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REVIEW

Evolution of intracytoplasmic sperm injection: From initial challenges to wider applications

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Abstract

Background: In vitro fertilization (IVF) has revolutionized infertility treatment. Nevertheless, male infertility requires more effective solutions. In 1992, the first-ever case of human birth via intracytoplasmic sperm injection (ICSI) was reported. ICSI involves microscopically injecting a sperm into an ovum. Successful ICSI has become a reliable therapy for couples facing infertility, a significant milestone. However, it has also introduced various challenges. This study also delves into ethical dilemmas arising from widespread ICSI use.

Methods: This review traces the history of ICSI, presenting pioneering attempts, first successful attempts, and critical reports on account of the initial skepticism toward the technology. The review also focuses on chronological progress until ICSI was recognized as effective and became widely applied.

Main findings: The review reveals that ICSI, although transformative, presents challenges. Successes include addressing male infertility and aiding fertilization. However, concerns arise regarding optimal sperm and embryo selection, genetic mutations, and long-term health implications. Ethical considerations surrounding ICSI's broad applications also surface.

Conclusions: Despite its success and effectiveness, ICSI is still evolving as a therapeutic method. By comprehensively evaluating the historical progress and the current status of ICSI and exploring its future prospects, this study highlights the importance of ICSI in infertility treatment.

KEYWORDS

assisted reproductive techniques, fertilization in vitro, intracytoplasmic sperm injections, male infertility, micromanipulation

1 | DEVELOPMENT OF MICROMANIPULATION SYSTEMS AND INITIAL ANIMAL EXPERIMENTATION

Micromanipulation, a technique for manipulating living cells using minuscule instruments, plays a critical role in cell research and experimentation. The prototype of this technique first appeared at the beginning of the 20th century.

In 1911, Kite reported a nuclear separation experiment where he separated the pronucleus of a fertilized ovum using a microneedle.[1] This was the first reported case of micromanipulation using a miniature instrument that penetrated a cell. Later in 1928, Emmerson designed a joystick-controlled micromanipulator, which reportedly made it possible to convey the operator's movements directly to the apparatus.[2] These are considered to be the prototypes of micromanipulation systems, and the development of these innovative systems allowed for more precise and accurate cell manipulations.

The primary goal of initial studies on sperm injection that were conducted from 1965 to 1980 was to investigate the early stages of fertilization, such as membrane fusion between intraspecific and interspecific gametes, the activation of ovum cytoplasm, and the formation of female and male pronuclei.[3] Experimental trials of ICSI were performed in various animal models before ICSI was successful in humans.

In 1962, the first attempt of ICSI was made on sea urchin eggs.[4] Although living sea urchin spermatozoa were injected into unfertilized sea urchin eggs, the nuclei of the spermatozoa did not decondense within the ovum. However, when further spermatozoa were injected into eggs that were activated by external stimuli after sperm injection, their nuclei exhibited behavior associated with the mitotic division of the fertilized eggs. These results indicated that the decondensation of sperm nuclei required activation of the ooplasm.

It was later reported that ICSI was performed in mammals (hamsters) and that pronuclei were formed within ova into which spermatozoa were injected.[5,6] The authors also reported that pronuclei were also formed when human spermatozoa were injected into hamster ova. Other cases of interspecific insemination have been reported for mice and rats,[7] frogs and humans,[8] and hamsters and mice.[9] These reports have revealed that factors within the ooplasm regulating the transformation of the sperm nucleus into the male pronucleus are not species-specific.

Later in 1988, ICSI was also reported to have been successfully performed in rabbit ova.[10,11] These reports serve as critical pieces of evidence in demonstrating the expanded applicability of ICSI to diverse animal species. Subsequently, in 1990, attempts of ICSI in bovine ova were reported.[12,13] These proved to be crucial studies on the application of ICSI in large animal models, thus prompting wider applications in reproductive technology for livestock.

Furthermore, the evolution of micromanipulation technology has been an important factor in the success of ICSI. In particular, the introduction of piezoelectric technology has resulted in a high success rate in ICSI of mouse oocytes.[14] In piezo-actuated micromanipulation, vibrations are induced by changing the voltage across piezoelectric elements, which are then transmitted to an injection needle attached to a piezo driver, allowing it to penetrate the zona pellucida without causing deformation. Similarly, after stretching the oolemma by inserting the needle, the oolemma can be punctured by applying a single piezo pulse. According to Kimura and Yanagimachi (1995), the oolemma of mouse oocytes has greater elasticity and extensibility and lower ooplasmic viscosity. This property leads to rapid dispersion of the ooplasm into the surrounding medium when the oolemma is punctured, often resulting in oocyte degeneration.[14] The piezo-driven technique effectively punctures the ooplasm of oocytes with minimal damage. The employment of piezoelectric technology enables ICSI with higher accuracy and reliability, with the technology playing particularly important roles in the research involving mice and other animals. Because mouse is an essential model animal in biological research, improving the success rate of ICSI has been an exceptionally important achievement in the fields of genetic manipulation and reproductive biology.

