ICSI FOR ALL?: A SWOT analysis

Running title: ICSI: a SWOT analysis

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ABSTRACT

Intracytroplasmatic sperm inyection (ICSI) is becoming a common practice to improve reproductive results, particularly among couples without male factor infertility. However, there is still no clear evidence on the possible advantages and drawbacks with regard to this procedure in patients with no formal indication for ICSI. In this discussion paper, based on a SWOT (strengths, weaknesses, opportunities, threats) analysis, the different aspects of this strategy are evaluated.

Current evidence suggests that ICSI it is not justified for non-male factor infertile couples requiring in vitro conception. One of the major strengths associated to the procedure is that cases complicated by total fertilization failure can almost be eliminated and a combination between IVF and ICSI on sibling oocytes has been advised in the literature. More technical difficulties, higher costs and performing an unnecessary invasive technique in some cases represent some weakness of the procedure and questions regarding safety issues cannot be ruled out. Additional large and well-designed RCT are needed to clarify definitive indications for ICSI in non-male factor infertility.

Keywords: intracytoplasmic sperm injection (ICSI), in vitro fertilization (IVF), severe non-male factor, pregnancy, assisted reproduction, infertility, implantation, live birth.

INTRODUCTION

Intracytroplasmatic sperm inyection (ICSI) is an assisted fertilization procedure that involves injection of single spermatozoa into a mature oocyte. ICSI was introduced in 1992 as a method to treat couples with severe male infertility (**Palermo et al., 1992**). However, in the last two decades, the use of ICSI has increased substantially, particularly among patients without male factor infertility, where ICSI use has increased from 15% in 1996 to 67% in 2012 (**Boulet et al., 2015**).

A recent report highlighted that, in some parts of the world (Egypt, Lebanon), ICSI is performed in 100% of IVF cycles and in 65% of IVF cycles in Europe (**Adamsom et al., 2018**). Despite its increase use, in cycles without male factor infertility, there is no clear evidence that ICSI improves reproductive outcomes and a scarcity of well-designed randomized controlled trials analyzing the possible benefits of ICSI in this population have been published (**van Rumste et al., 2003**).

For this reason, we designed a SWOT (strengths, weaknesses, opportunities, threats) analysis to assess the available published evidence on the possible recommendation of ICSI and, additionaly, assessed the scientific Oxford level of evidence for each of the reviewed papers to avoid subjectivity in their statements.

METHODS

In this study, a SWOT analysis was carried out to understand the perceived strengths and major pitfalls of the ICSI in non-male factor infertility, to identify the opportunities that can be taken and the key threats to this technology according to the bibliography analyzed, and to know the experts' point of view. The SWOT method is recently applied in fertility medical research when there is insufficient scientific evidence to assess the applicability or not of a particular technique, give light on specific issues and evaluate the possible pros and cons. (**Barrow, 2016; Blockeel et al. 2016;Engmann et al., 2016; Esteves et al. 2017**).

First, a bibliographic search was carried out aimed at "ICSI AND non-male factor infertility". The valuation of the bibliography has been carried out by two investigators independently and in case of non-agreement a third one was consulted. The primary review of the literature revealed 361 papers and 93 were classified as relevant articles. Then, a second manual bibliographic search was carried out to complete those matters of relevance included by the reasearches in the SWOT clinical outline and not resolved in the first general search. A division of the total number of references among the researchers and an Excel spreadsheet for each of the sections of the SWOT was proposed, which would be available on the **SISGtool.org platform** along with the corresponding references. In each of the tables the ideas/phrases proposed for each section was noted and each researcher added to each of them the studies classified by the degree of evidence. To unify the criteria for the evaluation of the evidence, the CEBM levels of quality of evidence were followed (Oxford CEBM, 2009) (**Table 1**).

Table 1. Oxford Centre for Evidence-based Medicine (CEBM)-Levels of Evidence (March 2009). Available at: http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/]

Levels of evidence	Type of study
1a	Systematic reviews (with homogeneity) of randomized controlled trials
1b	Individual randomized controlled trials (with narrow confidence interval)
1c	All or none randomized controlled trials
2a	Systematic reviews (with homogeneicity) of cohort studies
2b	Individual cohort study or low quality randomized controlled trials (e.g. <80% follow-up)
2c	"Outcomes" Research; ecological studies
3a	Systematic review (with homogeneity) of case-control studies
3b	Individual case-control study
4	Case-series (and poor quality cohort and case-control studies)
5	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"

Note: A minus sign "-" may be added to denote evidence that fails to provide a conclusive answer because it is *either* (a) a single result with a wide Confidence Interval; *OR* (b) a Systematic Review with troublesome heterogeneity.

RESULTS

STRENGTHS

1. **ICSI could increase fertilization rate and decrease fertilization failure in patients with unexplained infertility**

In conventional in-vitro fertilization (IVF), complete failure of fertilization occurs in 5-10% and live birth rates of IVF is in the range of 35-45% per embryo transfer (**SART, 2017)**. Some data suggest that ICSI may result in increased fertilization rate and decrease total fertilization failure with favorable clinical outcomes in couples with well-defined unexplained infertility (**Johnson et al., 2013; evidence 2a)** or long-term infertility (**Shi et al., 2010; evidence 2b**), but other studies contradict the finding of an increase in

fertilization rates with ICSI compared to IVF (see table 2). The largest meta-analysis published analyzing this matter included eleven studies with a total of 901 couples with unexplained infertility (female age range 30-35 years) with 11,767 sibling oocytes. In this meta-analysis the pooled relative risk (RR) of a mature oocyte fertilizing was higher with ICSI than with IVF (RR 1.49, 95% confidence interval [CI] 1.35-1.65.) and the pooled RR of total fertilization failure (TFF) was significantly higher with conventional IVF than with ICSI (RR 8.22, 95% CI 4.44-15.23) (**Johnson et al., 2013; evidence 2a)**. The number of subjects needed to treat with ICSI to prevent one case of TFF was five. This meta-analysis and other randomized controlled studies using sibling oocytes favors the use of ICSI to decrease the risk of TFF in couples with well-defined unexplained infertility (well-defined unexplained infertility was considered if the authors clearly documented that patients included in the analysis had no cause for infertility identified after a complete infertility evaluation) **(Johnson et al., 2013; evidence 2a).** Discordant results regarding the fertilization rate may be related to the low sample size and different characteristics of the populations analyzed.

2. **ICSI decreases fertilization failure in patients with mild male factor infertility or borderline semen**

In patients with borderline/subfertile semen (defined by the presence of at least one abnormal semen parameter, that is, concentration $<20 \times 10^6$ per milliliter and/or <40% motility) performing ICSI on at least some of the oocytes has demonstrated to avoid unnecessary fertilization failure and in these cases evidence supports that 25% of cycles could be rescued by ICSI (**van der Westerlaken et al., 2006; evidence 1b-; van Rumste et al., 2000; Plachot et al., 2002**). The risk ratio for an oocyte to become fertilized in patients with moderate male infertility has been estimated to be 1.9 (95% confidence interval of 1.4 to 2.5) in favor of ICSI, and 3.1 ICSI cycles may be needed to avoid one complete fertilization failure after conventional IVF (95% CI of 1.7 to 12.4) (**Tournaye et al., 2002; evidence 1b-).** Till more results are available, some authors suggest that it could be an option to apply the IVF–ICSI combined treatment in case of borderline semen when enough oocytes are available and to apply ICSI when the number of oocytes is too small for a fair chance of fertilization through IVF **(van der Westerlaken et al., 2006; evidence 1b**-).

