Is Intracytoplasmic Sperm Injection the Solution for all in Unexplained Infertility?

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Abstract

Intracytoplasmic sperm injection (ICSI) was first introduced as a supplemental method to conventional in vitro fertilization (c-IVF) for couples with severe male factor infertility to overcome the poor fertilization rate, while its indications expanded in current clinical practice and gained worldwide popularity. However, ICSI is invasive and crosses all natural barriers, raising several unresolved concerns regarding procedure-dependent and procedure-independent risks, as well as the characteristic of being labor-intensive and more expensive than c-IVF. This review is aimed to draw readers' attention, to the widespread use of ICSI worldwide, with its effectiveness in different indications of infertility, especially in those with unexplained infertility, as well as the cost-effectiveness of the ICSI-for-all strategy. Also, we covered current evidence on the short- and long-term safety of children born thanks to ICSI-aided conception. Further well-designed, adequately powered, and randomized controlled clinical trials are absolutely needed to arrive at a consensus on the use of ICSI over c-IVF in different populations.

Keywords
- intracytoplasmic sperm injection
- unexplained infertility
- assisted reproductive technology
- conventional in vitro fertilization
- infertility

Injection of a single sperm cell directly into the ooplasm, known as intracytoplasmic sperm injection (ICSI), is considered as one of the most dramatic technological breakthroughs in assisted reproductive technology (ART). It enabled many couples worldwide with compromised semen parameters or even azoospermia to have their own biological child. Also, the technique opened a wide door to several studies and interventions performed on the oocyte.

The first series of pregnancies and live births from ICSI was reported in 1992.[1] Interestingly enough, this technique was developed as early as the 1960s in nonmammalian gametes, not for the purpose of assisted fertilization but to prove that sperm nuclear decondensation and male pronuclear formation did not require prior interaction between the spermatozoon and the oocyte membrane.[2] These results were replicated in humans in 1988, using surplus immature oocytes and discarded sperm.[3] However, early attempts in applying this novel technique in clinical settings resulted in inconsistent fertilization rates, and they did not achieve any

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pregnancy.[4,5] In the meantime, Palermo and his colleagues experienced random success when human eggs were fully pierced during subzonal injection attempts. They pursued and refined the technique, which later became known as ICSI.[6] It is worth noting that the most important stipulation was the necessity for injection of the entire corpus of the spermatozoon, not solely the nucleus as it was in experimental animals.[7] The amazing attribute of ICSI is that no restriction to the source of the sperm used for fertilization exists. Soon after the first four live born babies from couples with severely impaired sperm, the world's first pregnancies obtained by using sperm aspirated from the epididymis of patients with obstructive azoospermia,[8] testicular sperm in obstructive azoospermia after failed epididymal aspiration,[9] and testicular sperm for nonobstructive azoospermia couples[10] were reported.

ICSI was first introduced to overcome the low and unpredictable fertilization rates encountered with conventional in vitro fertilization (c-IVF). However, the last two decades witnessed a rapid increase in the rate of use of ICSI, despite the fact that the rate of male infertility remains unchanged.[11] Since 2011, ICSI has been shown to be the predominant method of ART-assisted conception in many countries, resulting in over 2 million live births worldwide.[12] However, the real numbers are likely to be even higher, due to incomplete reporting of the initiated cycles and the incomplete follow-up data until delivery. Ongoing concerns exist about the extensive use of this technique both on the effectiveness and long-term outcomes of the children born from it. The purpose of this review is to draw readers' attentions to the widespread use of ICSI worldwide, its effectiveness in the different indications of infertility (especially in those with unexplained infertility), and the cost-effectiveness of the ICSI-for-all strategy. Also, we covered current evidence on the short- and long-term safety of children born from ICSI.

Current Practice of Intracytoplasmic Sperm Injection

When it was first introduced, ICSI was used in cases with severe male infertility to overcome the poor fertilization rate. However, this novel technique soon gained worldwide popularity, due to the broadening of its initial indications for several reasons. The world report on ART by the International Committee for Monitoring Assisted Reproductive Technology for the year 2012 showed that ICSI was used in approximately two-thirds of the aspiration cycles. However, major disparities existed in the use of ICSI worldwide, with nearly 97% in the Middle East against 55% in Asia and 69% in Europe.[13] The predominance of ICSI over c-IVF in European countries still continues to be more pronounced. The 19th annual report of the European IVF-Monitoring Consortium of ESHRE in 2015 showed that a rise of 6.5% in the use of ICSI occurred compared with the year 2014, with the proportion of ICSI being as high as 71.2% of the 541,632 cycles.[14] This figure also differs from country to country, with over 80% in Greece and Italy or up to 100% in the Czech Republic. In the United States, a steady increase from 34 to 76% also occurred, in the proportion of ART cycles involving ICSI performed from 1996 through 2012.[11] Interestingly, the greatest increase, from 15.4 to 66.9%, occurred in cycles without male factor infertility. The use of ICSI also differs by region, with the highest rates of ICSI occurring in Front Range (93.5%) and Gulf Coast (83.1%) megaregions. The lowest rates occurred in the Northeast (63.4%) and Florida (64.8%) megaregions.[15] The report from Australia and New Zealand showed that the rate of use of ICSI increased from 57.8% in 2005 to 67.5% in 2014 and then to 69.4% in 2016, whereas the proportion of male factor infertility was relatively stable over the same period.[16–18] It is worth noting that this figure was even higher, exceeding 80% in cycles using oocyte donors.[16] These data suggest that many clinics worldwide were using ICSI for non–male factor indications, often at their own discretion. In Vietnam, IVF was started in 1997, with the first baby being born from ICSI in 1999. Soon after that, legislations/regulations on ART were issued by the government. No official national data exist yet, but the proportion of ICSI use throughout the country is estimated to range from 70 to 80%. This figure could be more than 90% in centers serving as referral centers. In mainland China, the first national report on ART status showed that out of 530,718 oocyte retrieval cycles performed in 2016, 154,948 (29.2%) cycles used ICSI. This low proportion of use of ICSI could be due to female infertility, accounting for the majority of cycles and the technique being mainly indicated for only male factor infertility, as documented in ART guidelines in China.[19]