Moreover, a report in 1988 stated that the formation of pronuclei was confirmed after human spermatozoa were injected into animal ova.[15] In this study, morphologically aberrant spermatozoa from three male infertility patients were subjected to ICSI in hamster ova, and spermatozoa from all three patients reportedly formed pronuclei within the hamster ova. These results suggested that ICSI may function as a promising therapeutic option for patients with morphologically aberrant spermatozoa.

As such, attempts to use animal models before the success of human ICSI marked an important step toward the development and popularization of ICSI. These pioneering studies contributed to the establishment of the principles and techniques of ICSI and demonstrated the potential of ICSI as an innovative option for infertility treatment.

2 | ATTEMPTS BEFORE THE DEVELOPMENT OF ICSI: INVESTIGATION OF INSEMINATION BY MICROMANIPULATION

Since the birth of Louise Brown via in vitro fertilization (IVF) in 1978, IVF has greatly advanced infertility treatment. However, effective therapies for male infertility patients have still been called for. In the 1980s, with the rise in micromanipulation techniques, several novel methods were proposed to complement sperm–egg fertilization. This chapter presents insemination methods predating the development of ICSI, such as zona drilling, zona softening, partial zona dissection, and subzonal injection of a single spermatozoon into the perivitelline space (PVS). These attempts were pioneering approaches that constituted the archetypes of ICSI and are important pieces in understanding the background against which ICSI is widely practiced today.

First, zona drilling is a technique for prompting spermatozoa to penetrate the zona pellucida (ZP), which is a transparent external layer of an ovum. Since first proposed at the beginning of the 1980s, it was experimentally attempted on human and animal ova.[16,17] However, problems were encountered with this technique such as the defective formation and anomalous development of embryos due to damage to the ZP, and it was infeasible to achieve stable success rates.

Second, zona softening is a technique for physically reducing the ZP. It was aimed to improve the fertility of spermatozoa by further thinning down the ZP by chemical methods.[18] Although temporary successes were achieved in some experimental animals with this technique, it was never applied to human ova owing to concerns about the safety and embryo health of human eggs. Third, partial zona dissection is a technique for securing passage of spermatozoa by partially dissecting the ZP. First reported in 1988, the technique was successfully conducted in some experimental animals. However, in addition to problems such as embryonic abnormalities and implantation failure,[19] there have also been problems caused by zona rupture resulting from zona drilling, zona softening and zona dissection, which allows more sperm to pass through, leading to an increase in the polyspermy rate.[20]

In addition, the technique of subzonal injection of a single spermatozoon into the PVS was proposed in the mid-1980s.[21–25] It involved injecting a single spermatozoon (several spermatozoa according to some reports) into the PVS to achieve fertilization. Although some successes were reported in animal experiments,[26] concerns were raised over the difficulty in accurately injecting a spermatozoon and the low implantation rate. Because the zona-drilling procedure back then involved spraying acidic solution onto the ZP and direct zona-puncture was performed in the presence of cytochalasin D, it was likely that damage to ova was significant.[27]

These insemination techniques using micromanipulation technology served as invaluable experimental platforms toward the development of ICSI. However, a stable success rate could not be achieved with any of the techniques.

3 | SUCCESSFUL ICSI ATTEMPTS IN HUMANS

The history of successful ICSI attempts in humans was built on numerous trials, errors, and studies. This chapter explores the progress of human ICSI starting from failed attempts reported from 1988 to 1992 and then leading up to the first-ever successful case reported by Palermo et al. in 1992.

In 1988, an attempt was made at using human immature ova (MI stage) and spermatozoa that were scheduled to be discarded.[28] In this experiment, it was reported that insufficient force during sperm injection caused sperm to be pushed into the PVS and that sperm decondensation is hindered by the large amount of medium injected during sperm injection. The results of this attempt demonstrated that the formation of pronuclei may be triggered by the injection of sperm into human ova.