3. **Minimizing the risk for cycle cancellation due to total fertilization failure in couples with prior failed fertilization with conventional insemination (IVF).**

Unfortunately, some couples may not conceive at their first cycle of IVF and will need to consider a second cycle. In those couples with non-male factor infertility, two initial cohort studies suggested that the use of ICSI following prior total failed fertilization or poor fertilization rates in a prior IVF cycle could reduce the risk of subsequent failed fertilization. The first of them analyzed 662 sibling MII oocytes from patients with tubal disease and normozoospermic partners, and reported lower rates of total fertilization failure for ICSI (3.6%; 95% CI 0.4-12.3) compared to those obtained for IVF (12.5%; 95% CI 5.2-24.1) (**Staessen et al., 1999; evidence 2b**). In the other cohort study carried out by Hariprashad et al. (2002) similar findings were obtained and it was found that in these couples fertilization (77% vs 44,6%; $p<0.05$) implantation rates (21,6% vs 9,9%; $p<0.05$) and clinical pregnancy per transfer (42,1 vs 19.6; $p<0.05$) were significantly higher after the use of ICSI (**Hariprashad et al., 2002; evidence 2b**).

Other authors suggest that in these couples, if an amount of sibling oocytes are subjected to ICSI, total fertilization failure of an IVF can be prevented and fertilization can be improved maintaining high chances of achieving a pregnancy. At the same time, the optimal fertilization method for subsequent treatment cycles can be determined (**Bungum et al., 2004**). In a prospective randomized study where sibling oocytes were allocated to conventional IVF vs. ICSI/IVF combination after total failed fertilization resulted in 12/109 (11%) oocytes fertilized by IVF/conventional insemination and 78/162 (48%) fertilized with ICSI/IVF combination (**van der Westerlaken et al., 2005; evidence 2b**). In this study, ICSI/IVF reported higher fertilization rates (60% vs 22%) even in couples which showed a low fertilization rate (<25%) in a first IVF attempt. However, large well-designed randomized studies should be performed to definitively confirm this finding.

4. **ICSI could improve embryo quality**

Some authors suggest a superiority of ICSI embryos based on the fact that the ICSI procedure avoids oocyte and zygote culture with a lot of spermatozoa. This thereby reduces exposure to the reactive oxygen species produced by the spermatozoa that might contribute to embryonic damage (**Quinn et al. 1998**). To support the hypothesis of a possible improvement of embryo quality with ICSI a preliminary study that comprised 13 couples found that more grade A embryos were obtained by ICSI than by conventional

IVF performed on sibling oocytes (**Yang et al., 1996; evidence 2b**). Later on, a study using sibling oocytes from 35 couples with non-male-factor infertility, demonstrated higher formation of good-quality embryos per retrieved oocyte after ICSI than after conventional IVF (64.4 vs. 47.1%, respectively) **(Khamsi et al., 2001; evidence 1b)** whereas, Ruiz et al. (**Ruiz et al. 1997; evidence 2b**) failed to note any such difference. Compared fertility outcomes among ICSI and conventional IVF in couples with non-male factor infertility showed similar pronuclear morphology of zygotes (nucleoli, pronuclei and axis) derived from sibling oocytes **(Nicolai et al., 2010; evidence 2b)**, significantly lower rate of abnormal pronuclei formation (3.9% vs. 13.3%, P<0.01) and better cleavage stage embryo quality in the ICSI group (**Kim et al., 2014; evidence 2b**). Data collected and analyzed from patients with non-male factor infertility, aged ≥35, undergoing their first IVF/ICSI cycle attempt show higher top quality embryos (TQE) rate (defined as two to four, or six to eight blastomeres on day 2 or 3 respectively, with equally-sized blastomeres and < 15% fragmentation) following ICSI compared to conventional insemination (62.8% versus 45.5% respectively; $P < 0.001$) (**Farhi et al., 2019; evidence 2b**). Nevertheless, these differences in embryo quality should be confirmed in a large RCT as they could depend on intrinsic factors of the gametes involved rather than on the fertilization process per se.

5. **ICSI could increase pregnancy rates in patients with disordered zona pellucida-induced acrosome reaction.**

Some authors suggest that ICSI may probably provide some advantages in those cases with sperm-zona pellucida penetration and fertilization disturbances. In fact, up to a third of normozoospermic men have disordered ZP-induced acrosome reaction (AR) and, in patients with normal semen analysis, the sperm zona pellucida (ZP)- induced acrosome reaction is highly correlated with sperm-ZP penetration and fertilization rate (**Bastiaan et al., 2003**). In patients with unexplained infertility with ZP-induced AR <16%, average fertilizations rates are <30% with conventional IVF and, interestingly ICSI has been found to overcome these defects resulting in an improvement of pregnancy rates (**Liu et al., 1997; Liu and Baker, 2000; 2003; evidence 2c**).

6. **Avoid possible contamination with DNA from spermatozoa in cases where PGT is performed for monogenic conditions**

Testing errors can arise from many different problems in a PGT procedure but the most common and worrisome ones have been quoted to be the issues related to sample contamination. Utilizing ICSI rather than the conventional IVF in PGT cycles is recommended to prevent paternal contamination from excess sperm introduced into the zona pellucida, or maternal contamination from granulosa cells especially with PGT performed for single gene defects (**Harton et al., 2011; evidence 1a**).

7. **Sperm DNA fragmentation**

High sperm DNA fragmentation has adverse effects on ART outcomes (**Zini et al., 2011; Choi et al., 2017**). The results of a meta-analysis published in 2015 suggest that IVF treatment in men with high sperm DNA fragmentation is associated with lower live birth outcome compared with those with low DNA fragmentation but this detrimental effect has not been demonstrated when ICSI treatment was used. (**Osman et al., 2015**; **evidence 1a-)**. Nevertheless, well-designed randomized studies are required to confirm the merit of ICSI over IVF in the treatment of men with high sperm DNA fragmentation as the number of couples included in each study of the meta-analysis was relatively small and some methodological limitations were encountered

2. WEAKNESS

No standardized guidelines to define "Severe Male Factor" infertility

Male factor infertility is commonly defined using the conventional semen profile (number of spermatozoa present in the eyaculate, the proportion that are motile or progressively motile and the percentage of morphologically normal spermatozoa) but a standardized definition is still lacking (**WHO Laboratory Manual for the Examination and Processing of Human Semen, 2010**). Sperm dysfunction cannot be detected by the standard descriptive semen analysis, which is recognized as a relatively insensitive tool for predicting the fertilizing potential of sperm, except in extreme cases and, interestingly,

men can produce dysfunctional sperm even when their semen parameters (sperm concentration and motility) are normal (**Hamada et al., 2012; Alasmari et al., 2013; Cooper et al., 2009**).

Invasiveness and complexity of the technique

ICSI is a complex and invasive technique that requires micromanipulation where a single spermatozoon is injected centrally into the cytoplasm of the oocyte using a fine sharp tipped glass pipette (**Rahman et al., 2010**). The operator selects the spermatozoon for microinjection and therefore the natural selection barrier to fertilization is bypassed and may contribute to the creation of embryos with molecular disturbances and low implantation potential. One potential problem with ICSI micromanipulation is the oocyte damage (lisis) which is largely unpredictable and unsystematic in nature (**Rosen et al., 2015; Sfontouris et al., 2015; evidence 2a**).

Operator experience and technical factors are critical

Technical factors are critical for achieving high rates of fertilization and pregnancy with ICSI and include the use of standardized ICSI pipettes, the immobilization of the spermatozoon before injection, the aspiration of a minimal amount of ooplasm before reinjection with the sperm and substantial operator experience (**Mc Culloh et al. 2011**). In a prospective trial of 305 ICSI cycles, the oocyte degeneration rate ranged from 5 to 11% depending on the laboratory technician performing it and the volume of aspirated oocyte cytoplasm during the procedure affected the percentage of embryos reaching the blastocyst stage (**Dumoulin et al., 2001**). Precision in handling and manipulation, injection procedure and rigorous thermal control is critical for reducing adverse outcomes

ICSI complications: Defects in imprinting and DNA methylation, possible sex ratio modification

Imprinting and DNA methylation

Manipulation of spermatozoa and the duration of embryo culture are all thought to influence methylation changes and imprinting disorders, but reports of the effects of IVF/ICSI on imprinting have been heterogeneous (**Lazaravizute et al., 2015; evidence 1a**).Imprinting disorders and DNA methylation are more prevalent after human IVF or ICSI (**Whitelaw et al., 2014; evidence 3b)** but there is no proof of a causal relationship

between imprinted diseases and IVF or ICSI treatments (**Vermeiden et al., 2013; evidence 2a**).