Despite the fact that in 2012 the use of ICSI for unexplained infertility, low oocyte yield, and advanced maternal age has been shown not to improve clinical outcomes,[20] this technique is still being described as the "Santa Claus" of a fertility clinic.[21] Some possible explanations for this trend exist. An ICSI-for-all approach was implemented by some clinics to avoid failed fertilization and increase the number of available embryos.[22–24] The postulated high success rate of ICSI from previous data resulted in its increased rate of use in other populations, for whom c-IVF might have been a viable option, particularly in couples with non–male factor infertility,[25,26] unexplained infertility,[27] poor responders,[28] and women of advanced age.[29] Moreover, ICSI might exhibit a role in IVF cycles using preimplantation genetic testing,[30] in vitro maturation,[31,32] or previously cryopreserved oocytes.[20] The increased use of ICSI in the absence of any indication is also likely being encouraged by the availability of insurance coverage, laboratory efficiencies, and/or perceived competition among clinics within the country. In the United States, researchers showed that the rate of use of ICSI was higher in states without an IVF insurance mandate than in states with an insurance mandate for nearly all of the non–male factor infertility indications, especially low oocyte yield and unexplained infertility.[33] These findings suggest that insurance coverage for infertility treatment could demonstrate certain impacts on clinical practice. Hence, both physicians and infertile couples want to optimize the outcome of ART cycles, since treatments are often paid out-of-pocket by patients.

Effectiveness of ICSI versus c-IVF

Non–Male Factor Infertility

Since high fertilization rates and low rates of fertilization failure are associated with ICSI to some extent, ICSI is considered as another choice for patients who could be suited for c-IVF, particularly non–male factor infertility. Debates on the preferred option (ICSI or c-IVF) in non–male factor infertility (as well as the different indications within the diagnosis) have been ongoing for approximately 20 years. However, studies comparing ICSI and c-IVF showed inconsistent results. Early in 2001, a multicenter randomized controlled trial (RCT) compared clinical outcomes after ICSI or c-IVF in 415 couples with non–male factor infertility.[34] Among these patients, the implantation rate (30 vs. 22%) and pregnancy rate (33 vs. 26%) were both higher in the c-IVF group than in the ICSI group. The mean related laboratory time was also lower in the c-IVF group than in the ICSI group. In a nutshell, one of the major advantages of this study was randomizing patients rather than sibling oocytes, which were able to evaluate clinically relevant outcomes, as well as just excluding those who were obliged to receive ICSI due to severe male factor infertility for larger indicated populations. It can be deduced from this study that ICSI offers no advantages over c-IVF with regard to clinical outcomes for non–male factor infertility.

An evaluation was performed retrospectively on a population-based cohort made up of 14,693 women who experienced their first stimulated cycle. They presented with one oocyte at least through either ICSI or c-IVF between 2009 and 2014 in Australia.[35] Altogether, 13,092 and 7,980 embryo transfers among 8,470 women who underwent ICSI and 4,993 women who underwent c-IVF occurred, respectively. Compared with the ICSI group, the c-IVF group exhibited a higher fertilization rate in terms of oocyte retrieval. The ICSI group exhibited a general cumulative live birth rate of 36% as against 37% for the c-IVF group with no significant difference. Compared with c-IVF, ICSI led to a similar cumulative live birth rate for couples with non–male factor infertility. A retrospective multicenter analysis was performed in 15 European tertiary referral hospitals based on the individual patient data. This study aimed to determine if ICSI provides any benefits over c-IVF under different ovarian response categories in couples with non–male factor infertility.[36] The insemination method did not show any significant difference between the various ovarian response categories. Thirty Arab couples were present exhibiting normal semen concentrations and participating in the latest study.[37] An equal distribution existed between ICSI and c-IVF of the number of normal fertilizations per cycle, and c-IVF significantly increased the incidence of abnormal fertilization. On day 3, no difference was found between all fertilized oocytes in terms of the number of good-quality embryos. No difference occurred in the basculation rate on day 5. However, Kim et al from South Korea retrospectively reviewed 296 c-IVF/ICSI cycles performed in patients with non–male factor infertility and found no significant difference in the total fertilization failure (TFF) rate between these two groups (4 vs. 1%). On the contrary, they found that the ICSI group presented a significantly higher rate of normal fertilization (84 vs. 79%) and lower rate of abnormal PN formation (4 vs. 13%). In addition, the cleavage-stage embryo quality was better in the ICSI group.[38] Data from an RCT comparing ICSI and c-IVF in 1,064 couples with non–male factor infertility were recently reported at the 36th Annual Meeting of ESHRE by the team from My Duc Hospital in Vietnam.[39] The results showed that there is no advantage of ICSI over c-IVF, especially as far as clinical outcomes are concerned. The protocol of this trial has been published.[40]