In 1989, the following year, a technique called MIMIC (micro-insemination by microinjection into the cytoplasm) was reported.[29] This technique involves directly injecting spermatozoa into the ooplasm (or rather accidentally injecting spermatozoa sometimes into the ooplasm instead of the original target, which is the PVS) and observes morphological differences in the acrosome reaction of spermatozoa between subzonal sperm insemination (SUZI) and ICSI. SUZI is a technique for facilitating passage through the ovum membrane by injecting spermatozoa into the PVS. Because ICSI is an invasive technique, the need to investigate the mechanism of fertilization and post-insemination survival was advocated. In 1991, a report was published comparing the results of SUZI and ICSI performed for patients with severe teratozoospermia.[30] According to this report, the fertilization rate after sperm injection into the PVS in SUZI was either 14.9% or 16.6%, with pregnancy confirmed in five patients and delivery in one patient. In contrast, when spermatozoa were injected (single sperm heads were injected) into 38 ova in ICSI, two pronuclei were formed in only four fertilized ova, and no cases of pregnancy were noted after transplantation. At this point, SUZI was regarded as a more effective technique than ICSI for patients with severe teratozoospermia because it yielded higher fertilization rates and was less invasive.

In 1992, Palermo et al. reported the first-ever successful attempt at human ICSI.[31] They stated that of the 47 MII-stage ova used, 38 survived and 31 were fertilized following ICSI. Subsequently, 15 embryos were transplanted, and pregnancy was confirmed in four of the eight cycles. Thus, two male infants were born from single pregnancies, and one male infant and one female infant were born from a bigeminal pregnancy. These were the first successful cases of human delivery via ICSI.

Over the long history of attempts toward successful human ICSI, the principle and technology of ICSI have gradually become sophisticated through various animal models. Later, various cases, including ones where no human pregnancy was achieved, were analyzed. The report of successful ICSI in 1992 brought new hope to patients with infertility, marking a groundbreaking contribution in the evolution of infertility treatment.

4 | SUBSEQUENT SUCCESSFUL ICSI CASES

After the first-ever successful ICSI attempt was reported in 1992, a series of progress and success reports in ICSI technology ensued. This chapter focuses on relevant studies published around this period, presenting comparative studies on the fertilization rates of SUZI, ICSI, and partial zona dissection (PZD), as well as ICSI attempts on male infertility patients who were contraindicated for conventional IVF (C-IVF) or who repeatedly underwent C-IVF attempts that failed. These success stories include cases where urologists have performed testicular biopsies, leading to successful births through ICSI using sperm extracted directly from the testes.

Since the successful ICSI attempt in 1992, many studies have been published, comparing ICSI with other non-ICSI insemination methods using micromanipulation and discussing the efficacy of ISCI. Studies that compared the results of SUZI and ICSI reported that the normal fertilization rate of ICSI (which directly injects a spermatozoon into an ovum) was higher than that of SUZI, whereas the denaturation rate after insemination was higher in ICSI.[32,33] Back then, PZD was widely considered an adjuvant method for achieving insemination.[34,35] PZD opens a hole into the ZP by microscopically puncturing it with a glass needle, thereby facilitating the entry of a spermatozoon into an ovum. Unlike zona drilling and zona softening presented in Chapter 1, the nonuse of chemical agents apparently has less effect on the fertilized ovum. According to a comparative study on ICSI, SUZI, and PZD, 627 ova were collected from 58 patients who had underwent failed SUZI attempts, and 251 ova were inseminated with ICSI, of which 71 were fertilized (28%). Of 296 ova inseminated with SUZI, 29 were fertilized (10%). Of 70 ova inseminated with PZD, 2 were fertilized (3%). No ova were fertilized with IVF. Fishel et al. stated that ICSI may not only be a substitute for SUZI when it fails but also may function as a first-choice insemination method.[36]

In addition, the work of Schoysman et al. further underscores the potential of ICSI in male infertility treatment. They highlight a case in which testicular sperm obtained by testicular biopsy in patients with excretory azoospermia led to a successful pregnancy.[37] Their research is a testament to the viability of testicular sperm for fertilization and opens new avenues for the treatment of male infertility, particularly in cases of epididymal blockage or absence of the vas deferens.

Meanwhile, studies were published comparing the results of C-IVF and ICSI in male infertility patients who were contraindicated for C-IVF or who had undergone repeated C-IVF that failed.[38–42] Reports have been filed on successful ICSI attempts using semen collected from patients with infertility due to the congenital bilateral loss of seminal ducts by MESA (microsurgical epididymal sperm aspiration) or TESE (testicular sperm extraction) procedure directly from the epididymis or the testis.[43–47] Besides, a new report that followed stated that ICSI yielded high fertilization and implantation rates and that the abnormality rate of ICSI-born infants did not increase,[48] thus further emphasizing the efficacy of ICSI. In 1993, a review on ICSI and SUZI was published, summarizing ICSI-related phenomena and clinical results.[49] In the "QUESTIONS AND MORE QUESTIONS" section of this review, questions regarding ICSI were answered in a Q&A format based on previous articles (questions on subjects that were not clarified in humans were answered based on articles in the field of animal reproduction and husbandry), indicating the strong interest in ICSI back then.