Possible sex ratio modification

In 2009, a restrospective study of the Society for Assisted Reproductive Technology national database that included 15,164 singleton live births by IVF and ICSI indicated that use of ICSI, particularly with blastocyst-stage embryos, is associated with a decrease in the sex ratio of male infants. (**Luke et al., 2009; evidence** 3**b**). The impact of ICSI on sex ratio has also been observed in a recently published study that assessed a total of 59,628 singleton deliveries resulting from different assisted reproductive technologies (IVF, ICSI, intrauterine insemination, ovulation induction) from 101 IVF clinics in Germany. In this study, ICSI was associated with a lower sex ratio of male infants compared with the natural conception ($P < 0.001$), whereas IVF was associated with a higher sex ratio ($P = 0.015$) and this phenomenon was not influenced by maternal age (**Cirkel et al., 2018; evidence 2b**).

No benefit of ICSI on women-related factors of poor-prognosis

Oocytes of older women could contain structural damage that might reduce fertilization potential and ICSI could be the preferred insemination procedure to avoid fertilization failure. Nevertheless, several retrospective cohort studies and a meta-analysis suggest that, in the absence of male factor infertility diagnosis, ICSI did not improve fertilization rates compared with IVF in advanced maternal age **(Sunderan et al., 2018; evidence 2a; Tannus et al., 2017; evidence 2b),** poor-quality oocytes/low oocyte yield **(Luna et al., 2011; evidence 2b)** and poor responders **(Sunderan et al., 2018; evidence 2a; Tannus et al., 2017; Check et al., 2012; evidence 2b; Sfontouris et al., 2015; evidence 2b).**

No effect on reproductive outcomes

Although ICSI is capable of consistently overcoming unforeseen sperm cell dysfunction and provide increased fertilization rates in some cases, no significant effect on reproductive outcomes have been reported in several randomized studies yielding comparable reproductive outcome in comparison to conventional IVF (**Table 3**). A retrospective cohort study of 585 065 ART treatment cycles between 2002 and 2013

reports a lower live birth using ICSI compared to IVF in non-male factor patients in most years bringing into question its widespread use (**Chambers et al., 2016; evidence 2b**). Moreover, a recently published population-based cohort of 14,693 women (**Li et al., 2018; evidence 2b**), who had their first ever stimulated cycle with fertilization performed for at least one oocyte by either IVF or ICSI demonstrated that among couples with non-male factor infertility, ICSI resulted in a similar cumulative live birth rate compared with IVF (AHR: 0.96, 95% CI: 0.85-1.10).

No evidence of cost-benefit

In US, mandates for IVF coverage have been associated with lower ICSI use for non-male-factor infertility cycles, probably due to the elevated cost (**Dieke et al., 2018; evidence 2b).** ICSI cycle in average costs 8.5 – 30% more than an IVF cycle **(Kjellberg et al., 2006, Kovacs et al. 2004, Bouwmans et al., 2008).** Ola et al. (2001) found a cost difference of about £600 per fresh cycle between IVF and ICSI and estimated that £60 000 (cost needed to treat, CNT) would be needed to gain one additional live birth when ICSI was used for patients requiring IVF (**Ola et al., 2001**). Including consumables, personnel, and facility costs, the incremental cost per live birth (for patients unsuited for conventional IVF) has been estimated to be approximately three times the cost of standard IVF **(Hollingsworth et al., 2008**). For a patient who would have good fertilization without ICSI, performing ICSI will result in a higher cost. However, it could be cost-saving for a patient who may have very low fertilization or no fertilization.

Lack of well-designed RCT and inconsistencies in the results

The lack of large well-designed RCTs is one important limitation of ICSI and most of them have been criticized because of poor study design. Studies comparing IVF and ICSI have had varied methodology, outcome measures, and conclusions and inconsistencies among the results are equivocal at times. For example, although higher fertilization rates are described in most sibling oocytes studies carried out in infertile patients without a diagnosis of male factor infertility, no difference in pregnancy rates are observed in the RCT between those undergoing IVF with ICSI and those undergoing IVF without ICSI. Some authors suggest that one possible explanation for the lack of a positive effect of ICSI on outcomes is the limited quality of these studies or the fact that the patients

receiving ICSI have more severe infertility in ways that could be unobservable to the researchers (**Kim et al., 2007**).

3. OPPORTUNITIES

Selection of the oocytes

ICSI allows a mechanical selection of the oocytes as patient-specific oocyte quality has been significantly related to the incidence of lysis during the procedure **(Mc Cullogh et al., 2011; evidence 2b)**. Moreover, the removal of the cumulus cells provides a more direct feedback on the oocyte maturity (**Orief et al., 2004**). Human oocyte grading based on the triple factors (first polar body, size of perivitelline space and cytoplasmic inclusions) has been related significantly to fertilization rate and embryo quality after ICSI. (**Balaban et al., 2012; evidence 1c**). In non-male-factor infertility, ICSI could be considered when metaphase I (MI) oocytes are matured as the multi-pronuclei formation rate with ICSI is significantly lower than that with conventional insemination (**Park et al. 2017; evidence 2b**).

Technical improvements: Selection of a good quality semen with intact sperm genome (IMSI, PICSI)

1 Prior ICSI procedure, there is a potential to select good quality sperm (functional cells with intact sperm genome apparatus), other than the three basic sperm parameters and the development of diagnostic tools of dysfunctional sperm is likely to be a key component in improving ICSI outcomes for some infertile couples (**Alasmari et al., 2017**). Advanced sperm selection strategies include selection according to sperm morphology under ultra-high magnification (MSOME), ability to bind to hyaluronic acid, surface charge, sperm apoptosis and sperm birefringence. The analysis and selection of spermatozoa for ICSI using intracytoplasmic morphologically selected sperm injection (IMSI) had a theoretical potential to improve results in ART cycles through an increase in the number of grade A embryos formed and a decrease in the level of fragmentation in these embryos. **(Wilding et al., 2010; evidence 1a)** but results from RCTs with this technique do not support its clinical use. There is no evidence of effect on

live birth or miscarriage and the evidence that IMSI improves clinical pregnancy is of very low quality (**Mc Dowell et al., 2014; evidence 1a**).

The use of hyaluronic acid binding assays in ICSI cycles to improve clinical outcomes has been analyzed in three RCT that (**Parmegiani et al., 2012; Majumdar et al., 2013; Worrilow et al., 2013**; **evidence 1b-**).Results from these studies reported that ICSI with hyaluronan-selected sperm (so-called physiological ICSI [PICSI]) decreased miscarriage rates and improved embryo quality and livebirth rates compared with ICSI with sperm selected using standard methods. The couples who benefited most from PICSI had low hyaluronan–sperm binding scores. Nevertheless, the largest randomized trial of PICSI recently published provides a robust measure of livebirth and it shows that PICSI did not significantly increase the term livebirth rate compared with standard ICSI, but a significant decrease was observed in miscarriage rates among couples in the PICSI group (**Miller et al., 2019; evidence 1b**). According to these findings, the application of this technique may provide an improvement in embryo quality and implantation rate but evidence does not support its routine use in all ICSI cycles and the identification of patients that might benefit from this technique needs further study.

