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New Perspectives on Technical Aspects of Micromanipulation (ICSI) in Clinical Assisted Reproduction

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ABSTRACT

Intracytoplasmic Sperm Injection (ICSI) is presently performed for 70-80% of the ART (assisted reproductive technology) cycles [1]. This substantial use is partly due to the excessive stage of standardization through the procedure. The aim of this review was to consider the available publications dealing with the technical aspect of ICSI in assisted reproduction. The literature search was conducted employing PubMed and Google scholar. Some of the key words used: ICSI injection, ICSI outcomes, sperm immobilization, micro tools, commercial micropipette, home-made micropipette, injection speed, injection force, aspiration of cytoplasm, site of sperm deposition and laboratory set-up. The search strategy was limited to articles published in English involving human subjects, with the last search performed on June 2023.This overview provides modern-day knowledge on some technical factors to improve the ICSI efficacy in ART contexts.

Keywords: ICSI; Oolemma; Oocyte; Embryo; ART

Abbreviations: ICSI: Intracytoplasmic Sperm Injection; ART: Assisted Reproductive Technology; ZP: Zona Pellucids

Introduction

Intracytoplasmic sperm injection (ICSI) was first used in 1992 for overcoming the male factor infertility [2,3]. Today, it is also used in the treatment of cases with non-male factor [4]. Proposed indications for use of ICSI including the unexplained infertility, poor-quality oocyte, advanced maternal age, prior fertilization failure with IVF insemination, preimplantation genetic testing (PGT), fertilization after in vitro maturation (IVM), and cryopreserved oocytes [5,6]. International Committee for Monitoring Assisted Reproductive Technologies (ICMART) outlined an occurrence of ICSI ranging between 60% to 98% of the ART remedies carried worldwide [7]. Though, ICSI is an effective technique, complete fertilization failure following occurs in approximately 3% of the cases [8]. Fertilization rate from ICSI was once as low as 40%. However, it has progressively increased over the years to 70–80% per injected oocyte. This has been broadly speaking due to upgrades over the years in the technical method [9]. Conventional microinjection is performed by using two manual joysticks and it employs a spiked micropipette for facilitating penetration of the zona pellucida (ZP) and rupture of the oolemma to allow injection of sperm into the ovum [2]. This method is prone to mistakes and the performance of a microinjection technique can be evaluated in several contexts [10].

A significant amount of research investigated the technical details. As of today, many laboratory factors may influence the ICSI outcomes. These include the insemination procedure, oocyte denudation, and fine technical characteristics [11,12], polyvinylpyrrolidone

(PVP) [13], orientation of the first polar body(pb) [14-16], and oocyte integrity [17,18]. Several studies reviewed the sperm processing [19,20], sperm selection [21,22] and systems for ICSI [23]. However, there is a gap of knowledge on detail of micromanipulation with focus on injection procedure, such as micro tools, sperm immobilization, injection speed, aspiration of cytoplasm, site of sperm deposition and their influence on ICSI outcomes. The valuable laboratory phase post ICSI involves the oocyte survival rate, fertilization, and formation of good quality embryos. This review was designed to give a comprehensive investigation of the ICSI- technical related events and operators concerning the embryological outsight.

Methods

The exhaustive literature search was conducted employing PubMed and Google scholar. Some of the key words employed included: improving ICSI, injection, ICSI outcomes, sperm immobilization techniques, micro tools, micropipettes, injection speed, injection force, aspiration of cytoplasm, site of sperm deposition, injection funnel and injection procedure. The search strategy was limited to articles published in English involving human subjects, with the last search performed to June 2023.