5 | TRIAL AND ERROR FOR IMPROVING THE ICSI SUCCESS RATE

While the effectiveness of ICSI was reported, the rate of pronucleus formation following ICSI was around 50% back then, posing a challenge of overcoming the low chance of pronucleus formation. To address this issue and improve the ICSI success rate, various trials have been attempted. This chapter reviews the studies that attempted to improve the ICSI success rate, ranging from the calcium ionophore procedure, ovum membrane aspiration, sperm immobilization, to ICSI needle insertion methods.

Back then, the low pronucleus formation rate and several abnormalities associated with ICSI were considered to be caused by disruptions in calcium concentrations. According to a study observing changes in calcium concentrations in the ooplasm after ICSI using a confocal microscope, a short calcium spike occurred immediately after a microinjection needle entered the ooplasm. However, the fact that a similar change was also observed when a medium containing no spermatozoa was injected suggested that this phenomenon itself did not activate the ovum. Moreover, because two types of calcium changes (sperm-dependent and non-sperm-dependent) were observed, the release of sperm factors was reported to be required for ovum activation.[50] Edwards et al. argued that the concentrations of calcium injected along with spermatozoa might play an important role in ovum activation following insemination and hypothesized that calcium concentrations in the medium might be causing the low pronucleus formation rate after ICSI.[51] Based on this hypothesis, they performed ICSI using a medium containing no calcium and a medium to which was added EGTA (ethylene glycol tetraacetic acid) that chelates calcium and discussed the results. When an artificial activation procedure using a calcium ionophore was performed to change calcium concentrations, the pronucleus formation rate after ICSI was reported to be improved.[52] Furthermore, calcium concentrations were reported to differ depending on the puncture technique used during ICSI. A study stated that aspirating the ovum membrane during ICSI established an influx of calcium into the ooplasm and close contact between the sperm head and intracellular ovum calcium following injection, thereby improving the fertilization rate.[53] Opinions differ as to whether the ovum membrane should be aspirated or not for improving the ICSI success rate, and the discussion is still ongoing.[54,55]

It was reported that sperm pretreatment before injecting into the ooplasm may be important. Considerations were made on attempts to incubate spermatozoa in pentoxifylline or 2-deoxyadenosine before being subjected to ICSI. However, no differences in the fertilization and division rates were noted between the treated and nontreated spermatozoa.[56] Later, immobilization gained prominence as a pretreatment option for spermatozoa. Studies showed that immobilizing a spermatozoon by scraping its tail with an injection needle before being injected into the ooplasm resulted in an improved normal fertilization rate in ICSI.[57–59] The sperm immobilization technique is widely employed during ICSI even today. Damaging the sperm membrane was reported to immobilize the spermatozoon and introduce the centrosome within the spermatozoon into the ooplasm, thereby inducing a nuclear fusion after the formation of a pronucleus.[60–62] Laser-based techniques for immobilizing spermatozoa were developed and were reported as methods for easily immobilizing spermatozoa.[63–65] However, these apparently did not gain enough traction. The most common technique today still remains to be one that scrapes the tail of a spermatozoon with an injection needle.

Besides the effects of spermatozoa during ICSI, studies that also explored the effects of the position of inserting an injection needle on the spindle apparatus within an ovum have been reported. In a study exploring the effects of human spermatozoa on the morphology of the spindle apparatuses in hamster ova after ICSI, when a spermatozoon was injected while care was taken to distance the polar body from the injection site by fixating the polar body at the topmost or bottommost positions (at the 12:00- or 6:00-o'clock positions) with a holding pipette, damage to the spindle apparatus was minimized.[66] Another study reported a technique to confirm the spindle apparatus within an ovum by polarized light microscopy (Polscope).[67]

When ICSI first succeeded, the low pronucleus formation rate posed to be a challenge. To address this issue, various trials have been attempted. Through these trials, the survival rate and pronucleus formation rate in ICSI improved, thus leading to more successful ICSI attempts. The advancement of these techniques has arguably contributed to the current widespread adoption of ICSI and the evolution of infertility treatment.

6 | CONTINUATION OF OPINIONS AND DISCUSSIONS ON ICSI

Since the first successful human ICSI attempt in 1992, there are numerous reports on the efficacy and improved therapeutic results of ICSI. On the other hand, skeptical views on ICSI, along with reports on abnormalities in fertilized ova possibly caused by ICSI and negative effects on ICSI-born children, have also surfaced. Studies published in this period included many letters of rebuttal against certain reports, thus reflecting the arguments transpiring over ICSI. This chapter presents debates over fertilization rates in ICSI, reports and rebuttals on genetic abnormalities in ICSI-conceived babies, and the views of the European Society of Human Reproduction and Embryology (ESHRE) on the possible risks of spindle body damage by ICSI, thereby explaining the circumstances surrounding ICSI back then.