Artificial oocyte activation

The principal cause of failed fertilization has been attributed to an oocyte activation disruption (**Sousa et al., 1994**). In couples with a history of total fertilization failure in a previous cycle, using ICSI followed by artificial oocyte activation (AOA) has been associated with an increase in the proportion of cleavage stage embryos (RR 5.44, 95% CI 2.98-9.91), top/high quality cleavage stage embryos (RR 10.02, 95% CI 2.45-40.95) **(Sfontouris et al., 2015; evidence 1a-)** and higher fertilization rates (**Takihara et al., 1998**). Thus, oocyte activation failure can nowadays be overcome clinically by AOA such as calcium ionophores or injection of recombinant PLCζ protein. A number of cases have demonstrated that ICSI combined with AOA greatly increases the success rate of fertilization and subsequent pregnancy (**Rybouchkin *et al*., 1997; Kim *et al*., 2001; Tesarik *et al*., 2002; Heindryckx *et al*., 2005, 2008; Dirican *et al*., 2008; Tejara *et al*., 2008; Kyono *et al*., 2009; Taylor *et al*., 2010; evidence 3b**) even in patients that had a history of failed fertilization with ICSI (**Eldar-Geva et al., 2003; Heindryckx *et al*., 2005, 2008; Chi et al., 2004).** Several reports on the well-being of children born after ICSI with AOA have provided reassuring insights resulting with neonatal outcome within

normal limits in regard to birth weight, gestational age, neonatal malformations, occurrence of perinatal mortality motor skills, behavioural profile and early development (**Yanagida et al., 1999; Heindryckx et al., 2008; Kyono et al., 2012; Vanden et al., 2012**)

Genome editing (CRISP/Cas9)

New methods of genome editing, especially CRISPR/Cas 9, have been developed and new perspectives on germline interventions have arisen (**Vassena et al., 2016**). Supporters of germ line genome editing suggest that the procedure could be used as a means of disease prevention.

4. THREATS

Predisposition to use ICSI (indiscriminated use/intolerance to fertilization failure)

ICSI has become increasingly popular, and although it was intended primarily to treat male factor infertility**,** the procedure has spread alarmingly and is being adopted for standard in-vitro insemination in many cases for non-male factor indication. This has arisen, at least in part because of the intolerance to fertilization failure and because of the increasing expectation from infertile couples of obtaining a succesful pregnancy. Even more, the removal of the cumulus cells provides the physician with more direct feedback on the quality of their stimulation, giving the ICSI in patients with poor morphology oocytes a much higher chance of success. A database analysis conducted using the National Assisted Reproductive Technology Surveillance System (NASS) from the Centers for Disease Control and Prevention (CDC) in US reported an overutilization of ICSI with no improved outcomes, particularly when it is used for non-male factor infertility and supports the contention that it is being over applied. Practice patterns for ICSI differ by geographical region and possible explanations may relate to insurance coverage availability, laboratory efficiencies, and/or perceived competition among clinics in specific regions of the country. Another relevant consideration for further examination

is the specific criteria used to define male factor infertility and the indication for ICSI used by clinics in different regions of the country. (**Zagadailov et al., 2017; evidence 2a**)

ICSI "overuse" during preimplantation genetic testing

The adoption of preimplantation genetic testing (PGT) by an increasing number of centres have promoted the generation of embryos by ICSI to exclude the risk of interference of contaminating spermatozoa. Recently, ICSI "overuse" in non-male infertility has been doubted, since it does not offer an advantage over IVF and recent data suggest that IVF should be the preferred insemination methods in PGT cycles while ICSI should be indicated only in cases of male-factor infertility (**Feldman et al., 2017; evidence 2b**).

Improvement of IVF

A possible improvement in IVF procedures could decrease the use of ICSI. In particular, a recently published retrospective data analysis which summarizes the first 15 years of ART activity in Europe for 12 consecutive years (1997–2011) suggests that the proportion of ICSI versus IVF cycles showed a marked increase during this period but is reaching a plateau from 2008 (**Ferranetti et al. 2017**; **evidence 2a**).

Possible long-term effects of ICSI on children (infants, adults)

Contradictory results have been published about the possible long-term effects of ICSI on children. From a strictly medical perspective, an increased risk of adverse neonatal outcome has been documented for ICSI-born children (**Kim et al., 2010; Helmerhorst *et al*., 2004; Jackson *et al*., 2004; Lie *et al*., 2005**) and some meta-analysis have shown a increase in congenital malformations in children born after ICSI (**Hansen *et al*., 2002; Rimm *et al*., 2004; Kallen *et al*., 2005**) but no clustering of any specific major malformation and no increased risk for major malformations has been found in ICSI children. (**Wen et al., 2012; evidence 1a**)

Concerns refer mainly to potential changes in genetic material, the possible transmission of foreign genetic material, the use of immature or senescent germ cells and associations between genetic disorders and some forms of infertility (**Bowen *et al*., 1998; te Velde *et***

***al*., 1998; Tournaye, 2003**). Nevertheless, medical follow-up studies have failed to document detrimental outcomes for children born through the ICSI technique (**Kurinczuk, 2003; Devroey and Van Steirteghem, 2004; Bonduelle *et al*., 2005**).

Regarding children development, one of the first studies analyzing the possible impact of ICSI on children development showed that 1-year-old ICSI-born children compared with spontaneously conceived and IVF children, revealed an increased risk of mildly to significantly delayed development (**Bowen *et al*., 1998**). However, a concurrent single-centre follow-up study found no signs of delayed development in 2-year-old ICSI children (**Bowen *et al*., 1998; evidence 2b**). Later studies carried out in ICSI children aged up to 5 years show no delayed development (**Sutcliffe *et al*., 1999, 2001, 2003a,b; Neri *et al*., 2002; Bonduelle *et al*., 2003; Leslie *et al*., 2003; Place and Englert, 2003; Squires *et al*., 2003; Wennerholm *et al*., 2003; Papaligoura *et al*., 2004**) and although generalization of these findings should be treated with caution, given many methodological limitations, such as relatively small sample sizes, inclusion of ICSI procedures for male factor defficiency, lack of formal child assessment and demographic matching of samples.

Comparable or even better cognitive and motor development until the age of 8 years have been also demonstrated in two large-scale multicentre studies that compared developmental outcomes of 151 8-year-old singletons born through ICSI after 32 weeks of gestation with those of 153 singletons of the same age born after spontaneous conception (SC) (**Leunens et al., 2006; evidence 2b**). Authors did not find a higher resting blood pressure or higher stress response after psychological stress in ICSI-conceived pubertal children (**Belva et al., 2012**). Finally, a systematic review of 34 studies comparing ICSI- and IVF-conceived children suggest their physical health physical health, growth and neuro-development is comparable; however, studies are few and limited to childhood. ICSI-conceived children may be at increased risk of autism and intellectual impairment and no difference in risk of childhood cancer was reported in one study. These data are inconclusive and further research into health outcomes in adolescence and adulthood is required before conclusions can be drawn about the long-term safety of ICSI compared to IVF. Until then, ICSI might be better reserved for its original intended use, male-factor infertility (**Catford et al., 2017; evidence 2a**).

CONCLUSION (corroborar investigadores)

In summary, despite the widespread use of ICSI in patients without a diagnosis of male factor infertility, little evidence exists demonstrating its effectiveness in this population. The major advantage of performing ICSI is that cases complicated by total fertilization failure can almost be eliminated. However reproductive outcomes are similar to those obtained by IVF. These observations have to be taken with caution as most studies comparing IVF and ICSI in non-male factor infertility have low quality and varied methodology, outcome measures, and conclusions. In addition, many of these studies were based on small sample sizes with inadequate statistical power. Other studies were not randomized or were randomized by sibling oocytes rather than by patients or cycles.

This application has some disadvantages. They include more technical difficulties, higher costs and performing an unnecessary invasive technique in some cases. Moreover, questions regarding safety issues cannot be ruled out. Several studies have demonstrated that there is no increase in the incidence of congenital anomalies in children born after ICSI compared with standard IVF or with the general population. However, others showed that infants born after ICSI might indeed have an excess of birth defects and an increased incidence of chromosomal abnormalities. It is possible that the increases in congenital abnormalities observed in some ICSI children, are due to intrinsic factors of the gametes involved rather than on the fertilization process per se, but it is also possible that the technique itself plays a role. Furthermore, the long-term outcome of the ICSI procedure in terms of development and transfer of genetic disorders is still unknown. It may be concluded that it is not justified to perform ICSI for all couples requiring in vitro conception. In unexplained infertility and relative male factor infertility, a possible strategy might be to perform a diagnostic comparison between conventional IVF and ICSI in all first treatment cycles. This would be especially beneficial in preventing total fertilization failure. Additional large and well-designed RCT are needed to clarify definitive indications for ICSI in non-male factor infertility.

