] and even though deemed ready for ICSI, still contain an immature cytoplasm incapable of participating to the oocyte activation process.

While nuclear maturation is easily measured by the extrusion of the first polar body, cytoplasmic adequacy is not readily ascribable and is highly dependent upon the integrity of oocyte-cumulus-complex. We believe that an asynchronous nuclear-cytoplasmic maturation is responsible for the sporadic unexpected fertilization failure. This is not an anomaly but is simply a transient stage that can be easily corrected by lengthening the interaction between the oocyte with its cumulus oophorus. To prove our point, we correlated data between an unexpected failed fertilization cycle and a successful one within the same patients. In a four-year time span, we assessed ICSI patients who had presented both cycles with and without fertilization following ICSI including at least 3 MII oocytes. In this manner, the patient provided the study sample (failed fertilization cycles) and its own control (cycles with fertilization). We also compared the study population to patients who achieved fertilization in all oocytes injected considered as the gold standard [98].

A total of 8224 ICSI cases were reviewed, and of these, 6154 (74.8%) couples used their own gametes. We identified 183 (3.0%) couples that were characterized by complete failed fertilization and only 37 (0.6%) fulfilled the inclusion criteria. No differences were observed between the cycles that had successful fertilization and those that did not: maternal age, BMI, days of stimulation, or lead follicle size. While the hCG to oocyte retrieval interval (hCG-OPU) was similar between the study and control group, the hCG to decoronization (hCG-DEC) and hCG to ICSI intervals revealed a significantly shorter length in the failed fertilization cycles ($\Delta = -39$ min and $\Delta = -36$ min, respectively; $P = 0.01$) (Fig. 2). The increasing length in hCG → DEC in the study group *versus* its own control as well as the standard is depicted in Fig. 3 ($\Delta = -31$ min, $\Delta = -59$ min, and $\Delta = -65$ min, respectively; $P < 0.01$).

Here, we infer that cumulus cells facilitate signaling pathways instrumental in the acquisition of ooplasmic competence. This conclusion finds support in *in vitro* maturation studies, which indicate that the integrity of the cumulus–oocyte-complex plays a pivotal role in the expeditious completion of cytoplasmic maturation [146].

When all the different attempts to understand the reason for the failure of fertilization, and after dedicated counseling and related consent is obtained, the ultimate way to rule out gamete dysfunction is to inseminate the patient's oocytes with anonymous donor sperm. On the other hand, in resilient cases that fail to fertilize even with donor spermatozoa, the utilization of anonymous donor oocytes may be able to pinpoint the gamete responsible for the inability to generate a zygote.

5. Treatment Options

With the advent of standard *in vitro* fertilization procedures it became evident that infertile couples whose male partner displayed suboptimal semen parameters often fail to yield zygotes. Successful fertilization *in vitro* is usually affected by the number of motile spermatozoa available as well as their morphology. In some cases, the chances of fertilization can be enhanced by simply decreasing the medium volume and enhancing spatial interactions between the gametes such as in the microdrops insemination technique. This approach was capable of raising chances of fertilization to over 40% and providing a clinical pregnancy rate of 24% [147].

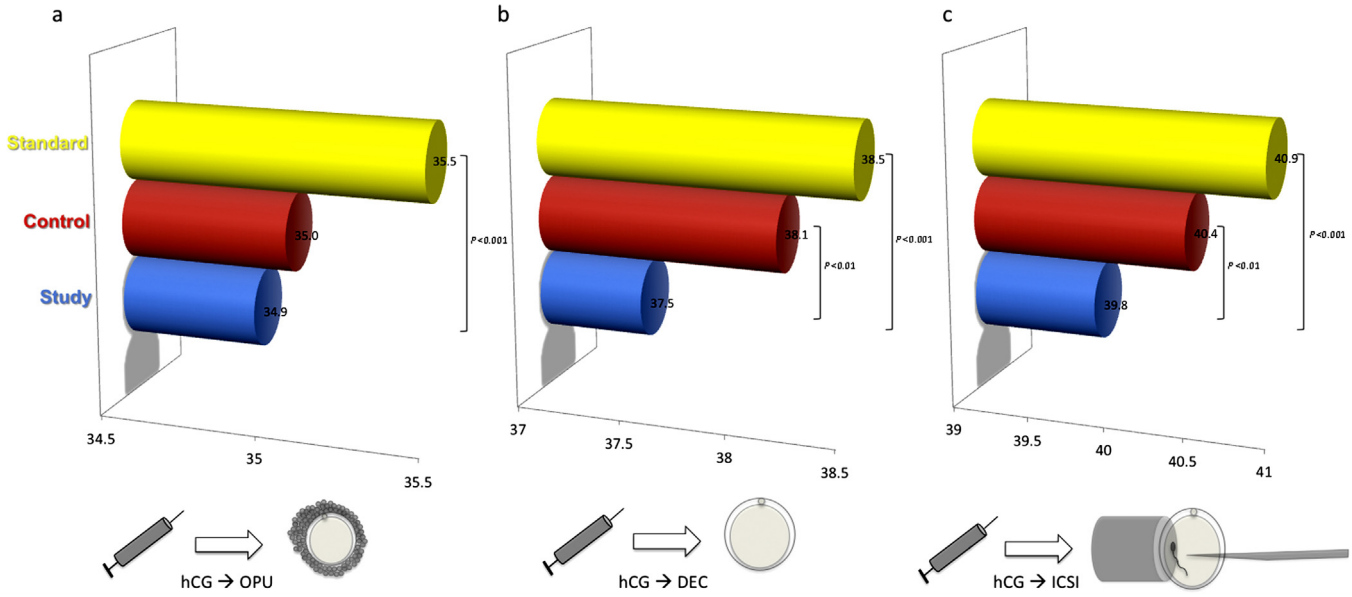

Fig. 2. ICSI cycles including study group (blue) and paired control (red) as well as the gold standard (yellow). The time interval from hCG administration to oocyte pick up (OPU) was shorter than the gold standard ($P < 0.001$) (a). The time interval from hCG administration to decoronization (DEC) was also shorter in the study group in comparison to their own control ($P < 0.01$) and standard ($P < 0.001$) (b). Finally, a similar pattern in observed in the hCG administration to ICSI injection between the study being the shortest in comparison to their own control ($P < 0.01$) and standard ($P < 0.001$) (c). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

With the adoption of assisted fertilization techniques based on bypassing the zona pellucida, such as zona drilling, partial zona dissection, and subzonal injection, there was an expectation for a superior outcome. However, these procedures still needed normal functioning and capacitated spermatozoa for sperm–oolemma fusion to occur, thereby resulting in unacceptably poor fertilization rates [148].

Assisted fertilization by ICSI has brought down the ratio of the two parental gametes to 1:1, whereas an individual spermatozoon is paired to an oocyte, paving the way to important clues

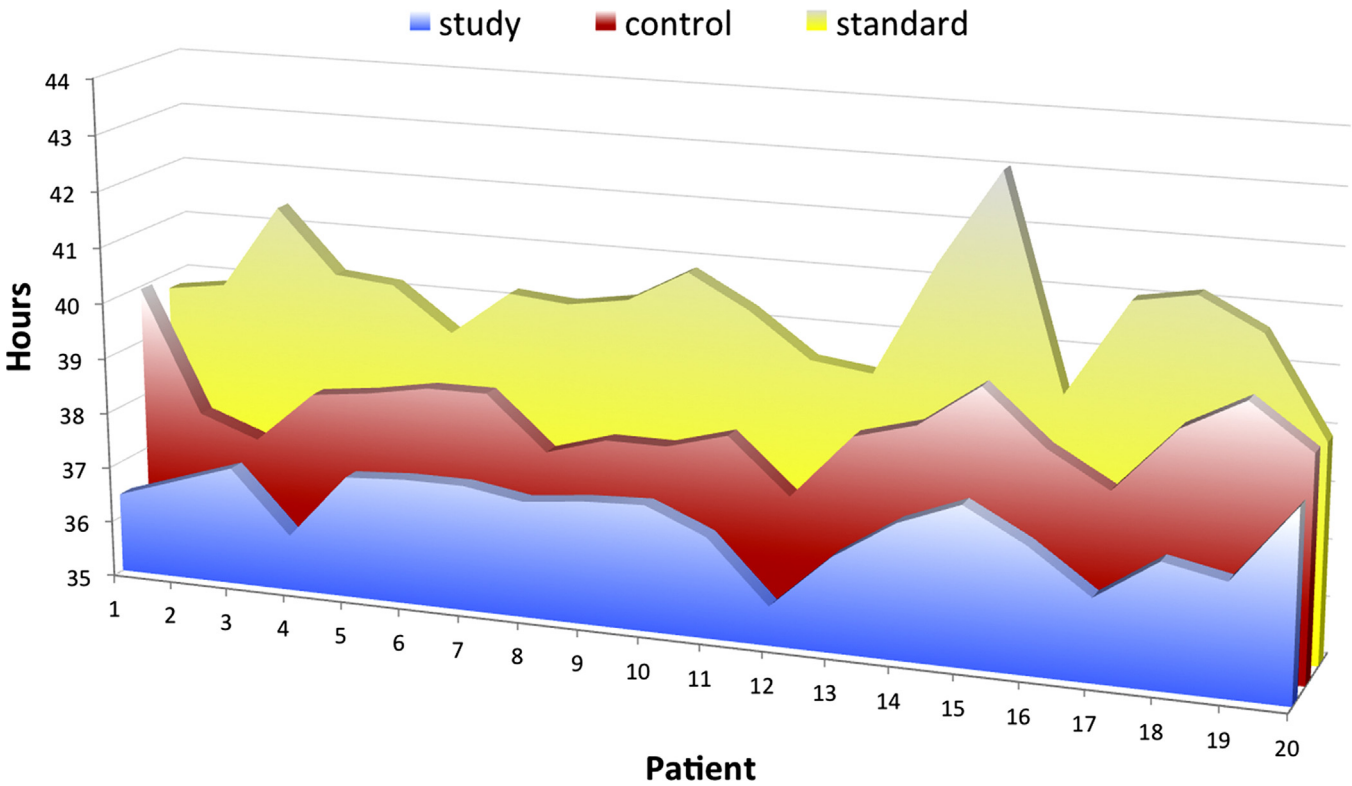

Fig. 3. Time interval between hCG administration and cumulus removal. An example of patients ($n = 20$) plotted according to the length of time from hCG administration to removal of the cumulus (DEC, decoronization). The patients in the study group (blue) had the shortest time interval (hCG → DEC) in comparison to their own cycle with fertilization (red) and the gold standard (yellow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

regarding the intimate interaction between the two gametes. ICSI has been comparably successful whether the sperm sample is fresh or frozen, with suboptimal semen parameters and other confounding factors such as the presence of anti-sperm antibodies. All these aspects of male infertility appeared insurmountable by other assisted fertilization techniques but appear to have a negligible impact on ICSI outcome [149,150]. Interestingly, ICSI performance is not related to whether spermatozoa were collected from ejaculate or, were retrieved surgically, from the epididymis or testis [151,152]. Moreover, ICSI's dependability has broadened its initial use from a technique capable of overriding the dysfunction of spermatozoa to one that may partly overcome issues related to the oocyte [153]. Even though ICSI has the ability to overcome many obstacles, we can only stress that ICSI does not guarantee successful fertilization to every patient, and complete fertilization failure can still occur. Such failures as previously have been reported to be between 1% [90] and 3% [154] and may be explained by different factors related to oocytes and/or spermatozoa [153]. The terminology "failed fertilization" typically refers to failure of all the available mature MII oocytes to fertilize. The principal cause of failed fertilization has been attributed to an oocyte activation disruption [94] whether related to female or male gamete dysfunction.