First, concerns were raised that ICSI might be less cost-effective than other insemination techniques because it essentially requires expensive micromanipulation systems and skilled operators who handle such systems. In 1993, Tucker et al. compared the fertilization rates of C-IVF, SUZI, and ICSI. They reported in the form of debates that the fertilization rate of ICSI was 19%, which was not significantly different from that of SUZI (17%). Therefore, they argued that C-IVF should be conducted as a first option because the cost-effectiveness of ICSI was still low and that ICSI and other techniques should be reserved as secondary options.[68]

ICSI was also reported to have a risk of damaging the spindle body.[69] In ICSI-conceived cases, the proportion of infants with genetic abnormalities reportedly increased.[70–72] Given these reports, concerns were raised about the overhasty spread of ICSI, with some espousing the need to assess the possibility of genetic abnormalities using model animals.[73,74]

While the causes of male infertility for which ICSI is indicated mostly remain unknown, some severe cases of oligospermia are associated with the deletion of the Y chromosome, and this genetic defect is transmitted to the next generation. It has been reported that Y chromosome deletions associated with severe male factor infertility have the potential to be transmitted to subsequent generations via ICSI. In particular, deletions in the AZF (azoospermia factor) region are frequently observed in men with infertility, and these genetic abnormalities can be transmitted on to offspring via ICSI.[75–79] Furthermore, it has been reported that AZF deletions can expand or cause new de novo deletions during the genetic transmission process via ICSI.[80–84] ICSI has become a widely used technique, despite the unresolved issue of potential transmission of AZF microdeletions to subsequent generations. A solution to this issue has yet to be found. These findings emphasize the importance of genetic screening for patients considering ICSI. To increase the understanding and awareness of the genetic risks associated with ICSI, it is important that information about the possibility of genetic abnormalities is shared transparently between clinicians and patients. This will enable patients to make informed treatment decisions.

Some researchers contended that the high risk of ICSI stems from the fact that spermatozoa that cannot be fertilized in vivo can be fertilized by this technique.[85–87] In some azoospermia patients, their sperm cells may be used in ICSI instead. In such cases, post-ICSI abnormalities may be caused by the differences in cell cycle between sperm cells and egg cells. Edwards et al. summarized precautions when using sperm cells in ICSI.[88] ICSI is an important approach for male factor infertility treatment and has rapidly been applied in practice. However, many concerns have been raised that its practical application might be premature. Moreover, a series of reports warned that ICSI-related abnormalities might be attributed to polyvinylpyrrolidone (PVP) used in the procedure. PVP is a water-soluble, high-molecular-weight synthetic polymer. It increases the viscosity of the solvent when dissolved. During ICSI, sperm is suspended in a PVP-added culture solution to decrease the motility of spermatozoa for ease of use. PVP is absorbed into an injection needle along with spermatozoa and then injected into the ooplasm along with spermatozoa in ICSI. There have been reports that the presence of PVP in the ooplasm resulted in a delay in the start of calcium oscillations after ICSI,[89] that PVP reduced the post-ICSI fertilization rate by affecting the sperm membrane and chromosomes,[90,91] and that the use of PVP in ICSI increased the rate of chromosomal abnormalities.[92] Some refuted these reports by stating that normal survival and fertilization rates were achieved even when PVP was used and that abnormalities may occur even from a normal karyotype.[93] Nevertheless, a series of articles were published, insisting that the long-term risks in children conceived from ICSI using PVP should be investigated[94] and proposing ICSI techniques without the use of PVP.[95–97]

In 1996, the ESHRE announced its views on ICSI. The ESHRE collects clinical and pregnancy-related results annually to provide reliable information on the efficacy and safety of ICSI and publish the findings biennially. From 1993 to 1994, the frequency of ICSI sessions increased substantially in Europe. Ovum denaturation rates were low (7.2–10.6%), and high fertilization rates were achieved (51.1–60.8%). In follow-up observations of ICSI-born children, the incidences of severe congenital malformations or chromosomal abnormalities did not increase, and the number of cases remained small. The reporters mentioned that these were very reassuring results.[98]

It is true that ICSI has been established as an important approach toward treating male factor infertility and has become globally widespread. On the other hand, discussions on ICSI have been manifold, and despite increasing successes, skeptical views and concerns over risks still linger. Against this backdrop, the focus of the discussion has shifted to the prognosis of ICSI-born children.

7 | RESULTS AND DISCUSSIONS OF ICSI PROGNOSTIC INVESTIGATIONS

As several years have elapsed since the rapid popularization of ICSI, the results of prognostic investigations in ICSI-born children have started to be published. Prognostic investigations are critical in evaluating the developmental and health status of ICSI-born children. This chapter presents major results on the prognosis of ICSI-born children, encompassing studies from both ICSI proponents and skeptics.