REFERENCES

1. Aboulghar MA, Mansour RT, Serour GI, Amin YM, Kamal A. Prospective controlled randomized study of in vitro fertilization versus intracytoplasmic sperm injection in the treatment of tubal factor infertility with normal semen parameters. Fertil Steril. 1996;66(5):753-6.
2. Aboulghar MA, Mansour RT, Serour GI, Sattar MA, Amin YM. Intracytoplasmic sperm injection and conventional in vitro fertilization for sibling oocytes in cases of unexplained infertility and borderline semen. J Assist Reprod Genet 1996;13:38–42.
3. Aboulghar MA, Mansour RT, Serour GI, Amin Y, Ramzy AM, Sattar MA, Kamal A. Management of long-standing unexplained infertility: A prospective study. Am J Obstet Gynecol. 1999;181(2):371-5.
4. Adamson GD, de Mouzon J, Chambers GM, Zegers-Hochschild F, Mansour R, Ishihara O, Banker M, Dyer S.International Committee for Monitoring Assisted Reproductive Technology: world report on assisted reproductive technology, 2011. Fertil Steril. 2018;110(6):1067-1080.
5. Alaa Hamada, Sandro Esteves, Ashok Agarwal. Unexplained male infertility—looking beyond routine semen analysis. Eur. Urol. Rev. 2012;7: 90-96.
6. Alasmari A. Importance of the assessment of intracellular Ca 2+ level as diagnostic tool of dysfunctional sperm, Middle East Fertil. Soc. J. 2017;22: 170–173.
7. Alasmari W, Barratt C, Publicover S, Whalley K, Foster E, Kay V, Da Silva SM, S. Oxenham.The clinical significance of calcium signalling pathways mediating human sperm hyperactivation. Hum. Reprod. 2013; 28: 866-876.
8. Antinori M, Licata E, Dani G, Cerusico F, Versaci C, d'Angelo D, et al. Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial. Reprod Biomed Online.2008;16:835–41.
9. Assisted Reproductive Technology. National Summary Report 2015. Accessed at: https://www.cdc.gov/art/pdf/2015-report/ART-2015-National-Summary-Report.pdf).
10. Balaban B, Barut T, Urman B. Assessment of oocyte quality. Practical Manual of In Vitro Fertilization 2012; 105-119

11. Balakier H, Sojecki A, Motamedi G, Librach C. Time-dependent capability of human oocytes for activation and pronuclear formation during metaphase II arrest. Hum Reprod. 2004;19:982–987.
12. Barrow, P. Revision of the ICH guideline on detection of toxicity to reproduction for medicinal products: SWOT analysis. Reprod Toxicol. 2016; 64: 57-63.
13. Belva F, Roelants M, De Schepper J, Roseboom TJ, Bonduelle M, Devroey P, Painter RC. Blood pressure in ICSI-conceived adolescents. Hum Reprod. 2012;27(10):3100-8.
14. Bhattacharya S, Hamilton MP, Shaaban M, et al. Conventional in vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. Lancet 2001;357(9274):2075–2079
15. Bingol B, Abike F, Gedikbasi A, Tapisiz OL and Gunenc Z. Comparison of chromosomal abnormality rates in ICSI for non-male factor and spontaneous conception. J Assist Reprod Genet. 2012;29(1):25-30
16. Blockeel C, Drakopoulos P, Santos-Ribeiro S, Polyzos N.P., Tournaye H. A fresh look at the freeze-all protocol: a SWOT analysis. Hum Reprod. 2016;31(3):491-7.
17. Bonduelle M, Camus M, de Vos A, Staessen C, Tournaye H, van Assche E, Verheyen G, Devroey P, Liebaers I, van Steirteghem A. Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. Hum Reprod. 1999;14(Suppl 1):243–264.
18. Boulet SL, Mehta A, Kissin DM, Warner L, Kawwass JF, Jamieson DJ. Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. JAMA. 2015;313(3):255-63.
19. Bouwmans CA, Lintsen BM, Eijkemans MJ, Habbema JD, Braat DD, Hakkaart L. A detailed cost analysis of in vitro fertilization and intracytoplasmic sperm injection treatment. Fertil Steril. 2008 Feb;89(2):331-41.
20. Bowen JR, Gibson FL, Leslie GI, Saunders DM. Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. Lancet , 1998; 351:1529-1534.

21. Bukulmez O, Yarali H, Yucel A, Sari T, Gurgan T. Intracytoplasmic sperm injection versus in vitro fertilisation for patients with a tubal factor as their sole cause of infertility: a prospective, randomized trial. Fertility and Sterility 2000;73(1):38-42.
22. Bungum, L., Bungum, M., Humaidan, P., and Andersen, C.Y. A strategy for treatment of couples with unexplained infertility who failed to conceive after intrauterine insemination. *Reprod Biomed Online*. 2004; 8: 584–589.
23. Catford SR, McLachlan RI, O'Bryan MK, Halliday JL. Long-term follow-up of intra-cytoplasmic sperm injection-conceived offspring compared with in vitro fertilization-conceived offspring: a systematic review of health outcomes beyond the neonatal period. Andrology. 2017;5(4):610-621.
24. Chambers GM, Wand H, Macaldowie A, Chapman MG, Farquhar CM, Bowman M, Molloy D, Ledger W.Population trends and live birth rates associated with common ART treatment strategies.Hum Reprod. 2016;31(11):2632-2641
25. Chi HJ, J.J. Koo, S.J. Song, J.Y. Lee, S.S. Chang, Successful fertilization and pregnancy after intracytoplasmic sperm injection and oocyte activation with
26. Chiamchanya C, Torudom P, Gamnarai N. Comparative study of intracytoplasmic sperm injection and in vitro fertilization with high insemination concentration in sibling oocytes in the treatment of unexplained infertility. J Med Assoc Thai. 2008;91(8):1155-60.
27. Cirkel C, König IR, Schultze-Mosgau A, Beck E, Neumann K, Griesinger G. The use of intracytoplasmic sperm injection is associated with a shift in the secondary sex ratio. Reprod Biomed Online. 2018;37(6):703-708.
28. Cooper TG, E. Noonan, S. Von Eckardstein, J. Auger, H.G. Baker, H.M. Behre, T.B. Haugen, T. Kruger, C. Wang, M.T. Mbizvo, K.M. Vogelsong, World Health Organization reference values for human semen characteristics, Hum. Reprod 2009: 048.
29. Liu DY, Baker HWG. Defective sperm–zona pellucida interaction: a major cause of failure of fertilization in clinical in-vitro fertilization, *Human Reproduction* 2000;15: 702–708.
30. Danan C, Sternberg D, Van Steirteghem A, Cazeneuve C, Duquesnoy P, Besmond C, Goossens M, Lissens W, Amselem S. Evaluation of parental mitochondrial inheritance in neonates born after intracytoplasmic sperm injection. Am J Hum Genet. 1999;65(2):463-73