Polycystic Ovarian Syndrome

During ART cycles for patients with polycystic ovarian syndrome (PCOS), the fertilization rate has been reported to be significantly lower and the fertilization failure rate higher in numerous studies.[41–43] As a technique originally intended to overcome the issues mentioned earlier for patients with severe male factor infertility, will ICSI be beneficial for patients with PCOS during their IVF cycles? A prospective study randomizing 1,089 sibling oocytes compared the fertilization rate and embryonic development between c-IVF and ICSI in patients with PCOS for the sole indication of infertility. Results showed that fertilization rate was significantly higher (73 vs. 45%), and no TFF occurred in the ICSI group.[25] However, this study did not compare the pregnancy outcomes, which was limited as randomizing sibling oocytes. Further RCTs on individual patients or cycles instead of sibling oocytes need to be considered to compare the pregnancy outcomes of ICSI to those of c-IVF in PCOS patients.

Tubal Ligation

The necessity also exists to explore what the outcomes are for couples with history of tubal ligation between c-IVF and ICSI. A total of 8,102 first autologous fresh ART cycles collected from women with a history of tubal ligation were present, and these women underwent the surgery in the United States between 2004 and 2012. A longitudinal cohort study was performed and found that no significant difference was found in the adjusted fertilization rate between these two groups (58 vs. 49%). The live birth rate and clinical intrauterine gestation rate were both lower in the ICSI group than in the c-IVF group. To conclude, the fertilization rate was not improved by the use of ICSI. Meanwhile, women who received tubal ligation exhibited lower live birth and pregnancy rates due to the use of ICSI.[44]

Endometriosis

Approximately 10% of women are affected by endometriosis in their reproductive ages. Currently, up to 50% of females exhibit infertility issues impacted by endometriosis.[45] To explore the preferred approach for endometriosis, a randomized study was performed on sibling oocytes only among people who present with endometriosis and received ART.[46] Researchers found that the ICSI group exhibited a significantly higher fertilization rate than the c-IVF group (74 vs. 55%), leading to a higher mean number of day 2 embryos (5.2 vs. 3.6). Meanwhile, the c-IVF group exhibited a significantly

higher triploid fertilization rate than the ICSI group (3.9 vs. 0.9%). To conclude, ICSI seemed to demonstrate a better effect than c-IVF on endometriosis-associated infertility. Its main advantages were lower triploid fertilization and total fertilization rates, a higher mean number of embryos, and a fertilization rate.

Poor Ovarian Response

Due to the high rate of cycle cancellation, patients with poor ovarian response always exhibit lower pregnancy and fertilization rates than normal couples. To maximize the success rate of pregnancy for these patients, choosing the most suitable fertilization approach is of great importance.[47,48] The outcomes of 194 patients were compared, and these patients were the ones retrieving only one or two oocytes. Researchers showed from the results that the fertilization rate was higher for patients who underwent ICSI than for those who underwent c-IVF (83 vs. 67%). No significant difference was found in terms of the rates of pregnancy and good-quality embryos.[49]

Advanced Maternal Age

One of the least explored groups is that of women taking infertility treatments at an advanced age. An increase in the number of women seeking infertility treatment occurs after the age of 35. Many of them need c-IVF because of their age-related infertility issues.[50] Thus, ICSI was identified as the preferred approach for clinicians. A total of 745 women aged 40 to 43 years participated in the retrospective study. These women underwent either c-IVF or ICSI because of the non–male factor infertility. Researchers showed from the results that c-IVF was better in terms of available transferred blastocysts and embryos and oocyte maturity. The two groups exhibited similar live birth and fertilization rates.[51] Data on patients aged over 35 years, with non–male factor infertility and experiencing their first ART cycle, were collected in another retrospective cohort study.[52] The rate of fertilization after ICSI was 71% compared with the rate of 50% after c-IVF. ICSI produced a top-quality embryo rate of 63%, as against 46% for c-IVF. However, the difference was nonsignificant among women between 40 and 45 years old. The effectiveness of ICSI was evaluated in the latest meta-analysis, which is also a systematic review, in terms of how it enhances the fertilization rate as compared with c-IVF for those females who were older than 38 years and exhibited non–male factor infertility.[53] No significant difference was found between ICSI and c-IVF among the 8,796 oocytes being retrieved. The mean age of these women was above 38 years. It was indicated by the author that more studies are required for the evaluation of ICSI in this group.