Positive reports on the prognosis of ISCI-born children from birth to age 8 stated that the premature birth rate, newborn weight, occurrence rates of major malformations, chromosomal abnormalities, karyotypes, and psychological, motor, and intellectual development in ICSI-born children were not significantly different from those in the general population.[99–112] Moreover, reports on the prognosis of children born via ICSI using TESE-collected spermatozoa and children born via round spermatid injection similarly indicated that their health state and developmental level did not differ significantly.[113,114] A study in 2017 reported the results of a long-term prognostic investigation in 18- to 22-year-old women who gave birth via ICSI owing to male infertility and stated that their hormone levels (including anti-müllerian hormone, follicle-stimulating hormone, luteinizing hormone, and dehydroepiandrosterone sulfate) and vesicular follicle count were almost comparable to those in similarly aged women who gave birth via natural conception.[115]

Meanwhile, ICSI skeptics raised concerns over the prognosis of ICSI-born children. There have been reports that congenital abnormalities increased in ICSI-born children,[116,117] that risks of developmental retardation and autism increased,[118,119] and that their sperm concentration, total sperm count, and total motile sperm count were significantly lower than men born via natural conception.[120]

Some of these prognostic investigations involved a sample size of more than 1000, with some being nationwide studies. However, most were single-center studies involving a sample size <1000 (some even falling short of 100), and the characteristics of patients undergoing ICSI might have been biased.[121]

There have been many conflicting reports on the ICSI prognosis, and debates are still ongoing about the health and development of ICSI-born children. Even today, some studies report positive results, whereas others express concerns. To accurately assess the risks and health condition of ICSI-born children and deepen the understanding of the safety of ICSI, it will be necessary to conduct more multi-center, multi-regional, and multi-national long-term follow-up investigations incorporating further data.

8 | CONFLICTING OPINIONS ON ICSI IN THE 2000S AND RECENT TRENDS

As successful ICSI attempts continued, the technique began to be widely adopted for male infertility and other types of infertility to reliably achieve insemination. Besides, both ICSI proponents and skeptics published studies on ICSI from various perspectives, discussing the efficacy and hazards of ICSI based on systematic reviews and meta-analyses. This chapter focuses on ISCI-related discussions from the 2000s onward and presents findings that were evaluated from various perspectives.

Originally, ICSI was intended for male infertility cases in which it was difficult to obtain a fertilized egg by IVF; however, at this time ICSI began to be used from a different perspective. Particularly, ICSI would often be selected in an increasing number of cases to reliably achieve insemination. Possible examples include split insemination, 1-day-old ICSI, and rescue ICSI, which appear to apply to this case. Because ICSI reliably achieves insemination compared with C-IVF, split insemination is considered to yield a higher fertilization rate. Split insemination involves dividing ova collected from patients with unknown causes of infertility into two groups and performing C-IVF and ICSI to avoid fertility impairments.[122] One-day-old ICSI involves collecting a non-fertilized ovum after C-IVF or an ovum that was not mature during collection but later became mature and then inseminating it the following day by ICSI. This is conducted to avoid complete non-fertilization by C-IVF or a failure in insemination due to the absence of a mature ovum.[123–125] Rescue ICSI involves inseminating an ovum by ICSI at an earlier stage (4–24 h after the first insemination) than 1-day-old ICSI to complete insemination before the ovum is aged by in vitro incubation.[126–130]

Regarding the current trend where ICSI is attempted for reliably achieving insemination in an increasing number of cases, some studies have criticized the over-reliance on this technique,[131–133] and others have highlighted that clinical outcomes may not necessarily be improved by ICSI in patients aged 40 or older and patients with non-male factor infertility.[134] ICSI is an effective approach strictly to male infertility patients and, as such, does not necessarily improve clinical outcomes in non-male factor infertility patients. Some even cautioned that the fact that ICSI is not for everyone should be accepted.[135]

Nevertheless, since the 2000s and even in recent years, a series of reports have been indicating no increase in the incidence of abnormalities in ICSI-born children.[136–140] Although some findings suggested that infertility treatments, including ICSI, might negatively affect the cognitive development of the child, a systematic review also reported that a consensus has not necessarily been reached.[141] Moreover, as the emergence of molecular biological analytic techniques led to the analysis of DNA methylation, a study reported that the state of DNA methylation did not differ between ICSI-born and naturally born children.[142] As the number of patients receiving infertility treatment has been increasing yearly, so has the number of patients with infertility of unknown origin where no definite male factors are identified. The application of ICSI to such cryptogenic patients is reportedly effective in avoiding unexpected cases of complete failure in fertilization and reduced fertilization (partial failure in fertilization).[143,144]

Since the turn of the 2020s, a series of reports have been filed on ISCI-related abnormalities and risks. While ICSI is still being increasingly performed, discussions are ongoing about the appropriate application of the technique.