31. Devroey P, Van Steirteghem A. A review of ten years experience of ICSI.Hum Reprod Update. 2004;10(1):19-28.
32. Dieke AC, Mehta A, Kissin DM, Nangia AK, Warner L, Boulet SL. Intracytoplasmic sperm injection use in states with and without insurance coverage mandates for infertility treatment, United States, 2000-2015. Fertil Steril. 2018;109(4):691-697.
33. Dumoulin JC, Land JA, Van Montfoort AP, et al. Effect of in vitro culture of human embryos on birthweight of newborns. Hum Reprod 2010;25(3):605–612
34. Dumoulin JM, Coonen E, Bras M, Bergers-Janssen JM, Ignoul-Vanvuchelen RC, van Wissen LC, Geraedts JP, Evers JL. Embryo development and chromosomal anomalies after ICSI: effect of the injection procedure. Hum Reprod. 2001;16(2):306.
35. Dyer S, Chambers GM, de Mouzon J, Nygren KG, Zegers-Hochschild F, Mansour R, Ishihara O, Banker M, Adamson GD.International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010.Hum Reprod. 2016 Jul;31(7):1588-609
36. Eldar-Geva T, Brooks B, Margalioth EJ, Zylber-Haran E, Gal M, Silber SJ. Successful pregnancy and delivery after calcium ionophore oocyte activation in a normozoospermic patient with previous repeated failed fertilization after intracytoplasmic sperm injection. Fertility and Sterility 2003;79 (Suppl. 3):1656–1658.
37. El-nashar IH, Nas A. The role of hysteroscopy before intracytoplasmic sperm injection (ICSI): a randomized controlled trial. Fertil. And Steril. 2011; 96(3)
38. Engmann L, Benadiva C., Humaidan P. GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis. Reprod Biomed Online. 2016; 32(3):274-85.
39. Esteves, S.C., Agarwal, A., Cho, C.L., Majzoub, A., 2017. A Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis on the clinical utility of sperm DNA fragmentation testing in specific male infertility scenarios. Transl Androl Urol., 6(Suppl 4):S734-S760.
40. Vanden Meerschaut F, E. D'Haeseleer, H. Roeyers, A. Oostra, K. Van Lierde, P. De Sutter. Neonatal and developmental outcome of children born following assisted oocyte activation (AOA), Fertility and Sterility 2012;98:S16.

41. Farhi J, Cohen K, Mizrachi Y, Weissman A, Raziel A, Orvieto R. Should ICSI be implemented during IVF to all advanced-age patients with non-male factor subfertility?. Reprod Biol Endocrinol. 2019;17(1):30.
42. Feldman B, Aizer A, Brengauz M[1], Dotan K[2], Levron J[1,3], Schiff E[1,3], Orvieto R[4] Pre-implantation genetic diagnosis-should we use ICSI for all? J Assist Reprod Genet. 2017;34(9):1179-1183.
43. Ferraretti A, Nygren K, Nyboe A, de Mouzon A, Kupka J, Calhaz-Jorge M, Wyns C, Gianaroli C, Goossens V (2017). Trends over 15 years in ART in Europe: an analysis of 6 million cycles. Human Reproduction Open. 2017. 10.1093/hropen/hox012.
44. Foong SC, Fleetham JA, Joseph A. O'Keane, Selma G. Scott, Suzanne C. Tough, and Calvin A. Greene.A prospective randomized trial of conventional *in vitro* fertilization versus intracytoplasmic sperm injection in unexplained infertility. J Assist Reprod Genet. 2006; 23(3): 137–140.
45. Gasca S, Reyftmann L, Pellestor F, Rème T, Assou S, Anahory T, Dechaud H, Klein B, De Vos J, Hamamah S.Total fertilization failure and molecular abnormalities in metaphase II oocytes. Reprod Biomed Online. 2008;17(6):772-81.
46. Gui J, Xu W, Yang J, Feng L, Jia J. Impact of local endometrial injury on in vitro fertilization/intracytoplasmic sperm injection outcomes: A systematic review and meta-analysis. J Obstet Gynaecol Res. 2019;45(1):57-68.
47. Hariprashad J, Liotta D, Cook, C *et al*. ICSI as a therapeutic measure for failed conventional in-vitro fertilization cycles. The Cornell Experience. *Fertility and Sterility 2002;*78, Abstracts O-43, S17.
48. Harton GL, De Rycke M, Fiorentino F, et al; European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium. ESHRE PGD consortium best practice guidelines for amplification-based PGD. Hum Reprod 2011;26(1):33–40.
49. Heindryckx B, S. De Gheselle, J. Gerris, M. Dhont, P. De Sutter. Efficiency of assisted oocyte activation as a solution for failed intracytoplasmic sperm injection. Reproductive Biomedicine Online 2008;17:662–668.
50. Heindryckx B, Van der Elst J, De Sutter P, Dhont M. Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI. Human Reproduction 2005;20: 2237–2241.

51. Hershlag A, Paine T, Kvapil G, Feng H, Napolitano B. In vitro fertilization-intracytoplasmic sperm injection split: an insemination method to prevent fertilization failure. Fertil Steril. 2002;77(2):229-32.
52. Hollingsworth BH, Duncan AM. The cost effectiveness of intracyctoplasmic sperm injection (ICSI). Journal of assisted reproduction and genetics. 2008; 24. 571-7.
53. IVF-patients with nonmale factor "to ICSI" or "not to ICSI" that is the question? J Assist Reprod Genet. 2001;18(4):205-8.
54. Jaroudi, K., Al-Hassan, S., Al-Sufayan, H., Al-Mayman, H., Qeba, M., and Coskun, S. Intracytoplasmic sperm injection and conventional in vitro fertilization are complementary techniques in management of unexplained infertility. *J Assist Reprod Genet*. 2003; 20: 377–381
55. Johnson LN, Sasson IE, Sammel MD, Dokras A. Does intracytoplasmic sperm injection improve the fertilization rate and decrease the total fertilization failure rate in couples with well-defined unexplained infertility? A systematic review and meta-analysis. Fertil Steril. 2013 Sep;100(3):704-11.
56. Jun JH, Lim CK, Kim JW, Son IP, Koong MK, Song IO, Song JH, Yoo KJ, Kang IS: Comparison of fertilization and embryonic development between conventional insemination and ICSI treatment in the sibling oocytes of non-male factor infertility [abstract no. O-014]. Am Soc Reproduct Med 1999;72(Suppl.1):S6
57. Khamsi F, Yavas Y, Roberge S, Lacanna IC, Wong JC, Endman M. The status of controlled prospective clinical trials for efficacy of intracytoplasmic sperm injection in in vitro fertilization for non-male factor infertility. J Assist Reprod Genet. 2000;17(9):504-7.
58. Khamsi F, Yavas Y, Roberge S, Wong JC, Lacanna IC, Endman M. Intracytoplasmic sperm injection increased fertilization and good-quality embryo formation in patients with non-male factor indications for in vitro fertilization: a prospective randomized study. Fertil Steril. 2001; 75(2):342-7.
59. Kim HH, Bundorf MK, Behr B, McCallum SW. Use and outcomes of intracytoplasmic sperm injection for non-male factor infertility. Fertil Steril. 2007;88(3):622-8.

60. Kim JW, Lee WS, Yoon TK, Seok HH, Cho JH, Kim YS, Lyu SW, Shim SH. Chromosomal abnormalities in spontaneous abortion after assisted reproductive treatment.BMC Med Genet. 2010;11:153.
61. Kim JY, Kim JH, Jee BC, Lee JR, Suh CS, Kim SH. Can intracytoplasmic sperm injection prevent total fertilization failure and enhance embryo quality in patients with non-male factor infertility? Eur J Obstet Gynecol Reprod Biol. 2014;178:188-91.
62. Kim MS, Kim J, Youm HW, Park JY, Choi HY, Jee BC. Embryonic development in human oocytes fertilized by split insemination. Obstet Gynecol Sci. 2015 ;58(3):217-22. doi: 10.5468/ogs.2015.58.3.217. Epub 2015 May 19.
63. Kjellberg AT, Carlsson P, Bergh C.Randomized single versus double embryo transfer: obstetric and paediatric outcome and a cost-effectiveness analysis. Hum Reprod. 2006;21(1):210-6.
64. Ko DS, Lee SH, Park DW, Yang KM, Lim CK. Pregnancy and fertilization potential of immature oocytes retrieved in intracytoplasmic sperm injection cycles. Clin Exp Reprod Med. 2015;42:118–125.
65. Kovacs P, Kovats T, Sajgo A, Szollosi J, Matyas S, Kaali SG. The role of hyaluronic acid binding assay in choosing the fertilization method for patients undergoing IVF for unexplained infertility. J Assist Reprod Genet 2011;28:49–54.
66. Kyono K, T. Takisawa, Y. Nakajo, M. Doshida, M. Toya. Birth and follow-up of babies born following ICSI with oocyte activation using strontium chloride or calcium ionophore A23187. Journal of Mammalian Ova Research 2012;29:35–40.
67. Lazaraviciute G, Kauser M, Bhattacharya S, Haggarty P, Bhattacharya S. A systematic review and meta-analysis of DNA methylation levels and imprinting disorders in children conceived by IVF/ICSI compared with children conceived spontaneously.Hum Reprod Update. 2015;21(4):555-7.
68. Leunens L, S. Celestin-Westreich, M. Bonduelle, I. Liebaers, I. Ponjaert-Kristoffersen; Cognitive and motor development of 8-year-old children born after ICSI compared to spontaneously conceived children, Human Reproduction 2006;21 (1): 2922–2929.