While recent studies aim at unveiling the intricate steps of the oocyte activation that trigger intracellular Ca^{2+} oscillations in the typical manner, in the clinical setting we currently observe complete and recurrent fertilization failure by enhancing the permeability of the oolemma and by modulating extracellular content to favor calcium influx.

Different protocols for assisted oocyte activation (AOA) have been established and are commonly divided into three subtypes—mechanical, electrical, and chemical stimuli—that elicit one or several calcium transients. During mechanical activation of oocytes, oolemmas are pierced using a microneedle to favor a calcium influx, and after a short period, ICSI is performed [60]. Another method for mechanical oocyte activation is the direct microinjection of calcium into an oocyte to increase intracellular calcium [155]. Electrical stimulation is another way to activate oocytes by allowing calcium influx through the formation of pores in the plasma membrane. The efficiency of this procedure depends on pore size, ionic content of the surrounding medium, and cell type. Electrical oocyte activation has been successfully used on bovine and human oocytes [110,156]. While it appears predictable and not noxious to the oocytes in terms of overall voltage and length of time exposure, it has been shown to induce reactive oxygen species [157]. Chemical compounds can also induce a calcium rise that initiates oocyte activation. It has been described with the use of compounds such as ethanol, calcium ionophore A213187, ionomycin, puromycin, strontium chloride, phorbol ester, and thimerosal [158]. These chemicals facilitate the influx of extracellular calcium ions into the oocyte due to the pores generated. Some of these compounds may induce a single or insufficient calcium rise in the oocyte while others may induce multiple and unpredictable calcium spikes [159]. However, human oocytes do not always respond to common mammalian oocyte activators such as most of the aforementioned agents. To mimic the natural pattern of calcium rise after sperm penetration or to optimize the rate of human oocyte activation, these agents are sometimes applied in combination [160].

A series of studies and case reports have focused on the use of assisted oocyte activation with a variety of agents [90] providing more consistent fertilization with term pregnancies. However, not all assisted oocyte activation studies have a successful outcome due to the small or no improvement in fertilization [161] or not being able to support a successful pregnancy. One of the earliest studies was a case report on ICSI couples characterized by poor fertilization, where the use of calcium ionophore post-injection lead to reasonable zygote formation [162]. Another cohort of 56 patients with a history of inconsistent fertilization due to severe sperm head abnormalities used ionomycin to enhance fertilization but failed to generate good quality embryos [163]. In another study where globozoospermic ($n = 10$) and oligo-astheno-terato-zoospermic ($n = 20$) men were treated by injecting $CaCl_2$ concurrently with ICSI followed by sequential exposure of calcium ionophore not only gained superior fertilization but provided clinical pregnancies and deliveries of healthy neonates [155]. In a larger group of patients with severe teratozoospermia ($n = 87$), couples without or with extremely low fertilization benefitted from 10 μm ionomycin which in turn positively affected implantation and pregnancy rates [164]. In another center, reproductive biologists utilized strontium chloride to help couples with previous failed fertilization ($n = 9$). The authors were able to achieve high fertilization rates with good quality embryos for transfer and delivery of healthy babies [164]. In the largest case series ($n = 246$), this team allocated half of the sibling oocytes to electrical oocyte activation and the other half to no treatment. They were able to increase fertilization in the electrical oocyte activation group to 68% *versus* 60% in the control ($P < 0.001$). However, this enhancement did not translate in a higher pregnancy rate [165].

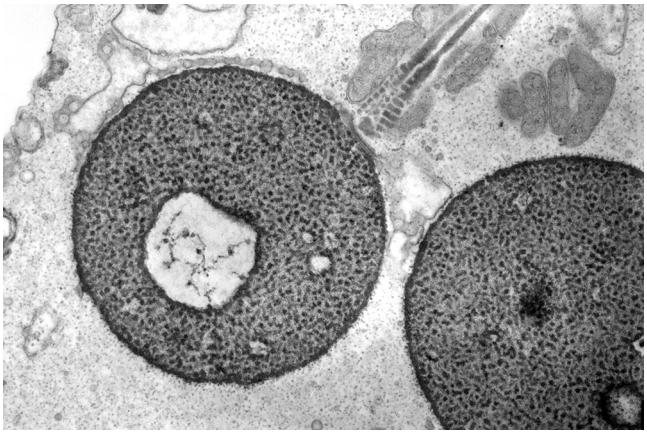

Fig. 4. Transmission electron microscopy (TEM) of spermatozoa was performed to diagnose globozoospermia in a man.

From September 1993 to June 2010, we have treated 11,390 couples by ICSI, representing 2.0% of the cycles with absolute fertilization failure. In 59 couples that had recurrent fertilization failure because of the lack of oocyte activating factor, only nine couples consented to undergo gamete pre-treatment. The couples (female age 37.4 ± 4 yr) had an average of 2.3 cycles with no fertilization with an average of 10.8 oocytes injected. They were subsequently treated in 18 cycles that produced a comparable number of oocytes (11.2). In one cycle, only a single MI oocyte was retrieved that did not mature. One man had a familial case of globozoospermia confirmed by TEM (Fig. 4). The semen specimens utilized for their gamete-pretreatment had an average concentration of $58.6 \pm 40 \times 10^6$ ml^{-1} with a mild impairment in motility of $21.6 \pm 11\%$, and morphology of $1.8 \pm 1\%$.

In all instances, the inability of the spermatozoa to induce oocyte activation was tested by injecting them into mouse oocytes [136]. In addition, prior to their AOA-ICSI treatment, we evaluated their spermatozoa for the presence of PLCζ by polyclonal antibody. PLCζ in these men was significantly lower ranging from 0 to no more than 14.4% of positivity in over 500 sperm cells/patient in comparison to 37–81% spermatozoa of fertile individuals ($P = 0.0001$) (Fig. 5).

To help facilitate the nuclear decondensation process without removing the sperm membrane and associated proteins, such as

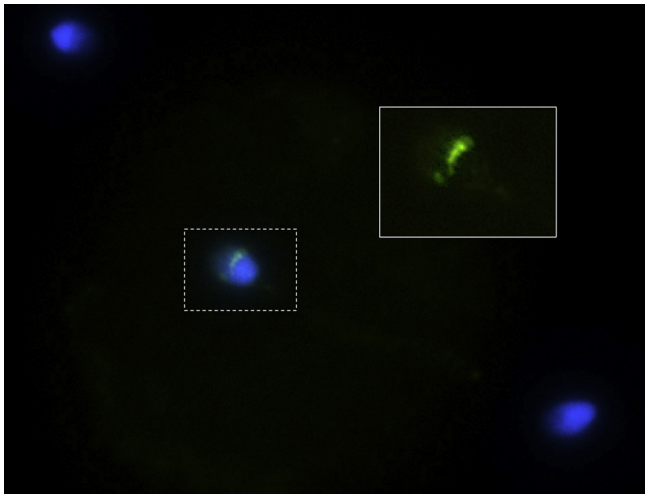

Fig. 5. Phospholipase C zeta (PLCζ) staining of spermatozoa where the central cell within the dotted box is positive (green fluorescence). The solid box indicated a magnified sperm nucleus with PLCζ localized in the equatorial segment. DAPI was used to counterstain the nuclei. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

PLCζ, we utilized a bacterial toxin–streptolysin O (SLO) [136,166]. Steptolysin O is a streptococcal secretory protein that permeabilizes cells by interacting with cholesterol in the plasmamembrane [167], aggregating within the lipid bilayer, and forming stable pores of 30 nm size [168]. The pores give continuous access to the intracellular milieu without stripping off the membrane, thus maintaining the integrity of the spermatozoon as well as its motility and the needed signal transduction pathways that maybe initiated through the constituents of the membrane such as the cell-surface receptors and sperm soluble factors. Spermatozoa were exposed to SLO (30 min) prior to ICSI to assist in sperm membrane permeabilization [169]. Following sperm injection, oocytes were sequentially treated with Ca^{2+} ionophore [119,136,155].

We were able to obtain a fertilization rate of 52.9% (41/79) with a cleavage rate of 87.7% with the mean number of blastomeres at day 3 of embryo development being 7.2 ± 1 and a mean fragmentation rate of $8.4\% \pm 4$. An average of 1.7 conceptuses were successfully replaced in all AOA cycles. A total 7 (38.9%) women had positive βhCG of which 3 (16.7%) progressed to clinical pregnancies and one couple delivered a healthy baby boy [166].