9 | NEW ICSI ATTEMPTS AND TECHNICAL SCRUTINY

Research on ICSI has been continuously advancing even in recent years, with many studies proposing novel methods and approaches. One such significant development is PIEZO ICSI, which represents a technological advancement in ICSI. The purposes of these studies are manifold and include improving the survival rate of fertilized ova and improving the success rate of conception in ICSI. This chapter presents the recent trends in ICSI-related research and explores how various techniques may contribute to infertility treatment by focusing on assessments and reviews, including meta-analyses, systematic reviews, and Cochrane reviews.

In recent years, the use of PIEZO ICSI in human reproductive medicine has progressed rapidly. In PIEZO ICSI, a smooth-tipped injection needle and a PIEZO drive are required. The needle vibrates back and forth due to piezo pulses. At this time, due to the large inertia of a heavy liquid such as mercury placed at the needle's tip, rapid changes in internal pressure occur in the lumen from the heavy liquid to the needle tip. This allows for the smooth penetration of the zona pellucida and the cell membrane.[145]

According to several studies, PIEZO ICSI reduces the rate of oocyte degeneration and abnormal fertilization in humans compared to conventional ICSI, which subsequently leads to improvements in oocyte survival, fertilization and pregnancy rates.[145–150] These improvements are believed to be due to the more refined technology offered by PIEZO ICSI compared to the conventional ICSI process. ICSI involves three main steps: sperm immobilization, zona pellucida penetration, and oocyte membrane puncture. While conventional ICSI requires extensive training to master these techniques, PIEZO ICSI is thought to facilitate these processes.[151–153] Despite the advantages of PIEZO ICSI, there are also disadvantages, such as the need for operating fluids such as mercury or fluorinert. The first application of PIEZO ICSI in human reproductive medicine was documented by Huang et al. in 1996.[151] In this report, mercury was not used to manipulate human oocytes, which may have limited the potential effectiveness of PIEZO ICSI at that time. Today, perfluoro-n-octane, a perfluorinated compound used in ophthalmic surgery as a heat transfer agent, dielectric fluid, and tamponade, is also used, which may improve safety compared to the use of mercury. However, the overall safety of PIEZO ICSI, including the use of these compounds for sperm injection into oocytes, requires long-term studies of children born from PIEZO ICSI.

ICSI techniques involve both mechanical and cellular aspiration methods to disrupt cell membranes. The mechanical method physically penetrates or disrupts the cell membrane, while the cellular aspiration method uses suction or negative pressure. In Japan, the widespread adoption of the cellular aspiration method may contribute to the ease of acceptance for PIEZO-ICSI, which simplifies cell disruption. It is important to recognize that while PIEZO ICSI may facilitate the ICSI process, this technology does not necessarily surpass the results achieved with well-practiced conventional ICSI techniques.

The second example is intracytoplasmic morphologically selected sperm injection (IMSI).[154,155] In ICSI, spermatozoa are generally screened at ×400 magnification. In IMSI, a high-magnification microscope (≥×6000) is used to more accurately observe minute characteristics and defects related to the morphology of spermatozoa at high resolution for screening morphologically favorable spermatozoa, which are then used for microscopic insemination. This procedure reportedly improves the success rate of conception.[154,155] Regarding Cochrane reviews, systematic reviews on IMSI were conducted in 2013[156] and then in 2020.[157] The 2020 review focused solely on the results of randomized controlled trials (RCTs) comparing ICSI with IMSI and reported that it was uncertain as to whether IMSI would improve clinical pregnancy rates, live birth rates, miscarriage rates per couple, and miscarriage rates per pregnancy.

The next example is hyaluronic acid–selected sperm intracytoplasmic sperm injection (HA-ICSI), which involves screening physiologically matured spermatozoa for ICSI. Based on its screening method, it is also referred to as physiological ICSI. Hyaluronic acid (HA) is a main component of the extracellular matrix of the cumulus oophorus. HA-binding sites on the spermatid membrane are seen in mature spermatozoa but not in immature ones. Based on this trait, sperm is introduced into an HA-added medium or seeded dish, and mature spermatozoa are screened by changes in sperm movement due to HA binding. The screened spermatozoa are then subjected to ICSI.[158] A Cochrane review stated that differences in the rates of pregnancy, miscarriage, and childbirth may be either negligible or nonexistent in previous RCTs comparing the effects of HA-ICSI and ICSI.[159]