Male Factor Infertility

For male infertility, the choice of c-IVF versus ICSI is usually based on the count, motility, and morphology of sperm, and their ability to penetrate the zona pellucida of oocyte. Over time, different definitions of male subfertility and infertility were used. Due to these different definitions that exist worldwide, estimating what proportion of fertility treatments are associated with this indication or how it affects the overall success rate is difficult. Also, no consensus exists on this selection in many previous pieces of literature.[54–56] Therefore, treatment for patients with male infertility is always empirical, which could lead to failure of c-IVF or to the unnecessary use of ICSI.[57] In most severe male infertility cases, including azoospermia, cryptozoospermia, and necrospermia, because the effects of empirical drug therapy and surgery are limited, ICSI might be the only viable option that enables a clear majority of infertile males to become the biological fathers of their children. As early as the 1990s, several specific studies all recommended ICSI as the first choice therapy for severe male infertility with different indications and types.[58–60] Currently, it is clear that the severe male infertility can be treated successfully only with ICSI.[61–63] However, whether or not ICSI comes with advantages regarding pregnancy outcomes as the powerful indications for effectiveness in patients with nonsevere male factor infertility remains controversial.

Sole Teratozoospermia

Till date, no widely accepted criteria exist for the treatment technique for isolated teratozoospermia. Thus, several studies were also conducted to determine if any therapeutic benefit for isolated teratozoospermia exists. In 1999, Pisarska et al[64] conducted a prospective experimental study to compare c-IVF with ICSI in males with isolated severe teratozoospermia (defined as morphology of $\leq$ 4% normal forms according to Kruger's strict criteria[65,66] along with normal count and motility) using randomized sibling oocytes. In these patients, ICSI resulted in higher fertilization rates than c-IVF, without significantly altering the embryo quality (66 vs. 48%). However, Fan et al[67] conducted a similar study which used the same eligibility criteria and recruited a total of 183 patients diagnosed as isolated teratozoospermia undergoing their first c-IVF/ICSI cycle in 2012. Data showed that no difference was found in the percentage of eggs fertilized, implantation rate, pregnancy rate, and spontaneous abortion rate between participants who underwent c-IVF and those who underwent ICSI regardless of the percentage of normal morphology. However, the heterogeneity between studies might be owing to different technology in laboratory as well as selection bias and the limitation of randomizing sibling oocytes.

Nonsevere Male Infertility

Different from severe male infertility, less clarity is present regarding whether or not a difference exists between ICSI and c-IVF among patients who suffer from nonsevere male infertility. In 2002, a comparison between c-IVF and ICSI was performed in an RCT for sibling oocytes based on the third edition of the WHO Laboratory Manual for Human Semen.[65,68] Researchers reported that patients who underwent c-IVF exhibited a significantly lower overall fertilization rate than those who underwent ICSI (37.4 vs. 64.3%), indicating that ICSI is more effective in fertilization for nonsevere male infertility.[69] However, in 2002 as well, similar standards of semen were used for another study,

which showed that no significant differences were found between ICSI and c-IVF in sibling oocytes for the variables being explored, including implantation, pregnancy, embryo morphology, rates of development, and the fertilization rate.[70] In the same year, another prospective cohort study with more strict standards[56] was performed on randomizing sibling oocytes. No significant difference was found between c-IVF (56.7%) and ICSI (58.1%), in terms of the fertilization rate.

In 2004, a study was performed by randomly classifying the sibling oocytes. Afterward, an evaluation was made regarding the performance of ICSI and c-IVF among couples who presented with mild oligoteratoasthenozoospermia.[65] According to the results of the study, compared with ICSI (64%), c-IVF exhibited a much lower fertilization rate (52%). c-IVF was found to exhibit a higher total fertilization rate (7%) than ICSI (0%).[71] van der Westerlaken et al[54] then performed a randomized research for sibling oocytes back in 2006 and found that unnecessary fertilization could be prevented by ICSI among people with borderline semen. Meanwhile, when compared with c-IVF, no significant difference was found.

From 2013 to 2015, Xie et al[57,72,73] performed several studies randomizing sibling oocytes among patients with the amount of 5 to 20×10^6 sperm/mL. The corresponding progressive motility was 10 to 32%. The ICSI and c-IVF groups did not differ from each other in terms of the pregnancy, implantation, and fertilization rates. However, ICSI group exhibited significantly more good-quality embryos than the c-IVF group. In terms of the nonsevere male factor infertility (both moderate and mild oligospermia without or with asthenospermia included), ICSI exhibited less clear pregnancy and fertilization outcomes.

