

POLICY AND PRACTICE

The use of ICSI in ART: Evidence for practice

Virginia N Bolton^a, Marta Jansa Perez^b, George Hughes^c, Therishnee Moodley^d, Morven Dean^e, Alejandro Fernandez-Ponce^e, George Southall-Brown^f and Jason Kasraie^f on behalf of the Executive Committees of the Association of Reproductive and Clinical Scientists and the British Fertility Society.

^aKing's College London, UK; ^bBritish Pregnancy Advisory Service Fertility, Chalton Street, London, UK; ^cAssisted Conception Unit, Ninewells Hospital, Dundee, UK; ^dThe Centre for Reproductive Medicine, St. Bartholomew's Hospital, London, UK; ^eHewitt Fertility Centre, Liverpool Women's Hospital, Liverpool, UK; ^fUniversity of Chester and University Centre Shrewsbury, Chester, UK

CONTACT: Virginia N Bolton; virginia.bolton@kcl.ac.uk

ABSTRACT

This article reviews the evidence regarding the safety and efficacy of intra-cytoplasmic sperm injection (ICSI) in order to provide evidence-based clinical and laboratory guidelines and recommendations for use of ICSI within an assisted reproductive technology (ART) service. The guidelines will address the evidence for the use of ICSI rather than conventional IVF insemination; the use of ART techniques supplementary to ICSI; and risks associated with ICSI. This article is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs and medical history of the patient, available resources, and institutional or clinical practice limitations. The Executive Committees of the British Fertility Society (BFS) and Association of Reproductive and Clinical Scientists (ARCS) have approved this report. It was reviewed by members of BFS and ARCS and their input was considered in the preparation of the final document. This article will not consider specific details concerning technical aspects of the ICSI procedure.

Keywords: ICSI, IVF, male factor infertility, unexplained infertility, fertilisation

Introduction

Subfertility affects 15% of heterosexual couples globally, but estimates of the contribution of male factor subfertility to this overall figure vary widely, from 7.5% to 70% of couples (Agarwal et al., 2015). Differences between countries, in the definition of male factor subfertility, and the scarcity of population-based reports make it difficult to establish an accurate estimate, but it is generally accepted that a male factor is implicated in around 50% of subfertility, with 20-30% of cases being exclusively due to male factor(s) (Agarwal et al., 2015).

Intracytoplasmic sperm injection (ICSI) is a laboratory technique for the management of male factor subfertility that aims to assist fertilisation using micromanipulation. A single spermatozoon is selected, immobilised and injected into a mature oocyte from which the surrounding cumulus cells have been removed. Successful pregnancy following ICSI was first described in 1992 (Palermo et al., 1992) and the technique was rapidly incorporated into routine ART practice worldwide, offering hope to a sub-group of men who would otherwise have little or no chance of achieving a pregnancy using their own gametes. The degree to which ICSI has revolutionised the treatment of severe male factor infertility cannot be overstated, and more than 5 million children have been born globally as a result of the technique (Adamson et al., 2018).

ICSI is now the accepted management for men with oligoasthenoteratozoospermia (OAT), a condition that includes oligozoospermia (low number of sperm), asthenozoospermia (poor sperm movement) and teratozoospermia (abnormal sperm shape), and with azoospermia, whether obstructive or non-obstructive. ICSI using surgically retrieved spermatozoa is currently the only available intervention for azoospermic men hoping to father their own

genetic offspring; in this sub-group, provided live spermatozoa can be retrieved from either the epididymis or the testes, ICSI provides the possibility of achieving fertilisation *in vitro*, and of pregnancy following transfer of resulting embryo(s). However, while ICSI was developed specifically for the management of male factor subfertility, there are numerous reports of its use where a male factor has not been identified. The potential risks associated with an invasive technique that requires *in vitro* manipulation of gametes (Retzloff & Hornstein, 2003), together with both the additional expense and concern that ICSI may be associated with an increase in the incidence of congenital malformations in resulting offspring (Lacamara et al., 2017; Esteves et al., 2018; Luke et al., 2021) make it essential to establish whether or not the use of ICSI, rather than conventional IVF, carries any benefit in cases where there is no identified male factor that is likely to impair fertilisation (Bodri et al., 2015).