A multitude of studies have been carried out in mammals that identified the need for a so called "sperm factor" to trigger oocyte activation and subsequent embryo development. Biochemical characterization of the sperm extracts revealed that the active key component of the sperm factor is PLCζ [170] that has the intrinsic ability to cause Ca^{2+} oscillations in the oocyte while other isoforms do not. With the prevalence of oocyte activation deficiency, many studies have used chemical and electrical activation but with inconsistent results [158]. This brought forth the idea that a physiologic approach would be to utilize PLCζ in some specific form. Several studies proposed to inject recombinant PLCζ RNA and protein into mouse oocytes and this induced Ca^{2+} oscillations similar to those observed at fertilization and triggered embryo development to the blastocyst stage [25,26,171]. Interestingly, human sperm exhibiting deficient PLCζ expression was rescued by co-injection of mouse PLCζ mRNA into mouse oocytes [117,118]. Another study showed that the microinjection of oocyte activation deficient spermatozoa along with exposure to calcium ionophore generated live offspring [133]. Furthermore, microinjection of oocytes, that failed to fertilize with ICSI, with PLCζ cRNA resulted in the appearance of prolonged Ca^{2+} oscillations where each transient Ca^{2+} wave was accompanied by a small coordinated cytoplasmic movement [172]. These studies further solidified the idea that PLCζ is a great candidate as a clinical therapeutic solution that could avoid potentially deleterious effects associated with AOA, which use the non-physiological stimuli. However, the therapeutic utilization of PLCζ cRNA is not likely viable since the uncontrollable transcription of PLCζ may be detrimental to normal pre-implantation development through gene expression irregularities, with eventual developmental defects observed in some embryos [173]. In addition, injected PLCζ RNA could potentially, by reverse transcription into cDNA, be incorporated into the genome [174]. Therefore, an active and purified human recombinant PLCζ protein would be the ideal alternative for therapeutic application, as it would function in a more physiologic fashion [175].

Despite the widely conserved role of the phosphoinositide pathway and Ca^{2+} release in oocyte activation, the mechanism(s) by which sperm induces IP_3 production remained unclear for quite some time. In mammals, growing experimental evidence supports the notion that, following fusion of the gametes, a factor from the spermatozoon is responsible for inducing Ca^{2+} oscillations and inducing IP_3 production [176]. Initial evidence stemmed from injection of cytosolic sperm extracts into oocytes that reproduced the Ca^{2+} responses associated with fertilization regardless of the oocytes' species of origin [18,72]. So in our laboratory, we purified the sperm cytosolic factor (SCF) from human spermatozoa of fertile donors by freeze–thawing and sonication. Human oocytes that were not activated with round spermatids were reinjected with the frozen-thawed sperm fraction with a final concentration of at least one sperm equivalent/pl allowing the delivery of 5 pl volume of SCF into each oocyte. Sham injections with culture medium did not induce long-lasting Ca^{2+} rises. The injection of 5 pl of SCF elicited persistent Ca^{2+} oscillations [72,177]. Pronucleus formation in 90% of the human oocytes that survived the procedure (19/21) suggested that the fertilization failure was due to lack of the oocyte activator within the injected male gamete. Cytogenetic analysis of the resulting zygotes confirmed the involvement of the male genome. The main limitation of this approach is that the SCF is not patient specific and was derived from pooled specimens.

Considering that during the process of oocyte activation there is always the need to first produce IP_3 to initialize the endogenous calcium release and sustain subsequent oscillation, we thought to directly inject IP_3 into the mouse oocytes [178]. In order to maintain the exact frequency of the release, we used caged-IP_3 that was injected into mouse oocytes to test the feasibility of highjacking the oocyte calcium release cascade and triggering calcium oscillation in a comparable manner to what occurs during normal fertilization. In response to the uncaging of IP_3 by exposure to UV light, a rise in cytosolic calcium generating an oscillation every 30 min that was comparable to the calcium influx occurring during physiologic fertilization (Fig. 6) (Lee and Machaca, unpublished data).

With these encouraging findings, we then attempted to simulate the physiological calcium cascade in humans. IP_3 (D-myo-inositol 1,4,5-triposphate potassium salt, Invitrogen) was directly injected into 15 donated human oocytes (IRB 9801003210) matured *in vitro*. The IP_3 concentrations injected ranged from 1 μM, 5 μM, 10 μM, and 100 μM but unfortunately, none of the oocytes showed any sign of activation. The concentration of 1–10 μM IP_3 was probably too low allowing immediate metabolization by the oocyte. While cells injected with 100 μM became vacuolated possibly due to non-specific activation of the calcium cascade unrelated to oocyte activation generally seen during fertilization [136].

An important aspect to identify spermatozoa capable of achieving fertilization is their viability. This, however, cannot be directly assessed on stage while performing ICSI and is conventionally extrapolated by a spermatozoon's kinetic characteristics. In fact, we know that samples from severe asthenospermic (extremely low motility) men can be successfully treated by ICSI but *conditio sine*

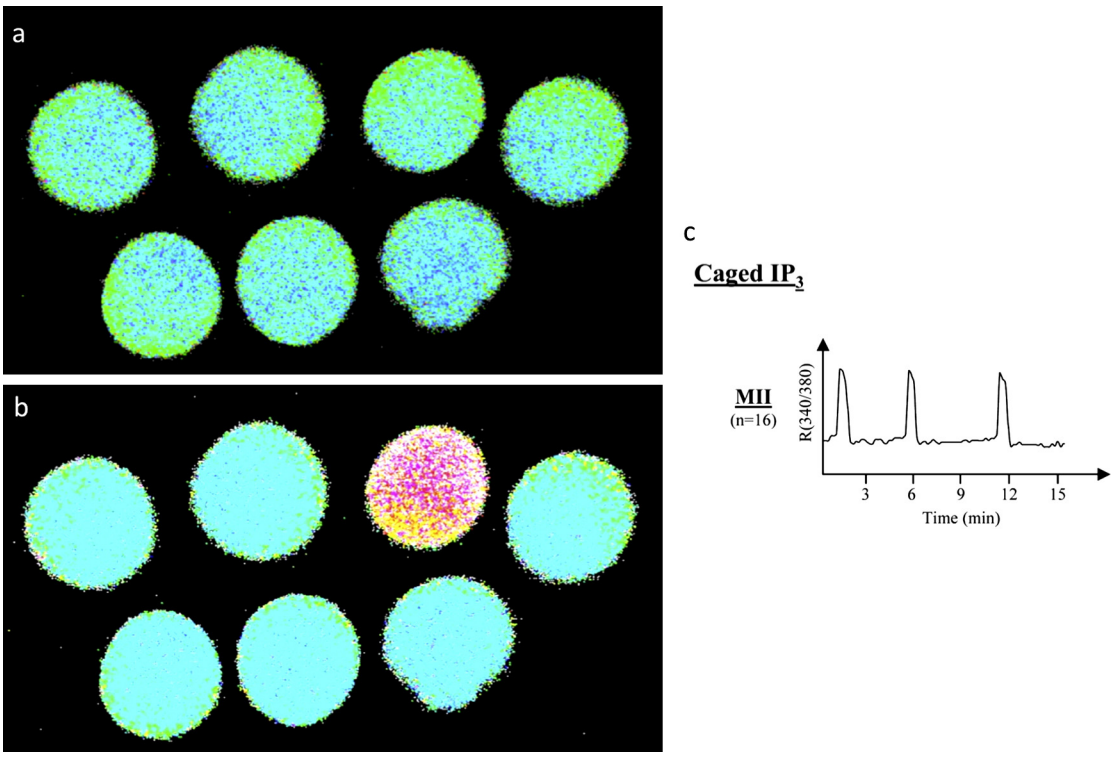

Fig. 6. Metaphase II oocytes treated with caged IP3 (a). The uncaging of IP3 through UV exposure enhanced calcium in-flux in one oocyte (red/pink) (b). The side graph depicts calcium oscillation observed at different time points following target release IP3 (c). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

qua non to achieve fertilization is sperm viability as detected by its motility.

Since early attempts of intra-ooplasmic injection, it was reported that completely immotile spermatozoa, referred to at the time as "dead" [179], would still actually achieve fertilization in animals. However, early experimentations of actual injection of immotile human spermatozoa into an oocyte, failed to achieve fertilization and to distinguish the native immotile possibly non-viable from the recently immotile obviously which brought to the introduction of sperm immobilization prior to ICSI (Palermo et al. 1999; [93]) aimed at answering the question of whether an immotile, but viable spermatozoon is capable of activating an oocyte. To prove this a progressively motile sperm cell was slashed on its flagellum with a glass pipette. The modification did indeed dramatically increase fertilization (Palermo, personal communication).

As a proof of this concept, we propose the use of ATP/$MgSO_4$ solution to stimulate the kinetic machinery to distinguish spermatozoa that possibly retain their ability to fertilize an oocyte. Upon exposure to ATP, 64.6% (296/458) of the spermatozoa evaluated were actually revived as detected by flagellar twitching. To confirm whether these spermatozoa can still activate an oocyte, we carried out a PLCζ assessment and evidenced that the putative soluble sperm cytosolic factor was maintained in the perinuclear theca. We plan to use this treatment on surgically retrieved spermatozoa and for those recovered after cryopreservation, where absence of motility is often observed and do not respond to motility enhancers.

We tested this procedure on a frozen-thawed epididymal specimen that although had a concentration of $34.0 \times 10^6\ ml^{-1}$, spermatozoa were all immotile and did not respond to motility enhancers with a viability of 33.2%. ATP incubation was able to elicit kinetic motion in 59.8% (79/132) of the exposed sperm cells [180]. Comforted by this outcome, in a one couple where the male partner was azoospermic post-chemotherapy, the only available specimen was accidentally thawed during intercontinental transport with obvious loss of motility, the immotile spermatozoa were unable to fertilize once injected into five oocytes. In a subsequent cycle, following exposure to ATP solution, we were able to "resuscitate" enough spermatozoa to inject 16 oocytes that after calcium ionophore yielded 37.5% pronuclear formation. Unfortunately, even though two conceptuses were placed in the female partner no implantation occurred. In a later attempt, only three oocytes fertilized but also in this implantation was also unsuccessful.

To treat the fertilization failure, due to nucleus-cytoplasmic asynchrony we identified two patients with apparently normal gametes and recurrent fertilization failure. In their subsequent ICSI cycle we changed their hormonal stimulation profile aiming at reducing the number of follicles to enhance the estrogen content at the same time hoping to increase the number of MII oocytes at retrieval. To allow enough time for the ooplasm to mature, we lengthened the post-retrieval *in vitro* incubation time by leaving the oocyte–cumulus-complexes undisturbed. We accomplished that by making sure that the patients were the first to have their oocytes retrieved and the last ones to have their cumulus cells removed. This facilitated the cumulus-enhanced post-retrieval *in vitro* maturation of the ooplasm. The fertilization rate was 4/4 (100%) and 6/10 (60%) that resulted in two viable intrauterine gestations.