Besides, a project for improving the quality of ICSI to increase its success rate was recently implemented.[160] The authors of this article found that the transplantation rate in ICSI at a certain infertility treatment clinic was below the key performance indicator and did not improve thereafter. Therefore, they implemented a quality-improvement project and reportedly made a series of changes to improve the transplantation rate in ICSI. The first approach was root cause analysis, which incorporates the opinions of outside observers, all systems and processes are reviewed, and the cause of the decreased transplantation rate in ICSI identified. Second, to implement changes, the recommended changes are implemented collectively, using quality-improvement methods and tools, such as statistical process control charts (BaseLine SAASoft). Third, for measurements and follow-up, standard clinical data were measured, and the trend in transplantation rate in

ICSI after the changes are tracked. Fourth, for the plan-do-study-act (PDSA) evaluation of improvement, small-scale PDSA cycles including every single change were designed to clearly visualize which change contributed to the improvement. Thus, they reported that the transplantation rate in ICSI significantly improved. This undertaking revealed that sufficiently accumulated data are necessary to clarify trends and concerns and that it is difficult to properly monitor whether the technical level satisfies a certain standard at a small-scale clinic.

In recent years, researchers have been exploring the application of artificial intelligence (AI) in ICSI. The image analysis capability of AI is unparalleled to the point of being able to recognize patterns that are unrecognizable to the human eye. The use of AI in sperm screening may provide solutions for subjectivity and efficiency challenges in the process.[161,162] AI-based systems for scoring spermatozoa based on their morphology, motility, and movement patterns have been developed.[163–175] However, some of them utilize existing AI platforms, almost all of which are proprietary, hence precluding them from being widely shared. As larger-scale and more robust datasets become available for training, continued improvements may be made over time.

To date, numerous novel agents, techniques, and methods have been attempted to improve the success rate of ICSI. However, some of them became obsolete as their efficacy was scrutinized over time. No internationally standardized protocol is available for ICSI. Consequently, the protocols and outcomes of ICSI may vary even among infertility treatment institutions that perform ICSI on a daily basis. Thus, it is important to understand the meaning behind the protocols and procedures for ICSI and maintain skill levels by always reflecting on daily clinical results.

10 | PROSPECTS FOR ICSI

In Japan, public insurance coverage was suddenly expanded, and assisted reproductive technologies became eligible for coverage in April 2022. IVF and microscopic insemination, which previously stirred up safety discussions, became generally accepted as they are covered by national insurance.

Thirty years have elapsed since the first successful ICSI attempt in the world in 1992, and ICSI has since become widely accepted by the general public. However, the technology of ICSI has hardly been standardized internationally, much less in Japan. Regarding the biopsy of preimplantation genetic testing for aneuploidy blastocysts, technological differences among institutions are reportedly significant. Similarly, fertilization and blastocyst-reaching rates also differ from one clinic to another.

Because manual micromanipulation skills are acquired from training embryonic culture specialists, they need to be educated extensively to achieve better efficiency. However, Japan has no qualification or education systems in place for embryonic culture specialists. To further develop assisted reproductive technologies and share them among many clinics, it is expected that education systems will soon be established not only for embryonic culture specialists but also for all personnel engaged in assisted reproductive medicine.

The introduction of ICSI was a major milestone in ART, similar to the groundbreaking introduction of IVF, revolutionizing the approach to the most challenging cases of male factor infertility. Recent scientific contributions have further elucidated the scope and efficacy of ICSI, shedding light on its profound implications for ART. For example, the study by Sandra Lara-Cerrillo et al. demonstrates how advances in microfluidic sperm sorting, when used in conjunction with ICSI, significantly improve clinical outcomes by facilitating the selection of sperm with lower DNA fragmentation rates, thus improving the potential for successful fertilization and pregnancy outcomes.[176] In addition, research by Sallam, Hassan et al. re-evaluates the use of ICSI in the context of non-male factor infertility, suggesting a nuanced understanding of its benefits beyond conventional use, thereby broadening the horizon of its applicability in ART.[177] These studies underscore the continued evolution and critical impact of ICSI in reproductive medicine, confirming its status as a transformative technology in ART.

Furthermore, the ongoing innovations in ICSI technique and its integration into ART protocols are carefully reviewed in works such as that of Fancsovits Peter et al., which critically evaluates the results of ICSI in different infertility scenarios, including those with advanced maternal age or low oocyte number.[178] Such research contributes to a comprehensive understanding of the parameters within which ICSI optimizes reproductive success and delineates its impact compared to traditional IVF methods.

Today, IVF and microscopic insemination are deemed essential for infertility treatment. Reproductive medicine has a history of gradual evolution while establishing its safety over many years. We must continue to improve gradually while confirming safety.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interests for this article.

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