To conclude, several inconsistent results were found by some prospective and retrospective studies regarding randomized sibling oocytes. Lower fertilization failure rates and higher fertilization rates among patients who underwent ICSI treatment are documented in some studies. The benefit of ICSI was not supported by other data in terms of the live birth rate, clinical pregnancy rate, implantation rate, embryo quality, and prevention of TFF. All these studies demonstrate some inherent weakness, such as no evaluation of live births, nonrandomized patients, and small sample size.

Previous Fertilization Failure

Among the couples who undergo ART, 5 to 15% of them present with TFF despite an obviously regular sperm quality.[22] To determine if ICSI should be performed for the couples with TFF in the prior IVF attempt, a prospective study was being performed while 228 sibling oocytes were randomized. The results showed that ICSI was significantly superior to an additional IVF attempt with further enhanced insemination concentrations.[74] van der Westerlaken et al later performed a prospective study that involved 38 couples who underwent c-IVF and ICSI on sibling oocytes after they made an attempt of IVF with TFF first or demonstrated a low fertilization rate ($< 25\%$).[22] Researchers showed from the results that during the second cycle, the sibling oocytes with c-IVF treatment were significantly lower than those with ICSI in terms of the fertilization rate. Besides, the c-IVF-treated oocytes exhibited a high occurrence rate of TFF. c-IVF and ICSI still exhibit differences in pregnancy outcomes.

In a nutshell, current available data are mostly based on cohort studies, which are prone to selection bias. However, an increasing body of literature exists showing that pregnancy outcomes could be compromised after the use of ICSI in cases without a firm indication. Therefore, well-designed, adequately powered RCTs are absolutely needed. Two ongoing RCTs exist from Asia in this field. To determine whether ICSI or c-IVF is more appropriate in couples with mild to moderate male factor infertility, researchers from the Peking University Third Hospital proposed a multicenter, open-label RCT in 10 Chinese hospitals.[75] This trial was designed to recruit 2,346 couples in total, of whom the male spouse was defined as a sperm concentrate of 5 to 15×10^6/mL or progressive motility of 10 to 32% undergoing their first or second ICSI or c-IVF cycle. The primary outcome to assess in this study was ongoing pregnancy leading to live birth after the first cycle with embryo transfer.

Intracytoplasmic Sperm Injection in Unexplained Infertility

Unexplained infertility is a diagnosis given to couples who demonstrate normal semen analysis, tubal patency, and standard tests (like the midluteal progesterone, serum luteinizing hormone surge, cervical mucus changes, and basal body temperature).[76] Due to the lack of a particular tests or misdiagnosis, the prevalence of unexplained infertility still remains debatable, which has been diagnosed in up to 30 to 40% of infertile couples.[77,78] Genetic and reproductive physiology, immunology, and hormonal balance were identified as the possible causes of unexplained infertility.[79]

Several studies exist proposing that couples demonstrating unexplained infertility issues might exhibit spontaneous conception.[80–82] Generally, each month, around 1 to 4% of the couples with explained infertility issues could conceive. In addition, women who are younger and demonstrate shorter infertility durations will exhibit a higher chance of conception through expectant management. More aggressive initial therapy is required when the wife is above 35 years of age and the couple has been trying to conceive for more than 2 years.[83]

By default, no correctable abnormality exists, and unexplained infertility therapy will be empiric. Treatment plans for this condition usually include ovarian stimulation (OS) with intrauterine insemination (IUI).[76] Patients needed to take CC and similar oral agents. Afterward, they needed injectable gonadotropins with IUI.[84] For the couples who still could not conceive after OS-IUI, IVF was performed.[85] Once the decision to perform IVF is made, one needs to decide the most beneficial approach through which the fertilization can be realized, either ICSI or c-IVF. The failure of fertilization happens in 5 to 10% of cases during IVF for unexplained infertility.[69,78] Reports of high live birth rates and high implantation rates expanded the spectrum of indications for ICSI beyond conventional male factor

diagnoses, making it possibly beneficial for couples with this condition. However, researchers found that no benefit was found in terms of adding ICSI to the treatment of non–male factor infertility over adding c-IVF.[34] Meanwhile, some controversial outcomes exist regarding the comparison of the efficacies of ICSI and c-IVF in unexplained infertility.

The TFF was reported to be 20% among 117 couples who failed to conceive over four to six cycles of superovulation and IUI in a retrospective case–control study.[86] To deal with this issue, sibling oocytes of 22 patients who underwent ICSI and c-IVF were recruited in a prospective study.[87] No significant difference was found between c-IVF (51%) and ICSI (63%) in terms of the fertilization rate per oocyte. However, data showed that 23% of the unexplained infertility patients would exhibit a total loss of their embryo transfer chance due to TFF if c-IVF was performed. Later, in 1997, a prospective randomized study was performed, in which the sibling oocytes of 63 patients were randomized into c-IVF and ICSI. According to the results, c-IVF and ICSI did not show a significant difference in the fertilization rate. No significant difference was found between the two groups in terms of the embryo quality. However, none presenting with TFF in the ICSI group were found, while 11% of those in the c-IVF presented with it.[24] It is demonstrated from the case–control study that a low fertilization rate and a high rate of failed fertilization were found for the couples who failed to conceive through the direct intraperitoneal insemination. However, through ICSI, they showed a good pregnancy rate.[88]

To decide if fertilization failure can be prevented by ICSI in unexplained infertility, a prospective cohort study was performed on the sibling oocyte cumulus complexes c-IVF and ICSI allocation in 60 couples with such an issue.[56] ICSI patients exhibited much higher fertilization rates per oocyte cumulus complex (65%) as against only 48% for c-IVF patients. Ten out of 60 couples with failed fertilization after c-IVF were present, whereas none for ICSI were found.