6. Safety and conclusions

The introduction of intracytoplasmic injection of a spermatozoon has alleviated the important aspect of human infertility related to the male partner. This procedure has clarified the different steps that result in the development of a new conceptus but has also allowed to pinpoint some dysfunctional aspects of the male gamete such as its inability to activate the oocyte. At the present time, cases with persistent failed fertilization can only

be solved by using assisted oocyte activation and the most widely adopted agents for human oocytes include Ca^{2+}-ionophore and ionomycin, at times strontium chloride, or electrical stimuli [155]. However, these methods represent indiscriminate attempts to activate the oocyte by flooding its ooplasm with calcium ions that do not follow the physiological cascade [117]. This practice fuels concerns as to how such chemicals may affect embryo viability and future offspring well-being due to potential cytotoxic, mutagenic and teratogenic effects they may have [2,158].

It has been postulated that inappropriate or insufficient Ca^{2+} oscillations may have long term consequences on embryo development and offspring well-being [181]. The literature shows that successful pregnancies have been achieved with the use of AOA in patients that had a history of failed fertilization with ICSI [107–110,155,164,182–188]. Several reports on the well-being of children born after ICSI with AOA have provided reassuring insights resulting with neonatal outcome within normal limits in regard to birth weight, gestational age, neonatal malformations, and occurrence of perinatal mortality [110,155,185]. In a cohort of 22 children born after AOA, regardless of the chemical used – $SrCl_2$ or A23187 – from birth to 4 years of age the activation process did not adversely affect height, weight, rate of malformation, and early development of these children [189]. In a larger cohort of 39 children evaluated at birth, it was found that birth weight and gestational age were not affected by the use of AOA. Some of the children were then reevaluated at the age of 3 and found that their IQ, motor skills, and behavioral profile were within normal limits [190].

There is now substantial evidence indicating that oocyte activation in mammals is triggered by the sperm specific PLCζ [25,117,118,191]. In fact, there is a growing interest in the possibility that defects in PLCζ function are responsible for certain types of male infertility. It has been proposed that the reduced levels or the existence of an inactive form of the oocyte activating factor in the sperm cell account for the rare cases of failed fertilization after ICSI [188]. The first observation was in the level and localization pattern of PLCζ in infertile men and healthy donors, even though variability in immunofluorescence was observed [29]. Subsequently, a report came out with the first genetic link between abnormal PLCζ expression and male factor infertility with the identification of the mutant PLCζ isoform [117]. A second PLCζ expression mutation was detected on the same patient manifesting as a substitution of histidine for leucine (H233L) within the X catalytic domain which was maternally inherited and was only treatable with the injection of the $PLC\zeta^{H233L}$ cRNA [191].

It appears that screening for PLCζ content allows the identification of couples that may benefit from assisted oocyte activation and provides them with a chance to conceive their own biological child. All the men included in our study had a lower content of PLCζ in the majority of their spermatozoa. This was more evident in the spermatozoa from globozoospermic men where the oocyte activating capacity is completely missing as shown by the absolute inability to yield fertilization with ICSI. Now our current predicament is that the large majority of our female patients needing assisted oocyte activation are over 35 years old, thus increasing their chance of generating aneuploid oocytes, and as such introducing a possible confounding factor into the process of embryo developmental competence following AOA.

Recurrent fertilization failure after ICSI can be successfully overcome by AOA treatment following sperm injection just triggering intracellular calcium oscillation in a calcium rich environment. The factitious exogenous calcium influx does not exclude the possibility that subtle effect on embryo development and gene expression regulation may occur in oocytes activated by ionophore treatment [181] and to examine this possibility, further studies will be needed. A more physiological intervention would be the injection PLCζ RNA or protein [175,192,193] at the time of ICSI, and may represent an important and novel physiological therapeutic strategy in these cases of failed fertilization. However, derivation of human recombinant PLCζ has proven to be problematic due to the need to use bacterial cell line, whole lysates, and at times the purified proteins are inactive [194].

At present, the current protocol to obviate human oocyte activation failure has been the indiscriminate flooding of the ooplasm with exogenous calcium. Although these efforts at correction appear safe there is a strong desire to devise more physiological methods to override the spermatozoal dysfunction to activate the oocyte for fertilization. Regardless of whether there is one or multiple proteins involved with oocyte activation, further work should be carried out since fertilization failure carries a high emotional and financial toll and finding new treatments would help alleviate affliction and distress to infertile couples.

Acknowledgements

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References

[1] K. Hardy, C. Wright, S. Rice, M. Tachataki, R. Roberts, D. Morgan, S. Spanos, D. Taylor, Future developments in assisted reproduction in humans, Reproduction 123 (2002) 171–183.
[2] M. Nomikos, K. Elgmati, M. Theodoridou, B.L. Calver, G. Nounesis, K. Swann, F.A. Lai, Phospholipase Czeta binding to PtdIns(4 5)P2 requires the XY-linker region, Journal of Cell Science 124 (2011) 2582–2590.
[3] P. Sutovsky, Sperm–egg adhesion and fusion in mammals, Expert Reviews in Molecular Medicine 11 (2009) e11.
[4] P.M. Wassarman, L. Jovine, H. Qi, Z. Williams, C. Darie, E.S. Litscher, Recent aspects of mammalian fertilization research, Molecular and Cellular Endocrinology 234 (2005) 95–103.
[5] Y. Lawrence, M. Whitaker, K. Swann, Sperm–egg fusion is the prelude to the initial Ca^{2+}increase at fertilization in the mouse, Development 124 (1997) 233–241.
[6] K. Swann, The soluble sperm oscillogen hypothesis, Zygote 1 (1993) 273–276.
[7] L.A. Jaffe, N.L. Cross, B. Picheral, Studies of the voltage-dependent polyspermy block using cross-species fertilization of amphibians, Developmental Biology 98 (1983) 319–326.
[8] D.S. Sun, S.J. Lo, C.H. Lin, M.S. Yu, C.Y. Huang, Y.F. Chen, H.H. Chang, Calcium oscillation and phosphatidylinositol 3-kinase positively regulate integrin alpha(IIb)beta3-mediated outside-in signaling, Journal of Biomedical Science 12 (2005) 321–333.
[9] S. Miyazaki, H. Shirakawa, K. Nakada, Y. Honda, Essential role of the inositol 1 and 4,5-trisphosphate receptor/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs, Developmental Biology 158 (1993) 62–78.
[10] K. Swann, M.J. Whitaker, Second messengers at fertilization in sea-urchin eggs, Journal of Reproduction and Fertility 42 (Suppl.) (1990) 141–153.
[11] B. Dale, L.J. DeFelice, G. Ehrenstein, Injection of a soluble sperm fraction into sea-urchin eggs triggers the cortical reaction, Experientia 41 (1985) 1068–1070.
[12] M.J. Berridge, Inositol trisphosphate and calcium signalling, Nature 361 (1993) 315–325.
[13] B. Lee, G. Palermo, K. Machaca, Downregulation of store-operated Ca^{2+} entry during mammalian meiosis is required for the egg-to-embryo transition, Journal of Cell Science 126 (2013) 1672–1681.
[14] L.L. Runft, L.A. Jaffe, L.M. Mehlmann, Egg activation at fertilization: where it all begins, Developmental Biology 245 (2002) 237–254.
[15] S.A. Stricker, Comparative biology of calcium signaling during fertilization and egg activation in animals, Developmental Biology 211 (1999) 157–176.
[16] J. Parrington, K. Swann, V.I. Shevchenko, A.K. Sesay, F.A. Lai, Calcium oscillations in mammalian eggs triggered by a soluble sperm protein, Nature 379 (1996) 364–368.
[17] S.L. Stice, J.M. Robl, Activation of mammalian oocytes by a factor obtained from rabbit sperm, Molecular Reproduction and Development 25 (1990) 272–280.
[18] K. Swann, A cytosolic sperm factor stimulates repetitive calcium increases and mimics fertilization in hamster eggs, Development 110 (1990) 1295–1302.
[19] Y.M. Wolny, R.A. Fissore, H. Wu, M.M. Reis, L.T. Colombero, B. Ergun, Z. Rosenwaks, G.D. Palermo, Human glucosamine-6-phosphate isomerase, a homologue of hamster oscillin, does not appear to be involved in Ca^{2+} release