Micro Tools

At the beginning of the ICSI introduction, microneedles were home-made, and each laboratory was required to have the fundamental equipment for needle preparation, such as pipette puller, grinder and micro forge. This gear was once expensive, complex and inclined to many blunders [24]. But, now, the majority of laboratories use ready-made commercial microneedles. There are quite a few research in this field, such as micropipette sharpness and hogging [25-28], inner diameter [29] and multiple pipette use [30]. Overall, the use of an injection pipette with the smallest feasible internal diameter and a lengthy taper increases the fertilization rate, decreases the incidence of degeneration and tripronuclear zygotes, and enhances embryo development [31]. Vanderzwalmen and co-workers studied the influence of the form and size of the pipettes' aperture on embryo quality and oocytes degeneration. They discovered that in instances where the diameter of the injection pipette was between 9 and 10 microns, there was a higher rate of degenerated oocytes and poor quality embryos [27]. Also, they concluded that the presence of a spike on the injection pipette facilitated passage through the ZP in ICSI cases. However, they did not look at the fertilization rate or injury when using the pipettes with or barring a spike. Svalander and associates additionally concluded that the first-class of micro tools also influences the consequence of the ICSI technique in quite a few ways; a blunt injection pipette can harm the oocyte using compression. Whereas, a pipette with large diameter causes an overload of injected fluid [28].

Routinely, a single injection pipette is used for sperm selection, immobilization, sperm aspiration, and impartial of the wide variety of oocytes for injection. The efficiency of the injection pipette ought to affect the outcome, both as a result of loss of sharpness due to repeated injection or deterioration in the pipette due to the accumulation of debris at the tip. Nagy and colleagues observed that breakage of the oolemma may additionally be due to differences in the oolemma or the sharpness of the injection pipette. This may have an effect on the developmental potential of a microinjected oocyte [32]. It was concluded that using one injection pipette for the ICSI procedure resulted in poor fertilization rates, but no impact was located when evaluating cleavage, pregnancy, and implantation rates [30]. Khalili and teammate indicated that pregnancy improves when commercial injection micropipettes are used in ICSI program. They compared ICSI outcome using commercial versus home-made injection micropipettes in a large population with severe male infertility [33].

Sperm Immobilization

Sperm immobilization prior to ICSI plays a vital role in high fertilization rates [27]. The sperm plasma membrane is disrupted after sperm immobilization. This disruption favors the sperm cytosolic factors that induce oocyte activation and sperm decondensation [34]. Also, immobilizing the spermatozoon before injection may prevent interference by the sperm with the cytoskeleton and metaphase spindle of the oocyte [35]. Injection of immobilized spermatozoa without tail breaking results in significantly lower fertilization [36]. In the conventional immobilization method, the sperm was transferred into a PVP drop, and spermatozoa were immobilized by compressing the tail or the sperm tail halfway between the head and the tip of the tail against the bottom of injection dish [27]. Palermo et al concluded that epididymal or testicular sperms had required aggressive immobilization [37]. Different techniques of sperm immobilization have been introduced to induce sperm membrane permeabilization with pipetting [38], or piezo-pulses [39], The squeezing method has involved touching [21], rubbing [40], stroking [41] or pressing [42,43]. When recent methods have compared to the conventional method, higher ICSI fertilization rates for ejaculated spermatozoa have been reported after more aggressive mechanical [12,37,44,45] or piezo method [46], but not after cutting the sperm tail at different places [47] or applying laser [41,48,49]. These different techniques have not been assessed in an adequately powered randomized study. In a trial study, no differences were noted between triple touch and single touch immobilizations [36].

Injection Speed

The piercing through the ZP and the membrane needs to be achieved with a minimal biological damage to facilitate a rapid healing of oocyte. During the injection procedure, the micropipette creates excessive deformation of the oocyte. Excessive deformation may induce emission of the cytoplasm into the perivitelline space (pvs) after injection [50]. The range of injection speeds investigated was between 0.05 mm/s and 1 mm/s. The results showed that a low injection speed is desirable to reduce the injection force; while, a high speed is used for reducing oocyte deformation. The previous method

was calibrated to be able to link injection speed, injection force, and deformation at the puncture. The latter proved to be able to predict the force-deformation trend over the whole range of injection speeds. Also, additional information was recorded on the stress and deformation fields over the oocyte during the injection. In particular, a stress concentration was observed in the contact area between the injection micropipette and the oocyte, which causes oocyte breakage [50]. A shorter time-to-piercing indicates a higher efficiency of a microinjection process [51]. The excessive manipulation inside the oocyte due to the spermatozoon remaining attached to the injection pipette at the moment of deposition, or the inadvertent oolemma damage at the opposite side of the injection hole is result of too deep penetration by the injection pipette [32].