69. Lewis SE, John Aitken R, Conner SJ, et al. The impact of sperm DNA damage in assisted conception and beyond: recent advances in diagnosis and treatment. Reprod Biomed Online 2013;27(4):325–337.
70. Li Z, Lin H, Xiao W, Wang Y. Fertilization of IVF/ICSI using sibling oocytes from couples with subfertile male or unexplained infertility. J Huazhong Univ Sci Technolog Med Sci 2004;24:365–8.
71. Li Z, Wang AY, Bowman M, Hammarberg K, Farquhar C, Johnson L, Safi N, Sullivan EA. ICSI does not increase the cumulative live birth rate in non-male factor infertility. Hum Reprod. 2018;33(7):1322-1330.
72. Liu H, Zhao H, Yu G, Li M, Ma S, Zhang H, Wu K.Conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI): which is preferred for advanced age patients with five or fewer oocytes retrieved? Arch Gynecol Obstet. 2018;297(5):1301-1306.
73. Liu DY, Bourne H and Baker HWG. High fertilization and pregnancy rates after intracytoplasmic sperm injection in patients with disordered zona pellucida-induced acrosome reaction. *Fertil. Steril.* 1997;67:955–958.
74. Liu, DY, Baker G. Disordered zona pellucida–induced acrosome reaction and failure of in vitro fertilization in patients with unexplained infertility. Fertility and Sterility 2003;79(1):74 - 80
75. Loke YJ, Galati JC, Saffery R, Craig JM. Association of in vitro fertilization with global and IGF2/H19 methylation variation in newborn twins. J Dev Orig Health Dis. 2015;6(2):115-24.
76. Lucettevan der Westerlaken M,Nico Naaktgeboren N, Verburg H, Dieben S, Helmerhorst F. Conventional in vitro fertilization versus intracytoplasmic sperm injection in patients with borderline semen: a randomized study using sibling oocytes. Fertil Steril. 2006;85(2):395-400.
77. Ludwig M & Geipel, A & Berg, Christoph & Gembruch, Ulrich & Schwinger, E & Diedrich, K. Is intracytoplasmic sperm injection itself an indication to perform preimplantation genetic diagnosis (PGD)? About PGD, invasive prenatal diagnosis and genetic sonography. Fetal diagnosis and therapy. 2001;16. 68-82.
81. Ludwig M, Schröder AK, Diedrich K. Impact of intracytoplasmic sperm injection on the activation and fertilization process of oocytes. Reprod Biomed Online. 2001;3(3):230-240.

82. Luke B, M. B. Brown, D. A. Grainger, V. L. Baker, E. Ginsburg, J. E. Stern and S. f. A. R. T. W. Group. The sex ratio of singleton offspring in assisted-conception pregnancies. Fertil Steril 2009; 92, Issue 5:1579–1585.
83. Luna M, Bigelow C, Duke M, Ruman J, Sandler B, Grunfeld L,Copperman AB. Should ICSI be recommended routinely in patients with four or fewer oocytes retrieved? J Assist Reprod Genet 2011;28(10):911–5.
84. Mahran A, Ibrahim M, Bahaa H.The effect of endometrial injury on first cycle of IVF/ICSI outcome: A randomized controlled trial. Int J Reprod Bio Med.2016;14:193–198.
85. Majumdar G, Majumdar A. A prospective randomized study to evaluate the effect of hyaluronic acid sperm selection on the intracytoplasmic sperm injection outcome of patients with unexplained infertility having normal semen parameters. *J Assist Reprod Genet.* 2013; 30: 1471-1475.
86. McCulloh D, Goorbarry BA, Shah MS, Ahmad K. Oocyte lysis following intracytoplasmic sperm injection:Association with measures of oocyte quality and technician performance. J Reprod Stem Cell Biotechnol 2011; 2(1):46-54.
87. McDowell S, Kroon B, Ford E, Hook Y, Glujovsky D, Yazdani A. Advanced sperm selection techniques for assisted reproduction. Cochrane Database Syst Rev. 2014;(10):CD010461.
88. Miller D, Pavitt S, Sharma V, Forbes G, Hooper R, Bhattacharya S, et al. Physiological, hyaluronan-selected intracytoplasmic sperm injection for infertility treatment (HABSelect): a parallel, two-group, randomised trial. Lancet. 2019 2;393(10170):416-422.
89. Moreno C, Ruiz A, Simon C, Pellicer A, Remohi J. Intracytoplasmic sperm injection as a routine indication in low responder patients. Hum Reprod 1998; 13:2126–9.
90. Morton PC, Yoder CS, Tucker MJ, Wright G, Brockman WD, Kort HI. Reinsemination by intracytoplasmic sperm injection of 1-day-old oocytes after complete conventional fertilization failure. Fertil Steril. 1997;68(3):488-91.
91. Nagy ZP, Verheyen G, Tournaye H et al. Special applications of intracytoplasmic sperm injection: the influence of sperm count, motility, morphology, source and sperm antibody on the outcome of ICSI. Human Reproduction 1998;13: 143–154.
92. Nelissen EC, Dumoulin JC, Daunay A, Evers JL, Tost J, van Montfoort AP. Placentas from pregnancies conceived by IVF/ICSI have a reduced DNA

methylation level at the H19 and MEST differentially methylated regions. Hum Reprod. 2013;28(4):1117-26.

93. Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from in vitro fertilisation: a prospective study of 144,018 treatment cycles. *PLoS Med*. 2011;8(1):e1000386.
94. Nicoli, A., Capodanno, F., Valli, B., Di Girolamo, R., Villani, M., Nucera, A., La Sala, G. (2010). Impact of insemination technique, semen quality and oocyte cryopreservation on pronuclear morphology of zygotes derived from sibling oocytes. *Zygote 2010; 18*(1), 61-68.
95. Ola B, Afnan M, Sharif K, Papaioannou S, Hammadieh N, Barratt C. Should ICSI be the treatment of choice for all cases of in-vitro conception. Considerations of fertilisation and embryo development, cost effectiveness and safety? Hum Reprod 2001;12:2485–90.
96. Orief Y, Dafopoulos, Konstantinos & Al-Hassani, Safaa. Should ICSI be used in non-male factor infertility?. Reproductive biomedicine online. 2004;9:348-56.
97. Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y.The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis.Reprod Biomed Online. 2015 Feb;30(2):120-7.
98. Palermo GD, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992;340(8810):17-8.
99. Palermo GD, Neri QV, Schlegel PN, Rosenwaks Z. Intracytoplasmic sperm injection (ICSI) in extreme cases of male infertility. PLoS ONE 2014;9(12):e113671
100. Park JH, Jee BC, Kim SH. Comparison of normal and abnormal fertilization of in vitro-matured human oocyte according to insemination method. J Obstet Gynaecol Res. 2016;42(4):417-21.
101. Parmegiani L, Cognigni GE, Bernardi S et al. Comparison of two ready-to-use systems designed for sperm–hyaluronic acid binding selection before intracytoplasmic sperm injection: PICSI vs Sperm Slow: a prospective, randomized trial. *Fertil Steril.* 2012; **98**: 632-637.
102. Plachot M, Belaisch-Allart J, Mayenga JM, Chouraqui A, Tesquier L, Serkine AM. Outcome of conventional IVF and ICSI on sibling oocytes in mild male factor infertility. Hum Reprod. 2002;17(2):362-9.