in mammalian oocytes, Molecular Reproduction and Development 52 (1999) 277–287.
[20] B. Dale, M. Marino, M. Wilding, Sperm-induced calcium oscillations. Soluble factor, factors or receptors? Molecular Human Reproduction 5 (1999) 1–4.
[21] K.T. Jones, Protein kinase C action at fertilization: overstated or undervalued? Reviews of Reproduction 3 (1998) 7–12.
[22] A. Rice, J. Parrington, K.T. Jones, K. Swann, Mammalian sperm contain a Ca(2+)-sensitive phospholipase C activity that can generate InsP(3) from PIP(2) associated with intracellular organelles, Developmental Biology 228 (2000) 125–135.
[23] J. Parrington, M.L. Jones, R. Tunwell, C. Devader, M. Katan, K. Swann, Phospholipase C isoforms in mammalian spermatozoa: potential components of the sperm factor that causes Ca^{2+} release in eggs, Reproduction 123 (2002) 31–39.
[24] H. Wu, J. Smyth, V. Luzzi, K. Fukami, T. Takenawa, S.L. Black, N.L. Allbritton, R.A. Fissore, Sperm factor induces intracellular free calcium oscillations by stimulating the phosphoinositide pathway, Biology of Reproduction 64 (2001) 1338–1349.
[25] C.M. Saunders, M.G. Larman, J. Parrington, L.J. Cox, J. Royse, L.M. Blayney, K. Swann, F.A. Lai, PLC zeta: a sperm-specific trigger of Ca(2+) oscillations in eggs and embryo development, Development 129 (2002) 3533–3544.
[26] L.J. Cox, M.G. Larman, C.M. Saunders, K. Hashimoto, K. Swann, F.A. Lai, Sperm phospholipase Czeta from humans and cynomolgus monkeys triggers Ca^{2+} oscillations, activation and development of mouse oocytes, Reproduction 124 (2002) 611–623.
[27] M. Aarabi, Y. Yu, W. Xu, M.Y. Tse, S.C. Pang, Y.J. Yi, P. Sutovsky, R. Oko, The testicular and epididymal expression profile of PLC zeta in mouse and human does not support its role as a sperm-borne oocyte activating factor, PLoS ONE 7 (2012) e33496, 1-12.
[28] L.T. Colombero, M. Moomjy, E.S. Sills, Z. Rosenwaks, G.D. Palermo, The role of structural integrity of the fertilising spermatozoon in early human embryogenesis, Zygote 7 (1999) 157–163.
[29] P. Grasa, K. Coward, C. Young, J. Parrington, The pattern of localization of the putative oocyte activation factor, phospholipase Czeta, in uncapacitated, capacitated, and ionophore-treated human spermatozoa, Human Reproduction 23 (2008) 2513–2522.
[30] S. Kuretake, Y. Kimura, K. Hoshi, R. Yanagimachi, Fertilization and development of mouse oocytes injected with isolated sperm heads, Biology of Reproduction 55 (1996) 789–795.
[31] L. Meng, D.P. Wolf, Sperm-induced oocyte activation in the rhesus monkey: nuclear and cytoplasmic changes following intracytoplasmic sperm injection, Human Reproduction 12 (1997) 1062–1068.
[32] Y. Kimura, R. Yanagimachi, S. Kuretake, H. Bortkiewicz, A.C. Perry, H. Yanagimachi, Analysis of mouse oocyte activation suggests the involvement of sperm perinuclear material, Biology of Reproduction 58 (1998) 1407–1415.
[33] L.T. Colombero, T. Takeuchi, E.S. Sills, W.G. Breed, Z. Rosenwaks, G.D. Palermo, A comparison of human spermatozoa immunolabeling features using xenogenic reagents for centrosomal proteins, Clinical and Experimental Obstetrics & Gynecology 26 (1999) 141–146.
[34] G. Palermo, S. Munne, J. Cohen, The human zygote inherits its mitotic potential from the male gamete, Human Reproduction 9 (1994) 1220–1225.
[35] R.H. CDC, Assisted Reproductive Technology Success Rates. National Summary and Fertility Clinic Reports, National Center for Chronic Disease and Prevention and Health Promotion, 2008.
[36] J. Cohen, R.G. Edwards, C.B. Fehilly, S.B. Fishel, J. Hewitt, G. Rowland, P.C. Steptoe, J. Webster, Treatment of male infertility by in vitro fertilization: factors affecting fertilization and pregnancy, Acta Europaea Fertilitatis 15 (1984) 455–465.
[37] J.W. Gordon, B.E. Talansky, Assisted fertilization by zona drilling: a mouse model for correction of oligospermia, Journal of Experimental Zoology 239 (1986) 347–354.
[38] Y. Tsunoda, T. Yasui, K. Nakamura, T. Uchida, T. Sugie, Effect of cutting the zona-pellucida on the pronuclear transplantation in the mouse, Journal of Experimental Zoology 240 (1986) 119–125.
[39] Y. Odawara, A. Lopata, A zona opening procedure for improving in vitro fertilization at low sperm concentrations: a mouse model, Fertility and Sterility 51 (1989) 699–704.
[40] J.W. Gordon, L. Grunfeld, G.J. Garrisi, B.E. Talansky, C. Richards, N. Laufer, Fertilization of human oocytes by sperm from infertile males after zona pellucida drilling, Fertility and Sterility 50 (1988) 68–73.
[41] J. Cohen, H. Malter, C. Fehilly, G. Wright, C. Elsner, H. Kort, J. Massey, Implantation of embryos after partial opening of oocyte zona pellucida to facilitate sperm penetration, Lancet 2 (1988), 162-162.
[42] S. Antinori, C. Versaci, P. Fuhrberg, C. Panci, B. Caffa, G.H. Gholami, Seventeen live births after the use of an erbium-yytrium aluminum garnet laser in the treatment of male factor infertility, Human Reproduction 9 (1994) 1891–1896.
[43] W. Feichtinger, H. Strohmer, P. Fuhrberg, K. Radivojevic, S. Antinori, G. Pepe, C. Versaci, Photoablation of oocyte zona pellucida by erbium-YAG laser for in-vitro fertilisation in severe male infertility, Lancet 339 (1992) 811.
[44] A. Laws-King, A. Trounson, H. Sathananthan, I. Kola, Fertilization of human oocytes by microinjection of a single spermatozoon under the zona pellucida, Fertility and Sterility 48 (1987) 637–642.
[45] G. Palermo, H. Joris, P. Devroey, A.C. Van Steirteghem, Induction of acrosome reaction in human spermatozoa used for subzonal insemination, Human Reproduction 7 (1992) 248–254.
[46] G. Palermo, A. Van Steirteghem, Enhancement of acrosome reaction and subzonal insemination of a single spermatozoon in mouse eggs, Molecular Reproduction and Development 30 (1991) 339–345.
[47] G. Palermo, H. Joris, P. Devroey, A.C. Van Steirteghem, Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte, Lancet 340 (1992) 17–18.
[48] Y. Hiramoto, Microinjection of the live spermatozoa into sea urchin eggs, Experimental Cell Research 27 (1962) 416–426.
[49] T.P. Lin, Microinjection of mouse eggs, Science 151 (1966) 333–337.
[50] T. Uehara, R. Yanagimachi, Microsurgical injection of spermatozoa into hamster eggs with subsequent transformation of sperm nuclei into male pronuclei, Biology of Reproduction 15 (1976) 467–470.
[51] S.J. Naish, S.D. Perreault, A.L. Foehner, B.R. Zirkin, DNA synthesis in the fertilizing hamster sperm nucleus: sperm template availability and egg cytoplasmic control, Biology of Reproduction 36 (1987) 245–253.
[52] S.D. Perreault, R.A. Wolff, B.R. Zirkin, The role of disulfide bond reduction during mammalian sperm nuclear decondensation in vivo, Developmental Biology 101 (1984) 160–167.
[53] N. Laufer, B.M. Pratt, A.H. DeCherney, F. Naftolin, M. Merino, C.L. Markert, The in vivo and in vitro effects of clomiphene citrate on ovulation, fertilization, and development of cultured mouse oocytes, American Journal of Obstetrics and Gynecology 147 (1983) 633–639.
[54] V.M. Thadani, Injection of sperm heads into immature rat oocytes, Journal of Experimental Zoology 210 (1979) 161–168.
[55] A. Iritani, K. Utsumi, M. Miyake, Y. Hosoi, K. Saeki, In vitro fertilization by a routine method and by micromanipulation, Annals of the New York Academy of Sciences 541 (1988) 583–590.
[56] K. Goto, A. Kinoshita, Y. Takuma, K. Ogawa, Fertilization of bovine oocytes by the injection of immobilized, killed spermatozoa, Veterinary Record 127 (1990) 517–520.
[57] S.E. Lanzendorf, M.K. Maloney, L.L. Veeck, J. Slusser, G.D. Hodgen, Z. Rosenwaks, A preclinical evaluation of pronuclear formation by microinjection of human spermatozoa into human oocytes, Fertility and Sterility 49 (1988) 835–842.
[58] Y. Nakano, H. Shirakawa, N. Mitsuhashi, Y. Kuwabara, S. Miyazaki, Spatiotemporal dynamics of intracellular calcium in the mouse egg injected with a spermatozoon, Molecular Human Reproduction 3 (1997) 1087–1093.
[59] M.S. Sato, M. Yoshitomo, T. Mohri, S. Miyazaki, Spatiotemporal analysis of $[Ca^{2+}]i$ rises in mouse eggs after intracytoplasmic sperm injection (ICSI), Cell Calcium 26 (1999) 49–58.
[60] J. Tesarik, M. Sousa, J. Testart, Human oocyte activation after intracytoplasmic sperm injection, Human Reproduction 9 (1994) 511–518.
[61] Y. Kimura, R. Yanagimachi, Intracytoplasmic sperm injection in the mouse, Biology of Reproduction 52 (1995) 709–720.
[62] A.C. Van Steirteghem, J. Liu, H. Joris, Z. Nagy, C. Janssenswillen, H. Tournaye, M.P. Derde, E. Van Assche, P. Devroey, Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles, Human Reproduction 8 (1993) 1055–1060.
[63] Y. Kimura, R. Yanagimachi, Mouse oocytes injected with testicular spermatozoa or round spermatids can develop into normal offspring, Development 121 (1995) 2397–2405.
[64] Y. Kimura, R. Yanagimachi, Development of normal mice from oocytes injected with secondary spermatocyte nuclei, Biology of Reproduction 53 (1995) 855–862.
[65] A. Sakurai, S. Oda, Y. Kuwabara, S. Miyazaki, Fertilization, embryonic development, and offspring from mouse eggs injected with round spermatids combined with Ca^{2+} oscillation-inducing sperm factor, Molecular Human Reproduction 5 (1999) 132–138.
[66] Y. Sato, S. Miyazaki, T. Shikano, N. Mitsuhashi, H. Takeuchi, K. Mikoshiba, Y. Kuwabara, Adenophostin, a potent agonist of the inositol 1,4,5-trisphosphate receptor, is useful for fertilization of mouse oocytes injected with round spermatids leading to normal offspring, Biology of Reproduction 58 (1998) 867–873.
[67] M. Sousa, C. Mendoza, A. Barros, J. Tesarik, Calcium responses of human oocytes after intracytoplasmic injection of leukocytes, spermatocytes and round spermatids, Molecular Human Reproduction 2 (1996) 853–857.
[68] J. Tesarik, Oocyte activation after intracytoplasmic injection of mature and immature sperm cells, Human Reproduction 13 (Suppl. 1) (1998) 117–127.
[69] A. Ogura, J. Matsuda, R. Yanagimachi, Birth of normal young after electrofusion of mouse oocytes with round spermatids, Proceedings of the National Academy of Sciences of the United States of America 91 (1994) 7460–7462.
[70] D. Dozortsev, A. Rybouchkin, P. De Sutter, C. Qian, M. Dhont, Human oocyte activation following intracytoplasmic injection: the role of the sperm cell, Human Reproduction 10 (1995) 403–407.
[71] L. Leybaert, H.T. Depypere, M. Dhont, A. de Hemptinne, Changes of intracellular free calcium during intracytoplasmic sperm injection, Molecular Reproduction and Development 43 (1996) 256–260.
[72] G.D. Palermo, O.M. Avrech, L.T. Colombero, H. Wu, Y.M. Wolny, R.A. Fissore, Z. Rosenwaks, Human sperm cytosolic factor triggers Ca^{2+} oscillations and overcomes activation failure of mammalian oocytes, Molecular Human Reproduction 3 (1997) 367–374.
[73] G. Harton, P. Braude, A. Lashwood, A. Schmutzler, J. Traeger-Synodinos, L. Wilton, J.C. Harper, ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening, Human Reproduction 26 (2011) 14–24.