Later, c-IVF and ICSI were compared in a RCT performed on 60 patients.[89] They were randomized to either ICSI or c-IVF. No significant difference was found in the fertilization rates between the patients of the ICSI and c-IVF groups (82 vs. 77%). The rates of implantation were 44 and 38%, while the clinical pregnancy rate was 50% for each group. The live birth rates were then 50 and 47%. Afterwards a meta-analysis and a systematic review was carried to decide if TFF is prevented and fertilization is improved among the couples with unexplained infertility.[26] ICSI exhibited higher fertilization (relative risk [RR]: 1.49, 95% confidence interval [CI]: 1.35–1.65), fertilization per allocated oocyte (RR: 1.27, 95% CI: 1.02–1.58), and pooled RRs of a mature oocyte than c-IVF. Meanwhile, it exhibited lower pooled RR of TFF (RR: 8.22, 95% CI: 4.44–15.23 for c-IVF compared with ICSI). A preference of the results existed for ICSI in terms of the decrease in TFF and the increase in fertilization rates. Additionally, authors also pointed out that the impact on the live birth rate and clinical pregnancy rate requires further studies.

Based on the aforementioned literature review, ICSI serves as a preferred choice for fertilization, which could minimize complete failure of fertilization while maximizing the number of embryos. However, a paucity of studies exist exploring pregnancy after ICSI taking into account other factors. For example, this process could not be confidently used to treat unexplained infertility considering time-consuming and extra costs and current knowledge of ICSI as an outcome procedure. Properly powered RCTs are still required to explore the role of ICSI in the treatment.[90]

Safety Concerns of Intracytoplasmic Sperm Injection

Surveillance of long-term outcomes of fertility treatments, including the health of children born from ICSI, remains of interest both to professional healthcare providers and infertile couples. This is of particular importance with children born from ICSI, a procedure that is considered invasive and crosses all natural barriers. Two types of risk associated with ICSI were suggested, namely, the procedure-dependent and procedure-independent risks.[90,91] The main risks associated with the procedure itself are related to both the physical and/or biochemical disturbance of the ooplasm or meiotic spindle, which might result in aneuploidy. Moreover, the use of polyvinylpyrrolidone or injection of foreign, sperm-associated exogenous DNA as well as the handling of oocytes outside the incubator could also be potential risks associated with the procedure. For procedure-independent risks, the causes of infertility and the use of sperm with structural defects should be considered. To facilitate the discussion of long-term outcomes, we divide the current section into six major areas: (1) perinatal outcomes, (2) aneuploidy and congenital malformations, (3) physical developmental outcomes, (4) reproductive outcomes, (5) psychological and neurodevelopmental outcomes, and (6) risk of cancers.

Perinatal Outcomes

ICSI is associated with a significantly higher risk of (very) preterm birth,[92] lower mean birth weight,[93] and an increased risk of rare imprinting disorders[94] compared with spontaneously conceived and non-IVF ART pregnancies. When ICSI is compared with c-IVF, most large-scale studies found similar or lower risks of (very) preterm birth and (very) low birth weight as well as peri/neonatal mortality in singletons.[95] These findings could be explained by the reproductively healthy status of women in couples undergoing ICSI, which could give a more favorable perinatal outcome.[96]

Aneuploidy and Congenital Anomalies

An abnormal karyotype was found in 3% of ICSI fetuses, of which 1.6% were *de novo* and 1.4% inherited. The frequency of aberrations was significantly higher in the *de novo* cohort compared with those in the general newborn population (1.6 vs. 0.45%) and was mostly due to an increased incidence of sex-chromosomal aberrations.[91] Researchers showed that these *de novo* chromosome anomalies in the offspring are linked to sperm concentrations, with a cut-off of 20 million sperm/mL.[91] Compared with c-IVF, the risk of presenting with chromosomal abnormalities, particularly sexual

chromosome aneuploidy, in children conceived through ICSI is higher.[97]

Some controversy exists regarding the risk of birth defects after ICSI. It has been established that ICSI does not lead to major abnormalities in offspring,[98] which is in line with a meta-analysis that indicated no significant increase was found in major birth defects in babies conceived through ICSI when compared with those conceived through c-IVF.[99] However, a large Australian study of babies born from assisted conception reported a significantly higher rate of congenital malformations in babies born from ICSI than that from c-IVF.[100]