103. Poehl M, Holagschwandtner M, Bichler K, Krischker U, Jürgen S, Feichtinger W.IVF-patients with nonmale factor "to ICSI" or "not to ICSI" that is the question? J Assist Reprod Genet. 2001;18(4):205-8.

104. Quinn P, Lydic ML, Ho M, et al. Confirmation of the beneficial effects of brief coincubation of gametes in human in vitro fertilization. Fertil Steril. 1998;69:399–402.

105. Rahman, A. Intracytoplasmic sperm injection-revolution in human and animal assisted reproduction: A review.. Biotechnology, 2010; 9: 392-410.

106. Rosen M P. Oocyte degeneration after intracytoplasmic sperm injection: a multivariate analysis to assess its importance as a laboratory or clinical marker. Fertility and Sterility , Volume 85 , Issue 6 , 1736 – 1743.

107. Ruiz A, Remohi J, Minguez Y, Guanes PP, Simon C, Pellicer A: The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. Fertil Steril 1997;68:171–173.

108. Sfontouris IA, Kolibianakis EM, Lainas GT, Navaratnarajah R, Tarlatzis BC, Lainas TG. Live birth rates using conventional in vitro fertilization compared to intracytoplasmic sperm injection in Bologna poor responders with a single oocyte retrieved.J Assist Reprod Genet. 2015;32(5):691-7.

109. Sfontouris IA, Nastri CO, Lima ML, Tahmasbpourmarzouni E, Raine-Fenning N, Martins WP. Artificial oocyte activation to improve reproductive outcomes in women with previous fertilization failure: a systematic review and meta-analysis of RCTs. Hum Reprod. 2015;30(8):1831-41.

110. Shi XY, Wu FR, Chen SL, Wang QL, Luo C, Ni YP, Zheng HY, Qiu ZL, Zhang WQ, Yang J, Chen X. In vitro fertilization versus intracytoplasmic sperm injection for primary and secondary infertility using sibling oocytes: clinical analysis of the outcomes. Nan Fang Yi Ke Da Xue Xue Bao. 2010;30(10):2263-6.

111. Shu Y, Gebhardt J, Watt J, Lyon J, Dasig D, Behr B. Fertilization, embryo development, and clinical outcome of immature oocytes from stimulated intracytoplasmic sperm injection cycles. Fertil Steril. 2007;87:1022–1027.

112. Sousa M, Tesarik J. Ultrastructural analysis of fertilization failure after intracytoplasmic sperm injection, Human Reproduction 9 (1994) 2374–2380.

113. Staessen C, Camus M, Clasen K *et al*. 1999 Conventional in-vitro fertilization versus intracytoplasmic sperm injection in sibling oocytes from couples with tubal infertility and normozoospermic semen. *Human Reproduction 1999;*14: 2474–2479.

114. Strassburger D, Friedler S, Raziel A, Kasterstein E, Schachter M, Ron-El R. The outcome of ICSI of immature MI oocytes and rescued in vitro matured MII oocytes. Hum Reprod. 2004;19:1587–1590.

115. Sunderan S, Boulet SL, Kawwass JF, Kissin DM. Comparing fertilization rates from intracytoplasmic sperm injection (ICSI) to in vitro fertilization (IVF) in women older than 38 years with no male factor infertility: a meta-analysis. Fertility and Sterility 2018; 110:e221.

116. Takihara H. The treatment of obstructive azoospermia in male infertility--past, present, and future. Urology. 1998;51:150–5.

117. Tournaye H, Verheyen G, Albano C, et al. Intracytoplasmic sperm injection versus in vitro fertilization: a randomized controlled trial and a meta-analysis of the literature. Fertil Steril 2002;78(5):1030–1037

118. Ubaldi F, Liu J, Nagy Z et al. Indications for and results of intracytoplasmic sperm injection (ICSI). International Journal of Andrology 1995; 18, 88–90.

119. van der Westerlaken L, Helmerhorst F, Dieben S, Naaktgeboren N. Intracytoplasmic sperm injection as a treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization. Fertil Steril. 2005 ;83(3):612-7.

120. van der Westerlaken L, Naaktgeboren N, Verburg H et al. Conventional in-vitro fertilization versus intracytoplasmic sperm injection in patients with borderline semen: a randomized study using sibling oocytes. Fertility and Sterility 2006; 85, 395–400.

121. 1 van Rumste MM, Evers JL, Farquhar CM, Blake DA. Intra-cytoplasmic sperm injection versus partial zona dissection, subzonal insemination and conventional techniques for oocyte insemination during in vitro fertilisation. Cochrane Database Syst Rev. 2000;(2):CD001301.

122. van Rumste MM, Evers JL, Farquhar CM.ICSI versus conventional techniques for oocyte insemination during IVF in patients with non-male factor subfertility: a Cochrane review. Hum Reprod. 2004;19(2):223-7.

123. Vassena R, Heindryckx B, Peco R, Pennings G, Raya A, Sermon K, Veiga A. Genome engineering through CRISPR/Cas9 technology in the human germline and pluripotent stem cells. Human Reproduction Update 2016*;*22 (4): 411–9.

124. Vermeiden JP, Bernardus RE. Are imprinting disorders more prevalent after human in vitro fertilization or intracytoplasmic sperm injection? Fertil Steril. 2013;99(3):642-51.

125. Wen J, Jiang J, Ding C, Dai J, Liu Y, Xia Y, Liu J, Hu Z. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. Fertil Steril. 2012;97(6):1331-7.e1-4.

126. Whitelaw N, Bhattacharya S, Hoad G, Horgan GW, Hamilton M, Haggarty P. Epigenetic status in the offspring of spontaneous and assisted conception. Hum Reprod. 2014;29(7):1452-8.

127. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed. Cambridge: Cambridge University Press; 2010).

128. Wilding M, Coppola G, di Matteo L, Palagiano A, Fusco E, Dale B. Intracytoplasmic injection of morphologically selected spermatozoa (IMSI) improves outcome after assisted reproduction by deselecting physiologically poor quality spermatozoa. *J Assist Reprod Genet*. 2010;28(3):253-62.

129. Wong EC, Hatakeyama C, Robinson WP, Ma S.DNA methylation at H19/IGF2 ICR1 in the placenta of pregnancies conceived by in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril. 2011 Jun 30;95(8):2524-6.e1-3.

130. Worrilow KC, Eid S, Woodhouse D, et al. Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): significant improvement in clinical outcomes—multicenter, double-blinded and randomized controlled trial. *Hum Reprod.* 2013; **28**: 306-314.

131. Wyns C, Vandermonde J, Pirard C, Demylle D, Vanabelle B, Donnez J. IVF and ICSI outcome in couples with unexplained infertility: a randomized study of 60 cases. J Bras Reprod Assist 2004;8:16–24.

132. Yanagida K, Katayose H, Yazawa H, Kimura Y, Sato A, Yanagimachi H, Yanagimachi R. Successful fertilization and pregnancy following ICSI and electrical oocyte activation, Human Reproduction 14 (1999) 1307–1311.

133. Yang D, Shahata MA, Al-Bader M, Al-Natsha SD, Al-Flamerzia M, Al-Shawaf T. Intracytoplasmic sperm injection improving embryo quality: comparison of the sibling oocyte of non-male-factor couples. J Assist Reprod Genet 1996;13:351–5.

134. Yoeli R, Orvieto R, Ashkenazi J, Shelef M, Ben-Rafael Z, Bar-Hava I. Comparison of embryo quality between intracytoplasmic sperm injection and in vitro fertilization in sibling oocytes. *J Assist Reprod Genet*. 2008;25(1):23-8.

135. Zagadailov P, Hsu A, Seifer DB, Stern JE. Differences in utilization of Intracytoplasmic sperm injection (ICSI) within human services (HHS) regions and metropolitan megaregions in the U.S.Reproductive Biology and Endocrinology 2017;15:45.