[74] G.D. Palermo, Q.V. Neri, D. Monahan, Z. Rosenwaks, Micromanipulation: Intracytoplasmic Sperm Injection and Assisted Hatching, Springer, New York, 2012.
[75] B. Bartoov, A. Berkovitz, F. Eltes, Selection of spermatozoa with normal nuclei to improve the pregnancy rate with intracytoplasmic sperm injection, New England Journal of Medicine 345 (2001) 1067–1068.
[76] B. Bartoov, A. Berkovitz, F. Eltes, A. Kogosowski, Y. Menezo, Y. Barak, Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome, Journal of Andrology 23 (2002) 1–8.
[77] M. Antinori, E. Licata, G. Dani, F. Cerusico, C. Versaci, D. d'Angelo, S. Antinori, Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial, Reproductive Biomedicine Online 16 (2008) 835–841.
[78] B. Bartoov, A. Berkovitz, F. Eltes, A. Kogosovsky, A. Yagoda, H. Lederman, S. Artzi, M. Gross, Y. Barak, Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection, Fertility and Sterility 80 (2003) 1413–1419.
[79] A. Berkovitz, F. Eltes, S. Yaari, N. Katz, I. Barr, A. Fishman, B. Bartoov, The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm, Human Reproduction 20 (2005) 185–190.
[80] A. Hazout, M. Dumont-Hassan, A.M. Junca, P. Cohen Bacrie, J. Tesarik, High-magnification ICSI overcomes paternal effect resistant to conventional ICSI, Reproductive Biomedicine Online 12 (2006) 19–25.
[81] L. Zamboni, The ultrastructural pathology of the spermatozoon as a cause of infertility: the role of electron microscopy in the evaluation of semen quality, Fertility and Sterility 48 (1987) 711–734.
[82] D.W. Fawcett, S. Ito, Observations on the cytoplasmic membranes of testicular cells, examined by phase contrast and electron microscopy, Journal of Biophysical and Biochemical Cytology 4 (1958) 135–142.
[83] B. Baccetti, A.G. Burrini, G. Collodel, A.R. Magnano, P. Piomboni, T. Renieri, C. Sensini, Crater defect in human spermatozoa, Gamete Research 22 (1989) 249–255.
[84] O. Kacem, C. Sifer, V. Barraud-Lange, B. Ducot, D. De Ziegler, C. Poirot, J. Wolf, Sperm nuclear vacuoles, as assessed by motile sperm organellar morphological examination, are mostly of acrosomal origin, Reproductive Biomedicine Online 20 (2010) 132–137.
[85] S. Peer, F. Eltes, A. Berkovitz, R. Yehuda, P. Itsykson, B. Bartoov, Is fine morphology of the human sperm nuclei affected by in vitro incubation at 37 degrees C? Fertility and Sterility 88 (2007) 1589–1594.
[86] A. Tanaka, M. Nagayoshi, S. Awata, I. Tanaka, H. Kusunoki, S. Watanabe, Are crater defects in human sperm heads physiological changes during spermiogenesis? Fertility and Sterility 92 (2009) S165.
[87] S. Watanabe, A. Tanaka, S. Fujii, H. Misunuma, No relationship between chromosome aberrations and vacuole-like structures on human sperm head, Human Reproduction 24 (2009) i94–i96.
[88] G.D. Palermo, J.C.Y. Hu, L. Rienzi, R. Maggiulli, T. Takeuchi, A. Yoshida, A. Tanaka, H. Kusunoki, S. Watanabe, Q.V. Neri, Z. Rosenwaks, Thoughts on IMSI, Springer, New York, 2011.
[89] G.D. Palermo, Q.V. Neri, T. Takeuchi, Z. Rosenwaks, ICSI: where we have been and where we are going, Seminars in Reproductive Medicine 27 (2009) 191–201.
[90] N. Esfandiari, M.H. Faved, L. Gotlieb, R.F. Casper, Complete failed fertilization after intracytoplasmic sperm injection – analysis of 10 years' data, International Journal of Fertility and Women's Medicine 50 (2005) 187–192.
[91] P. Devroey, J. Liu, Z. Nagy, A. Goossens, H. Tournaye, M. Camus, A. Van Steirteghem, S. Silber, Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia, Human Reproduction 10 (1995) 1457–1460.
[92] S.P. Flaherty, D. Payne, N.J. Swann, C.D. Mattews, Aetiology of failed and abnormal fertilization after intracytoplasmic sperm injection, Human Reproduction 10 (1995) 2623–2629.
[93] G.D. Palermo, P.N. Schlegel, L.T. Colombero, N. Zaninovic, F. Moy, Z. Rosenwaks, Aggressive sperm immobilization prior to intracytoplasmic sperm injection with immature spermatozoa improves fertilization and pregnancy rates, Human Reproduction 11 (1996) 1023–1029.
[94] M. Sousa, J. Tesarik, Ultrastructural analysis of fertilization failure after intracytoplasmic sperm injection, Human Reproduction 9 (1994) 2374–2380.
[95] K. Yanagida, Complete fertilization failure in ICSI, Human Cell 17 (2004) 187–193.
[96] M. Moomjy, L.T. Colombero, L.L. Veeck, Z. Rosenwaks, G.D. Palermo, Sperm integrity is critical for normal mitotic division and early embryonic development, Molecular Human Reproduction 5 (1999) 836–844.
[97] H. Kang, Q.V. Neri, T. Takeuchi, F. Moy, Z. Rosenwaks, G.D. Palermo, Investigating unexpected complete fertilization failure with ICSI, Fertility and Sterility 84 (2005) S257.
[98] J. Kocent, D. Monahan, Q.V. Neri, Z. Rosenwaks, G.D. Palermo, Untimely cumulus removal affects ooplasmic competence, Fertility and Sterility 94 (2010) S252.
[99] G.D. Palermo, Q.V. Neri, D. Monahan, J. Kocent, Z. Rosenwaks, Development and current applications of assisted fertilization, Fertility and Sterility 97 (2012) 248–259.
[100] P. Sundstrom, B.O. Nilsson, Meiotic and cytoplasmic maturation of oocytes collected in stimulated cycles is asynchronous, Human Reproduction 3 (1988) 613–619.
[101] J. Kashir, B. Heindryckx, C. Jones, P. De Sutter, J. Parrington, K. Coward, Oocyte activation, phospholipase C zeta and human infertility, Human Reproduction Update 16 (2010) 690–703.
[102] K. Swann, M.G. Larman, C.M. Saunders, F.A. Lai, The cytosolic sperm factor that triggers Ca^{2+} oscillations and egg activation in mammals is a novel phospholipase C: PLC zeta, Reproduction 127 (2004) 431–439.
[103] J.E. Swain, T.B. Pool, ART failure: oocyte contributions to unsuccessful fertilization, Human Reproduction Update 14 (2008) 431–446.
[104] V.Y. Rawe, S. Brugo Olmedo, F.N. Nodar, A.D. Vitullo, Microtubules and parental genome organisation during abnormal fertilisation in humans, Zygote 10 (2002) 223–228.
[105] V.Y. Rawe, Y. Terada, S. Nakamura, C.F. Chillik, S.B. Olmedo, H.E. Chemes, A pathology of the sperm centriole responsible for defective sperm aster formation, syngamy and cleavage, Human Reproduction 17 (2002) 2344–2349.
[106] B. Kovacic, V. Vlaisavljevic, Configuration of maternal and paternal chromatin and pertaining microtubules in human oocytes failing to fertilize after intracytoplasmic sperm injection, Molecular Reproduction and Development 55 (2000) 197–204.
[107] T. Eldar-Geva, B. Brooks, E.J. Margalioth, E. Zylber-Haran, M. Gal, S.J. Silber, Successful pregnancy and delivery after calcium ionophore oocyte activation in a normozoospermic patient with previous repeated failed fertilization after intracytoplasmic sperm injection, Fertility and Sterility 79 (Suppl. 3) (2003) 1656–1658.
[108] B. Heindryckx, J. Van der Elst, P. De Sutter, M. Dhont, Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI, Human Reproduction 20 (2005) 2237–2241.
[109] Y. Murase, Y. Araki, S. Mizuno, C. Kawaguchi, M. Naito, M. Yoshizawa, Y. Araki, Pregnancy following chemical activation of oocytes in a couple with repeated failure of fertilization using ICSI: case report, Human Reproduction 19 (2004) 1604–1607.
[110] K. Yanagida, H. Katayose, H. Yazawa, Y. Kimura, A. Sato, H. Yanagimachi, R. Yanagimachi, Successful fertilization and pregnancy following ICSI and electrical oocyte activation, Human Reproduction 14 (1999) 1307–1311.
[111] B. Van Wissen, C. Eisenberg, P. Debey, G. Pennehouat, J. Auger, O. Bomsel-Helmreich, In vitro DNA fluorescence after in vitro fertilization (IVF) failure, Journal of Assisted Reproduction and Genetics 9 (1992) 564–571.
[112] C. Racowsky, M.L. Kaufman, R.A. Dermer, S.T. Homa, S. Gunnala, Chromosomal analysis of meiotic stages of human oocytes matured in vitro: benefits of protease treatment before fixation, Fertility and Sterility 57 (1992) 1026–1033.
[113] B.E. Rosenbusch, Frequency and patterns of premature sperm chromosome condensation in oocytes failing to fertilize after intracytoplasmic sperm injection, Journal of Assisted Reproduction and Genetics 17 (2000) 253–259.
[114] U. Eichenlaub-Ritter, H. Schmiady, H. Kentenich, D. Soewarto, Recurrent failure in polar body formation and premature chromosome condensation in oocytes from a human patient: indicators of asynchrony in nuclear and cytoplasmic maturation, Human Reproduction 10 (1995) 2343–2349.
[115] F. Miyara, F.X. Aubriot, A. Glissant, C. Nathan, S. Douard, A. Stanovici, F. Herve, M. Dumont-Hassan, A. LeMeur, P. Cohen-Bacrie, P. Debey, Multiparameter analysis of human oocytes at metaphase II stage after IVF failure in non-male infertility, Human Reproduction 18 (2003) 1494–1503.
[116] WHO, WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed., Cambridge University Press, Cambridge, 2010.
[117] E. Heytens, J. Parrington, K. Coward, C. Young, S. Lambrecht, S.Y. Yoon, R.A. Fissore, R. Hamer, C.M. Deane, M. Ruas, P. Grasa, R. Soleimani, C.A. Cuvelier, J. Gerris, M. Dhont, D. Deforce, L. Leybaert, P. De Sutter, Reduced amounts and abnormal forms of phospholipase C zeta (PLCzeta) in spermatozoa from infertile men, Human Reproduction 24 (2009) 2417–2428.
[118] S.Y. Yoon, T. Jellerette, A.M. Salicioni, H.C. Lee, M.S. Yoo, K. Coward, J. Parrington, D. Grow, J.B. Cibelli, P.E. Visconti, J. Mager, R.A. Fissore, Human sperm devoid of PLC, zeta 1 fail to induce Ca(2+) release and are unable to initiate the first step of embryo development, Journal of Clinical Investigation 118 (2008) 3671–3681.
[119] Q.V. Neri, D. Monahan, J. Kocent, J.C.Y. Hu, Z. Rosenwaks, G.D. Palermo, Assessing and restoring sperm fertilizing ability, Fertility and Sterility 94 (2010) S147.
[120] S. Aghajanpour, K. Ghaedi, A. Salamian, M.R. Deemeh, M. Tavalaee, J. Moshtaghian, J. Parrington, M.H. Nasr-Esfahani, Quantitative expression of phospholipase C zeta, as an index to assess fertilization potential of a semen sample, Human Reproduction 26 (2011) 2950–2956.
[121] J. Kashir, C. Jones, G. Mounce, W.M. Ramadan, B. Lemmon, B. Heindryckx, P. de Sutter, J. Parrington, K. Turner, T. Child, E. McVeigh, K. Coward, Variance in total levels of phospholipase C zeta (PLC-zeta) in human sperm may limit the applicability of quantitative immunofluorescent analysis as a diagnostic indicator of oocyte activation capability, Fertility and Sterility 99 (2013) 107–117.
[122] A. Rybouchkin, D. Dozortsev, P. de Sutter, C. Qian, M. Dhont, Intracytoplasmic injection of human spermatozoa into mouse oocytes: a useful model to investigate the oocyte-activating capacity and the karyotype of human spermatozoa, Human Reproduction 10 (1995) 1130–1135.
[123] F. Vanden Meerschaut, L. Leybaert, D. Nikiforaki, C. Qian, B. Heindryckx, P. De Sutter, Diagnostic and prognostic value of calcium oscillatory pattern analysis for patients with ICSI fertilization failure, Human Reproduction 28 (2013) 87–98.