Aspiration of Cytoplasm

Aspiration of the ooplasm into the pipette resulting in breakage of the oolemma is regarded as a proof that the spermatozoa stay in the cytoplasm. Also, cytoplasmic aspiration may provide a mechanical stimulus to induce an important release of intracytoplasmic calcium stores [50,52]. However, in a prospective randomized study on sibling oocytes, the cytoplasmic aspiration before sperm injection in ICSI was not essential for oocyte activation. The findings showed similar data on the rate of oocyte damage, fertilization, and embryo quality with or without cytoplasmic aspiration. In some cases, a considerable volume is aspirated into the injection pipette and it can disturbed the cytoplasmic structure [53]. Dumoulin et al. found that in vitro development to the blastocyst was compromised in a group of embryos originating from oocytes in which >6 pL cytoplasm ascended into the injection pipette during ICSI. This would mean that oocytes with more aqueous texture have a worse prognosis than oocytes with a more viscous cytoplasm. In a prospective study, blastocyst development rate was found to be significantly different among four ICSI operators according to the volume of aspirated cytoplasm [54].

It was shown that in the groups of oocytes in which either a sudden or no breakage of the oolemma occurred, survival and fertilization rates were lower than in the group of oocytes which the membrane broke after application of suction. Furthermore, immediate membrane breakage was associated with high degeneration rates [32,54,55]. Also, a difficulty to breach the oocyte membrane leads to the application of stronger suction that cannot be reversed in time at the moment the membrane breaks. It is also possible that the type of membrane breakage and the volume of aspirated cytoplasm are not related to the injection technique, but they are oocyte-related phenomena [56]. Although, little information is available about the effect of volume of cytoplasm aspiration on ICSI outcomes, but two studies reported that zygotes from a large volume cytoplasm aspiration have a lower potential for blastocyst formation [54,57]. Volume of cytoplasm aspirated is associated with the timing of oolemma breakage. Some studies proposed that sudden breakage has an effect on oocyte degeneration [32,55,58].

Site of Sperm Deposition

MII spindle is located in the periphery of the ooplasm subjacent to the first pb [59]. Therefore, first pb marks the approximate spindle location, and historically, the injection procedure was performed with the pb positioned at 6 or 12 o'clock [60]. The site of sperm deposition in the oocyte has been examined by several researchers. Nagy et al compared injections of oocytes between 6 and 12 o'clock pb orientation. They found high-quality embryos when the pb oriented at 6 o'clock [32]. In natural fertilization, oocyte permits sperm fusion and penetration anywhere over the plasma membrane [61]. In ICSI, a sperm injected into the ooplasm in the proximity of the spindle moved to orient itself appropriately next to the spindle. When the sperm was deposited contralateral to the spindle, the sperm head first rotated to orient the centrosome-containing midpiece toward the spindle before it migrated into the interior portion of the ooplasm. The contralaterally deposited sperm took 2.5 to 3 to reach the proximity of the developing female pronucleus [62]. It seems that there is a preferential area within the oocyte to deposit the sperm, while still avoiding any disruption of the spindle. In evaluating the orientation of the opening of the injection pipette, some reported better embryonic development [63] and pregnancy rates [64] when the opening was directed towards the animal pole, thus injecting the sperm towards the direction of the meiotic MII spindle.

Hardarson, et al. [65] found that in 93% of oocytes, the MII spindle was located in the same hemisphere as the pb (animal pole). They concluded that it might be advantageous for the sperm to be injected at the midline between the two hemispheres of the oocyte in the direction of the vegetal pole [65]. Also, Yanaihara and co-workers showed that when the spermatozoa remained near the injection site after ICSI, the fertilization rate was significantly lower. Once again, better fertilization rate was obtained when injected spermatozoa remained on the center or in the left of the oocytes [66]. Both manipulation and stress caused during oocyte denudation represent the main factors responsible for the spindle deviation, also oocyte ageing. The displacement of the spindle away from the pb could make the spindle and the cytoplasm more susceptible to disturbances caused by the ICSI needle by causing an alteration of the position of the polarized molecules in the cytoplasm [67,68]. So, by choosing an injection angle of 900 (corresponding to the 3 o'clock position), it is still possible to disturb the spindle apparatus, but this is highly unlikely.