Physical Developmental Outcomes

A recent meta-analysis was conducted to compare 3,972 c-IVF/ICSI children with 11,012 spontaneously conceived children up to a mean follow-up time of 22 years. The results showed that compared with spontaneously conceived controls, c-IVF singleton children exhibited lower body weights, although the difference between theirs and the weights of the children in the c-IVF/ICSI group was not statistically significant. Similarly, the mean weight of children following ICSI treatment was comparable with that of the controls. It is worth noting that the mean weight of c-IVF/ICSI children was significantly lower than that of the controls, but only up to preschool age. These results indicate that c-IVF/ICSI children exhibit an increased postnatal growth rate. However, these results must be interpreted with caution, as very few studies included children beyond preschool age.[101]

Reproductive Outcomes

The first study attempting to assess semen quality in the oldest ICSI young adult was recently reported. Compared with men born from spontaneous conception, those born from ICSI aged between 18 and 22 years were nearly three times more likely to have sperm concentrations below 15 million per milliliter of semen and four times more likely to have total sperm counts below 39 million per millimeter of semen.[97] The significant difference between these findings remains even after adjusting for relevant confounders. However, no clear correlation between semen parameters of the young ICSI men and their fathers was found.[102] In another study with a limited sample size, the same group showed that reproductive hormone levels in these ICSI young adults were comparable to those in young adults conceived spontaneously.[103] For young adult women conceived by ICSI due to male factor infertility, antral follicle count and circulating reproductive hormone levels were found to be similar to those in their peers born after spontaneous conception.[104] It should be noted that patients in this cohort were conceived after the use of ICSI with ejaculated sperm in couples with male infertility. Reassuring data on the pubertal development of the ICSI offspring cohort with a mean age of 16.5 years at the time of investigation were recently reported in the largest prospective controlled trial.[105] The mean age at menarche was comparable between both groups and was also in line with data from a nationwide survey. The pubertal development determined by Tanner stages of both female and male ICSI offspring was similar to that of the spontaneously conceived adolescents.[105] The aforementioned data suggest that male fertility might be compromised in ICSI offspring, most likely owing to paternal inheritance. However, for female fertility, the existing information is insufficient to draw any conclusions.

Psychological and Neurodevelopment Outcomes

The neurodevelopment of children born from ART (with 52.5% from c-IVF/ICSI) was assessed in a prospective cohort study, involving 175 children in the ART group (62.9%) and 1,345 in the natural-conception group (64.4%) at 24 months of age.[106] After adjusting for relevant confounders, researchers found that the neurodevelopmental outcomes in children born from ART were not significantly different from those of naturally conceived children. Also, no significant difference was found between ICSI versus c-IVF and other treatment techniques.[106] However, it should be noted that the study was not powered for this outcome. In children ranging from toddlers to teenagers, a systematic review on 79 cohort or case–control studies indicated that overall, the neurodevelopment of children born from ICSI and c-IVF was comparable to that of children born from spontaneous conception.[107] Furthermore, the results of the first nationwide study in adolescents aged 15 to 16 years showed that ICSI and IVF adolescents exhibited similar academic performances in both the crude and adjusted analyses for both singletons and twins.[108] These reassuring data were recently confirmed in a pragmatic study, where children born after ICSI ($n = 6{,}953$) were compared with children born after c-IVF ($n = 11{,}713$) and with children born after spontaneous conception ($n = 2{,}022{,}995$). Of children born after ICSI, those born with nonejaculated sperm ($n = 462$) were compared with those born with ejaculated sperm ($n = 6{,}491$).[109] The authors concluded that children born after ICSI exhibited similar school performances as those born after c-IVF. Although a small difference was found in favor of the spontaneously conceived children in the national tests in third grade, no difference was found between these groups in the ninth grade. Interestingly, children born from nonejaculated ICSI achieved significantly lower scores in Swedish and English. They also exhibited a significantly higher risk of poor performance than children born from ejaculated ICSI.[109]

Cancers

As with any new techniques in the field of reproduction, a general awareness of the risks from the introduction of new tools and chemical substances should be present, for example, the safety of using polyvinylpyrrolidone which has long been considered as a carcinogen. A large nationwide historical cohort study recently showed that after a median follow-up of 21.0 years, the overall cancer risk in ART-conceived children is not significantly increased, neither when compared with the general population nor when compared with the population of naturally conceived children born to subfertile women. A nonsignificantly increased risk of cancer was found in children conceived from ICSI compared with that in naturally conceived children (adjusted hazard ratio

[aHR] = 1.52, 95% CI: 0.81–2.85) or with c-IVF-conceived children (aHR = 1.60, 95% CI: 0.87–2.93).[110]