[124] A.F. Holstein, C.G. Schirren, C. Schirren, J. Mauss, Round headed spermatozoa: a cause of male infertility, Deutsche Medizinische Wochenschrift 98 (1973) 61–62.
[125] G. Singh, Ultrastructural features of round-headed human spermatozoa, International Journal of Fertility 37 (1992) 99–102.
[126] B. Dale, M. Iaccarino, A. Fortunato, G. Gragnaniello, K. Kyozuka, E. Tosti, A morphological and functional-study of fusibility in round-headed spermatozoa in the human, Fertility and Sterility 61 (1994) 336–340.
[127] R.J. Aitken, L. Kerr, V. Bolton, T. Hargreave, Analysis of sperm function in globozoospermia – implications for the mechanism of sperm-zona interaction, Fertility and Sterility 54 (1990) 701–707.
[128] A. Rybouchkin, D. Dozortsev, M.J. Pelinck, P. De Sutter, M. Dhont, Analysis of the oocyte activating capacity and chromosomal complement of round-headed human spermatozoa by their injection into mouse oocytes, Human Reproduction 11 (1996) 2170–2175.
[129] A.H. Dam, I. Feenstra, J.R. Westphal, L. Ramos, R.J. van Golde, J.A. Kremer, Globozoospermia revisited, Human Reproduction Update 13 (2007) 63–75.
[130] E. Moretti, G. Collodel, G. Scapigliati, I. Cosci, B. Sartini, B. Baccetti, 'Round head' sperm defect. Ultrastructural and meiotic segregation study, Journal of Submicroscopic Cytology and Pathology 37 (2005) 297–303.
[131] A.H. Dam, I. Koscinski, J.A. Kremer, C. Moutou, A.S. Jaeger, A.R. Oudakker, H. Tournaye, N. Charlet, C. Lagier-Tourenne, H. van Bokhoven, S. Viville, Homozygous mutation in SPATA16 is associated with male infertility in human globozoospermia, American Journal of Human Genetics 81 (2007) 813–820.
[132] G. Liu, Q.W. Shi, G.X. Lu, A newly discovered mutation in PICK1 in a human with globozoospermia, Asian Journal of Andrology 12 (2010) 556–560.
[133] S.L. Taylor, S.Y. Yoon, M.S. Morshedi, D.R. Lacey, T. Jellerette, R.A. Fissore, S. Oehninger, Complete globozoospermia associated with PLCzeta deficiency treated with calcium ionophore and ICSI results in pregnancy, Reproductive Biomedicine Online 20 (2010) 559–564.
[134] J. Kashir, N. Sermondade, C. Sifer, S.L. Oo, C. Jones, G. Mounce, K. Turner, T. Child, E. McVeigh, K. Coward, Motile sperm organelle morphology evaluation-selected globozoospermic human sperm with an acrosomal bud exhibits novel patterns and higher levels of phospholipase C zeta, Human Reproduction 27 (2012) 3150–3160.
[135] E. Heytens, T. Schmitt-John, J.M. Moser, N.M. Jensen, R. Soleimani, C. Young, K. Coward, J. Parrington, P. De Sutter, Reduced fertilization after ICSI and abnormal phospholipase C zeta presence in spermatozoa from the wobbler mouse, Reproductive Biomedicine Online 21 (2010) 742–749.
[136] Q.V. Neri, Tweaking Human Fertilization, in: Reproductive Medicince, Clinical & Translation Science Center, Weill Cornell Medical College, New York, 2010, pp. 24.
[137] D.F. Albertini, A. Sanfins, C.M. Combelles, Origins and manifestations of oocyte maturation competencies, Reproductive Biomedicine Online 6 (2003) 410–415.
[138] J.J. Eppig, M. O'Brien, K. Wigglesworth, Mammalian oocyte growth and development in vitro, Molecular Reproduction and Development 44 (1996) 260–273.
[139] R.M. Moor, Y. Dai, C. Lee, J. Fulka Jr., Oocyte maturation and embryonic failure, Human Reproduction Update 4 (1998) 223–236.
[140] A. Trounson, C. Anderiesz, G. Jones, Maturation of human oocytes in vitro and their developmental competence, Reproduction 121 (2001) 51–75.
[141] J.J. Eppig, R.M. Schultz, M. O'Brien, F. Chesnel, Relationship between the developmental programs controlling nuclear and cytoplasmic maturation of mouse oocytes, Developmental Biology 164 (1994) 1–9.
[142] P. Sundstrom, O. Nilsson, Sequential changes in cytoplasmatic features during maturation of the human oocyte, Progress in Clinical and Biological Research 296 (1989) 327–333.
[143] L. De Santis, I. Cino, E. Rabellotti, F. Calzi, P. Persico, A. Borini, G. Coticchio, Polar body morphology and spindle imaging as predictors of oocyte quality, Reproductive Biomedicine Online 11 (2005) 36–42.
[144] L. Rienzi, F. Ubaldi, M. Iacobelli, M.G. Minasi, S. Romano, E. Greco, Meiotic spindle visualization in living human oocytes, Reproductive Biomedicine Online 10 (2005) 192–198.
[145] M. Montag, T. Schimming, H. van der Ven, Spindle imaging in human oocytes: the impact of the meiotic cell cycle, Reproductive Biomedicine Online 12 (2006) 442–446.
[146] C.S. Hyun, J.H. Cha, W.Y. Son, S.H. Yoon, K.A. Kim, J.H. Lim, Optimal ICSI timing after the first polar body extrusion in in vitro matured human oocytes, Human Reproduction 22 (2007) 1991–1995.
[147] A.O. Trounson, The choice of the most appropriate microfertilization technique for human male factor infertility, Reproduction, Fertility, and Development 6 (1994) 37–43.
[148] G.D. Palermo, J.M. Bedford, Micromanipulation of Human Gametes, Zygotes, and Embryos, CRC Press LLC, Boca Raton, Florida, 2000.
[149] G. Palermo, H. Joris, M.P. Derde, M. Camus, P. Devroey, A. Van Steirteghem, Sperm characteristics and outcome of human assisted fertilization by subzonal insemination and intracytoplasmic sperm injection, Fertility and Sterility 59 (1993) 826–835.
[150] G.D. Palermo, J. Cohen, M. Alikani, A. Adler, Z. Rosenwaks, Intracytoplasmic sperm injection: a novel treatment for all forms of male factor infertility, Fertility and Sterility 63 (1995) 1231–1240.
[151] P.N. Schlegel, Nonobstructive azoospermia: a revolutionary surgical approach and results, Seminars in Reproductive Medicine 27 (2009) 165–170.
[152] H. Tournaye, P. Devroey, J. Liu, Z. Nagy, W. Lissens, A. Van Steirteghem, Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens, Fertility and Sterility 61 (1994) 1045–1051.
[153] R. Maggiulli, Q.V. Neri, D. Monahan, J. Hu, T. Takeuchi, Z. Rosenwaks, G.D. Palermo, What to do when ICSI fails, Systems Biology in Reproductive Medicine 56 (2010) 376–387.
[154] S. Bhattacharya, M.P. Hamilton, M. Shaaban, Y. Khalaf, M. Seddler, T. Ghobara, P. Braude, R. Kennedy, A. Rutherford, G. Hartshorne, A. Templeton, Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial, Lancet 357 (2001) 2075–2079.
[155] B. Heindryckx, S. De Gheselle, J. Gerris, M. Dhont, P. De Sutter, Efficiency of assisted oocyte activation as a solution for failed intracytoplasmic sperm injection, Reproductive Biomedicine Online 17 (2008) 662–668.
[156] J. Zhang, C.W. Wang, A. Blaszcyzk, J.A. Grifo, J. Ozil, E. Haberman, A. Adler, L.C. Krey, Electrical activation and in vitro development of human oocytes that fail to fertilize after intracytoplasmic sperm injection, Fertility and Sterility 72 (1999) 509–512.
[157] O.J. Koo, G. Jang, D.K. Kwon, J.T. Kang, O.S. Kwon, H.J. Park, S.K. Kang, B.C. Lee, Electrical activation induces reactive oxygen species in porcine embryos, Theriogenology 70 (2008) 1111–1118.
[158] M.H. Nasr-Esfahani, M.R. Deemeh, M. Tavalaee, Artificial oocyte activation and intracytoplasmic sperm injection, Fertility and Sterility 94 (2010) 520–526.
[159] R. Alberio, V. Zakhartchenko, J. Motlik, E. Wolf, Mammalian oocyte activation: lessons from the sperm and implications for nuclear transfer, International Journal of Developmental Biology 45 (2001) 797–809.
[160] S. Yamano, K. Nakagawa, H. Nakasaka, T. Aono, Fertilization failure and oocyte activation, Journal of Medical Investigation 47 (2000) 1–8.
[161] J.H. Check, M.C. Levito, D. Summers-Chase, J. Marmar, H. Barci, A comparison of the efficacy of intracytoplasmic sperm injection (ICSI) using ejaculated sperm selected by high magnification versus ICSI with testicular sperm both followed by oocyte activation with calcium ionophore, Clinical and Experimental Obstetrics & Gynecology 34 (2007) 111–112.
[162] J. Tesarik, M. Sousa, More than 90% fertilization rates after intracytoplasmic sperm injection and artificial induction of oocyte activation with calcium ionophore, Fertility and Sterility 63 (1995) 343–349.
[163] M.N. Moaz, S. Khattab, I.A. Foutouh, E.A. Mohsen, Chemical activation of oocytes in different types of sperm abnormalities in cases of low or failed fertilization after ICSI: a prospective pilot study, Reproductive Biomedicine Online 13 (2006) 791–794.
[164] M.H. Nasr-Esfahani, S. Razavi, Z. Javdan, M. Tavalaee, Artificial oocyte activation in severe teratozoospermia undergoing intracytoplasmic sperm injection, Fertility and Sterility 90 (2008) 2231–2237.
[165] R. Mansour, I. Fahmy, N.A. Tawab, A. Kamal, Y. El-Demery, M. Aboulghar, G. Serour, Electrical activation of oocytes after intracytoplasmic sperm injection: a controlled randomized study, Fertility and Sterility 91 (2009) 133–139.
[166] T. Fields, Q.V. Neri, J.C.Y. Hu, Z. Rosenwaks, G.D. Palermo, A qualitative assay for sperm fertilization competence, Human Reproduction 19 (2011) i349.
[167] J.E. Alouf, Streptococcal toxins (streptolysin O, streptolysin S, erythrogenic toxin), Pharmacology & Therapeutics 11 (1980) 661–717.
[168] L. Buckingham, J.L. Duncan, Approximate dimensions of membrane lesions produced by streptolysin-S and streptolysin-O, Biochimica et Biophysica Acta 729 (1983) 115–122.
[169] E.J. Sullivan, S. Kasinathan, P. Kasinathan, J.M. Robl, P. Collas, Cloned calves from chromatin remodeled in vitro, Biology of Reproduction 70 (2004) 146–153.
[170] K. Swann, C.M. Saunders, N.T. Rogers, F.A. Lai, PLCzeta(zeta): a sperm protein that triggers Ca^{2+} oscillations and egg activation in mammals, Seminars in Cell & Developmental Biology 17 (2006) 264–273.
[171] Z. Kouchi, T. Shikano, Y. Nakamura, H. Shirakawa, K. Fukami, S. Miyazaki, The role of EF-hand domains and C2 domain in regulation of enzymatic activity of phospholipase Czeta, Journal of Biological Chemistry 280 (2005) 21015–21021.
[172] K. Swann, S. Windsor, K. Campbell, K. Elgmati, M. Nomikos, M. Zernicka-Goetz, N. Amso, F.A. Lai, A. Thomas, C. Graham, Phospholipase C-zeta-induced Ca^{2+} oscillations cause coincident cytoplasmic movements in human oocytes that failed to fertilize after intracytoplasmic sperm injection, Fertility and Sterility 97 (2012) 742–747.
[173] N.T. Rogers, E. Hobson, S. Pickering, F.A. Lai, P. Braude, K. Swann, Phospholipase Czeta causes Ca^{2+} oscillations and parthenogenetic activation of human oocytes, Reproduction 128 (2004) 697–702.
[174] C. Spadafora, Endogenous reverse transcriptase: a mediator of cell proliferation and differentiation, Cytogenetic and Genome Research 105 (2004) 346–350.
[175] S.Y. Yoon, J.H. Eum, J.E. Lee, H.C. Lee, Y.S. Kim, J.E. Han, H.J. Won, S.H. Park, S.H. Shim, W.S. Lee, R.A. Fissore, D.R. Lee, T.K. Yoon, Recombinant human phospholipase C zeta 1 induces intracellular calcium oscillations and oocyte activation in mouse and human oocytes, Human Reproduction 27 (2012) 1768–1780.
[176] S. Miyazaki, M. Ito, Calcium signals for egg activation in mammals, Journal of Pharmacological Sciences 100 (2006) 545–552.
[177] R.A. Fissore, C.R. Long, R.P. Duncan, J.M. Robl, Initiation and organization of events during the first cell cycle in mammals: applications in cloning, Cloning 1 (1999) 89–100.
[178] H. Shirakawa, S. Miyazaki, Spatiotemporal analysis of calcium dynamics in the nucleus of hamster oocytes, Journal of Physiology 494 (Pt 1) (1996) 29–40.
[179] C.L. Markert, Fertilization of mammalian eggs by sperm injection, Journal of Experimental Zoology 228 (1983) 195–201.