Injection Funnel

Albeit marked oocyte abnormalities have been observed in oocytes such as ZP thickness, spindle birefringence and extracellular dimorphisms [69-71]. In addition, response to penetration with an ICSI pipette is useful determinant of oolemma quality the plasma membrane oocyte [72]. During the ICSI process, the so-called "injection funnel" is usually formed and disappears spontaneously after a certain time. Sometimes, the injection funnel is not formed (i.e., ICSI

the injection needle passes through the oolemma without any change in oolemma shape). In another situation, the injection funnel is maintained for a long time even 3–4 min later. Sudden rupture of oolema does not form an injection funnel. Therefore, sudden rupture is associated with a higher rate of degeneration. The injection funnel formed at some stage in the ICSI process is a shielding property that helps the oocyte prevents cytoplasmic leakage [54,73,74]. Inn cases where the water content of the cytoplasm is lower, the injection funnel is smaller and exhibit a greater tendency to restore its spherical form after ICSI owing to a greater intracellular pressure [75]. In human ICSI, intracellular pressure and/or cytoplasm fluidity can be estimated by the extent to which the ooplasm rises within the injection pipette immediately earlier than penetration of the oolemma before aspiration of cytoplasm [74].

Khalilian et al. postulated that the success of ICSI is mostly dependent on the dimension of the created funnel as properly as the mode by way of which the ZP is breached at some point of ICSI [76]. Ebner et al. stated, the injection funnel disappears for 2-3 min after ICSI pipette withdrawal to be associated with better embryo quality. They suggested that persistence of injection funnel even 2–3 min later may be associated with higher viscosity of the oocytes, thereby result in poor embryo quality [77]. Danfour and co-workers divided oocytes into three corporations in accordance to the degree of oolemma resistance and injection funnel form at some point of ICSI; "no resistance and no funnel," "mild resistance and curve-shaped," and "good resistance and funnel shaped". They discovered higher fertilization and higher proportion of good-quality embryo in "mild resistance and curve-shaped" group [78]. Also, Krause et al. calculated the injection funnel from the point of view of volume during ICSI, but observed no relation between the injection funnel volume and embryo quality. In their study, the injection funnel persisted 2–3 min later in 35% of the oocytes, but they still did not found out individual injection funnel persistence time as well as oolemma resistance [79]. Recently "oolemma score" (each from injection funnel persistence time and character oolemma resistance) has been established, in which an "oolemma score"> 14.5 predicted the formation of high-quality embryos [80].

Discussion

The lower incidence of degeneration and the higher normal fertilization rate and better embryo development and faster cleavage from ICSI using the smallest-diameter (3–5 μm) injection pipette was likely a result of minimized degree of the mechanical trauma exerted on the oolemma, minimized amount of ooplasm aspirated into the injection pipette prior to injection, and minimized the amount of PVP injected into the oocyte along with the spermatozoon [33]. At the time of oolemma breakage, reducing the volume of aspirated cytoplasm into the injection pipette can improve the ICSI success rate. During ICSI orientation of the pb at 8, 9, or 10 o'clock could partially disrupt the spindle and the pb at 5, 6, 12, or 1 o'clock could delay pronuclear syngamy. So, position of the pb at 7 or 11 o'clock results in a high fertilization rate and the development of the greatest proportion of high-quality embryos. Very resistant oolemma indicates higher ooplasmic viscosity and this would hamper cytokinesis during the development of embryo, or favor to generate fragmentations. No human studies have been done on holding needle yet, and two available studies have been related to animal specimens [81,82]. Also, comparison of piezo-ICSI to ICSI is in order to clarify whether piezo-ICSI can improve the results of ICSI (Table 1).