Safety of Intracytoplasmic Sperm Injection in Non–Male Factor

Another important question is whether the ICSI procedure, in particular, poses any risk for congenital malformations and long-term health of the offspring when used for non-male indications. However, limited evidence exists regarding this issue, most likely because the use of ICSI for non-male indications has been broadened only in the past two decades. Data from the U.S. National ART Surveillance System from 1996 to 2012 reported that ICSI in non–male factor infertility was associated with lower birth weight than with c-IVF.[11] In a population-based retrospective cohort study, after a 5-year observation period of all live born ART-conceived children in California between 1997 and 2006 ($n = 42,383$), the authors reported that ICSI in non–male factor infertility was associated with a higher risk of autism than c-IVF (adjusted HR: 1.57; 95% CI: 1.18–2.09).[111]

Limitations

All aforementioned data on the safety of ICSI should be interpreted with caution. A key limitation of the existing literature is the overall small sample size of the ICSI group. Given the low incidence of specific congenital anomalies, a large number of children are obviously required to detect a true increase in incidence, if any. Another major challenge is the difficulty in separating the contribution of infertility per se from the technique itself, the so-called the chicken or the egg.[95] Furthermore, selecting appropriate comparison groups for children born through ART is vital and challenging. In most studies, children conceived spontaneously are used as the control group, but whether this is the correct group is debatable, since parental factors such as infertility per se might influence the health of the offsprings.[112] It is worth noting that the long-term developmental outcomes of children, especially intelligence and cognitive function, are closely associated with parents' level of education and socioeconomic status.[113] Last, existing studies are also subject to bias as a result of incomplete follow-up arising from attrition.

Cost-Effectiveness

ICSI is considered as being extensive given that public funding usually would not subsidize it. Therefore, the increased costs exhibit a substantial scope throughout the society. Meanwhile, ICSI is time- and resource- consuming but actually increases success rate among specific patients. Introducing these services comes with costs and also effects, which are also assessed thoroughly. Hollingsworth et al[114] once assessed the incremental costs of the ICSI and also the available practice comparators for every patient group in Australia and made an assessment of this service economically. According to the estimation based on the results, authors pointed out that the additional cost of ICSI over the relevant target populations in Australia gives potential total financial implications of over A$31 million per annum. Moolenaar et al[115] from the Netherlands made an assessment of the TMSC (total mobile sperm count) and used it to assess the cost-effectiveness of interventions for male subfertility. A study was performed targeting subfertile women who were 30 years old and their partners presented with a pre-wash TMSC, which was between 0 and 10 million. An evaluation was made regarding the expected live birth rate among IUI, c-IVF, and ICSI. Significant differences were found in the cost per live birth, the range of which could be between €14,986 for a 0.1 million pre-wash TMSC and €11,811 for a 10 million one. There was a cost of €12,783 per live birth of the ICSI cycle irrespective of pre-wash TMSC. When the pre-wash TMSC was lower than 3 million, ICSI could generate less cost than c-IVF, which started to exhibit a lower cost than 3 million. The incremental cost-effectiveness ratio (ICER) varies greatly, which could be between €4,598 for a 0.1 million pre-wash TMSC of 0.1 million and €4,873,646 for a 10 million one. In light of this, it can be seen that ICSI could generate more costs and be more effective.

An abundant amount of cost analyses exists regarding infertility treatment, even though very few of them explored unexplained infertility. Besides, the cost-effectiveness is analyzed by few cost analyses, such as the incremental cost per live birth when there is an alternative treatment. To measure the cost-effectiveness of split c-IVF-ICSI, Vitek et al adopted an adaptive decision model for the prediction of the costs among the cohort in which women were younger than 35 years and underwent c-IVF and also among 154 couples presenting with a split c-IVF/ICSI cycle.[116] c-IVF, split c-IVF/ICSI, or all ICSI, and adapting treatment are used as the modeling insemination method in the first IVF cycle according to fertilization outcomes. All c-IVF is preferred in a single cycle, given that the increased live birth rate (3%) cannot be justified by ICER of split c-IVF/ICSI or all ICSI ($58,766). Split c-IVF/ICSI is preferred under the circumstances where two cycles are required given that the increased cumulative live birth rate (3.3%) is obtained at an ICER of $29,666. With regard to the single cycle, due to which the increased cost per live birth did not justify all ICSI and the enhanced live birth rate with split c-IVF/ICSI, a preference on all c-IVF was found. However, a preference over the split c-IVF/ICSI approach was found when it requires two IVF cycles due to the comparison of the lesser cost per live birth with all ICSI and also the higher cumulative live birth rate with all IVF. According to the results of the study, the increased cost of 50:50 could not be justified by the minimal growth of the live birth rate (3%) as c-IVF:ICSI, though this was a statistical modeling study.

Conclusion

In conclusion, a paucity of evidence of a clear benefit of ICSI use over c-IVF exists, except for severe male infertility. However, its use continues to increase, particularly among couples without male factor infertility. Also, ICSI might bring additional costs and potential health risks, although current results are to be interpreted with caution. Further evidence-based studies should be undertaken to determine whether

ICSI is more beneficial for different indications of infertility than c-IVF regarding effectiveness, safety, and cost-effectiveness. With such certain results, it will bring new knowledge to offer future guidance to clinicians treating infertile patients.

Conflict of interest

The authors declare no conflict of interest.

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