[180] Q.V. Neri, J. Kocent, Z. Rosenwaks, G.D. Palermo, Attempts to revive non-motile spermatozoa resilient to motility enhancers, Fertility and Sterility 94 (2010) S15–S16.
[181] J.P. Ozil, B. Banrezes, S. Toth, H. Pan, R.M. Schultz, Ca^{2+} oscillatory pattern in fertilized mouse eggs affects gene expression and development to term, Developmental Biology 300 (2006) 534–544.
[182] A. Ahmady, E. Michael, Successful pregnancy and delivery following intracytoplasmic injection of frozen-thawed nonviable testicular sperm and oocyte activation with calcium ionophore, Journal of Andrology 28 (2007) 13–14.
[183] H.J. Chi, J.J. Koo, S.J. Song, J.Y. Lee, S.S. Chang, 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, Fertility and Sterility 82 (2004) 475–477.
[184] E.K. Dirican, A. Isik, K. Vicdan, E. Sozen, Z. Suludere, Clinical pregnancies and livebirths achieved by intracytoplasmic injection of round headed acrosomeless spermatozoa with and without oocyte activation in familial globozoospermia: case report, Asian Journal of Andrology 10 (2008) 332–336.
[185] A. Egashira, M. Murakami, K. Haigo, T. Horiuchi, T. Kuramoto, A successful pregnancy and live birth after intracytoplasmic sperm injection with globozoospermic sperm and electrical oocyte activation, Fertility and Sterility 92 (2009) e2035–e2039, 2037.
[186] S.T. Kim, Y.B. Cha, J.M. Park, M.C. Gye, 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, Fertility and Sterility 75 (2001) 445–447.
[187] K. Kyono, S. Kumagai, C. Nishinaka, Y. Nakajo, H. Uto, M. Toya, J. Sugawara, Y. Araki, Birth and follow-up of babies born following ICSI using SrCl2 oocyte activation, Reproductive Biomedicine Online 17 (2008) 53–58.
[188] A.V. Rybouchkin, F. Van der Straeten, J. Quatacker, P. De Sutter, M. Dhont, Fertilization and pregnancy after assisted oocyte activation and intracytoplasmic sperm injection in a case of round-headed sperm associated with deficient oocyte activation capacity, Fertility and Sterility 68 (1997) 1144–1147.
[189] K. Kyono, T. Takisawa, Y. Nakajo, M. Doshida, M. Toya, Birth and follow-up of babies born following ICSI with oocyte activation using strontium chloride or calcium ionophore A23187, Journal of Mammalian Ova Research 29 (2012) 35–40.
[190] F. Vanden Meerschaut, E. D'Haeseleer, H. Roeyers, A. Oostra, K. Van Lierde, P. De Sutter, Neonatal and developmental outcome of children born following assisted oocyte activation (AOA), Fertility and Sterility 98 (2012) S16.
[191] J. Kashir, M. Konstantinidis, C. Jones, B. Lemmon, H.C. Lee, R. Hamer, B. Heindryckx, C.M. Deane, P. De Sutter, R.A. Fissore, J. Parrington, D. Wells, K. Coward, A maternally inherited autosomal point mutation in human phospholipase C zeta (PLCzeta) leads to male infertility, Human Reproduction 27 (2012) 222–231.
[192] K. Swann, F.A. Lai, PLCzeta and the initiation of Ca(2+) oscillations in fertilizing mammalian eggs, Cell Calcium 53 (2012) 55–62.
[193] M. Nomikos, Y. Yu, K. Elgmati, M. Theodoridou, K. Campbell, V. Vassilakopoulou, C. Zikos, E. Livaniou, N. Amso, G. Nounesis, K. Swann, F.A. Lai, Phospholipase Czeta rescues failed oocyte activation in a prototype of male factor infertility, Fertility and Sterility 99 (2013) 76–85.
[194] K. Swann, F.A. Lai, PLCzeta and the initiation of Ca(2+) oscillations in fertilizing mammalian eggs, Cell Calcium 53 (2013) 55–62.