Table 1: The technical aspects in ICSI program.

ICSI-related apparatuses & events	Characteristics	Effects	References
Micro tools	Inner diameter	Micro tools with small inner diameter increases fertilization rate and embryo development and decrease tripronuclear zygotes formation and degeneration.	Yavas et al. 2001 [29]
	Long tapper	Normal fertilization rate and embryo development increases, degeneration and tripronuclear zygotes formation decreases.	Vanderzwalmen et al. 1996 [27]
	Spike & Blunt	Presence of a spike facilitates passage through the ZP.	Svalander et al., 1995 [28], Ivakhnenko et al, 2008 [25],; Svalander et al., 1995 [28]
		Presence and absence of spike did not change fertilization or damage rates	
		Damage the oocyte via compression.	
	Single use	A single injection pipette should be used because sharpness loss causes debris accumulation and breakage of the oolemma and using one injection pipette causes poor fertilization rates	Vanderzwalmen et al., 1996 [27]; & Zhang et al,.2012 [26]; & Kastelic et al., 2002 [30] ; & Nagy et al., 1995 [32]
		Improves clinical pregnancy	
	Commercial	Commercial injection micropipettes improved pregnant compared to home-made micropipettes	Khalili et al, 2017 [33]
Sperm immobilization	Conventional method	Sperm immobilized by compressing the sperm tail with lower fertilization occurs when immobilized spermatozoa injected without tail breaking	Vanderzwalmen et al., 1996 [27]
	Aggressive mechanical method	For epididymal or testicular spermatozoa, higher fertilization rates than ejaculated spermatozoa	Palermo, P. N. Schlegel, et al., 1996 [37], Fishel et al., 1995 [45]; Gerris et al., 1995 [44]
	Pipetting, piezo-pulses, the squeezing method	higher post ICSI fertilization rates for ejaculated spermatozoa reported after more aggressive mechanical	Palermo, et al., 1996 [37]; M Van den Bergh, et al, 1995 [36]
	Triple touch immobilization and single touch immobilization	A randomized trial study showed no differences between triple touch immobilization and single touch immobilization	M Van den Bergh, et al, 1995 [36]
Injection Speed	0.05 mm/s and 1 mm/s	low injection speed is desirable to reduce the injection force, high speed be used for reducing oocyte deformation	Hajiyavand et al., 2019 [50]

Cytoplasm Aspiration	Aspiration before sperm injection	Not essential for oocyte activation, since differences in the rate of oocyte damage, fertilization rate, and embryo quality were not found.	Mansour et al., 1996 [53]
	Volume is aspirated into the injection pipette	Disturbed cytoplasmic structure, following cell injury	Mansour et al., 1996 [53]& Dumoulin et al., 2001 [54]
	Type of membrane breakage	Immediate membrane breakage was associated with high degeneration rates compared to sudden membrane breakage	Carrillo et al., 1998 [55].; Dumoulin et al., 2001 [54]; Nagy et al., 1995 [32] , Carrillo et al., 1998 [55]; Fernández et al., 2020 [58] & Nagy et al., 1995 [32]
Site of sperm deposition	Pb positioned at 6 or 12 o'clock	When the pb was oriented at 6 o'clock. high quality embryos were achieved.	Nagy et al., 1995 [32]
	The displacement of the spindle away from pb	Manipulation, stress caused during oocyte denudation and intrinsic factors, such as oocyte aging cause alteration of position of polarized molecules in the cytoplasm, consequently, the embryo development. So, choose injection angle of 900 (corresponding to 3 o'clock position)	Cooke et al, 2003 [68] & Taylor et al., 2008 [67].

Conclusion

The primary reasons for ICSI popularity stem from its effectiveness, the standardization of the procedure, which can easily be incorporated into the routine practice of fertility. This review provided the current knowledge on some controversial technical aspects of the ICSI procedures in order to advance its efficacy in ART program. However, this area of expertise still demands a greater number of well-designed studies, in order to solve issues about the safety of these procedures.

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Conflict of Interest

The authors declare no conflict of interests.

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