As an ART procedure, ICSI is more labour-intensive and technically challenging than conventional IVF and its cost is reported to be 8.3 % higher in four Dutch centres (Bouwman et al., 2008). The additional cost is frequently borne by fertility patients. A decision to offer ICSI rather than conventional IVF in a given treatment cycle must be evidence-based, and ultimately rests with appropriately qualified Clinical Scientists (clinical embryologists/andrologists).

Current Guidelines

In the UK, the recognised indications for ICSI as listed in the National Institute for Health and Care Excellence (NICE) Guidelines are:

- Severe deficits in semen quality
- Obstructive azoospermia
- Non-obstructive azoospermia

In addition, NICE guidance recognises that treatment using ICSI should be considered for patients in whom a previous IVF treatment cycle has resulted in failed or very poor fertilisation, and recommends that “*couples should be informed that ICSI improves fertilisation rates compared to IVF alone, but once fertilisation is achieved the pregnancy rate is no better than with IVF*” (NICE, 2004).

The fertility sector in the UK is regulated by the Human Fertilisation and Embryology Authority (HFEA), whose 9th Code of Practice (2019; updated in 2021) states that before treatment using ICSI is offered, centres should provide specific information about its risks including the risks of:

- *a reduced number of eggs being available for treatment (compared to IVF), due to eggs being immature or damaged by the process of ICSI*
- *children conceived having inherited genetic, epigenetic or chromosomal abnormalities (including cystic fibrosis gene mutations, imprinting disorders, sex chromosome defects and heritable sub-fertility).*

While the HFEA makes no specific recommendations with respect to clinical criteria for selecting ICSI rather than conventional IVF in ART treatment, it does require that the reason(s) for performing ICSI should be recorded in the patient’s medical notes.

In 2020, the American Society for Reproductive Medicine (ASRM) advised that:

- *ICSI without male factor subfertility may be of benefit for selected patients undergoing IVF with preimplantation genetic testing for monogenic disease and previously cryopreserved oocytes.*

- *The additional cost burden of ICSI for non–male factor indications, where data on improved live-birth outcomes over conventional insemination are limited or absent, must be considered.*

The guidelines presented here have been drafted according to levels of evidence as outlined by NICE (2004) (Table 1).

Trends in the use of ICSI

Despite an absence of conclusive evidence to support its use in ART treatment other than for the management of severe male factor subfertility, or where there has been previous fertilisation failure, ICSI is currently used globally more frequently than conventional IVF; in some countries it is used exclusively in place of IVF for all treatment indications (Dyer et al., 2016). The observed rise in the use of ICSI is neither consistent internationally, nor reflects the reported rise in the incidence of male factor subfertility (Andersen et al., 2008; Povey & Stocks, 2010). For example, in the USA, the use of ICSI between 1996 and 2012 almost doubled, from 36.4% to 76.2%, with the greatest increase found in cycles where no male factor had been identified, in which the use of ICSI increased from 15.4% to 66.9% of cycles (Boulet et al., 2015).

It is clear that the use of ICSI in couples with non-male factor subfertility may be beneficial where a previous IVF attempt resulted in low fertilisation or total fertilisation failure (Van der Westerlaken et al., 2005). However, its use in the management of couples with unexplained infertility, advanced female age, low ovarian reserve or low oocyte yield, presumably in attempts to increase fertilisation rates in these groups, has contributed to the reported progressive overall rise in the use of ICSI compared with conventional IVF (Andersen et al., 2008). This increase is taking place despite evidence that while ICSI may indeed increase fertilisation rates compared with conventional IVF in these patient groups, implantation and

pregnancy rates, and overall reproductive outcomes are not improved (Sfontouris et al., 2015; Boulet et al., 2015; Haas et al., 2021). The incidence of total fertilisation failure with ICSI remains 2% per cycle, compared with 5% after conventional IVF (Bhattacharya et al., 2001).

Numerous studies indicate that the use of ICSI in non-male factor subfertility does not yield better clinical outcomes than IVF; rather, it functions as a normaliser of fertilisation in reducing the number of cases of failed or low fertilisation (Palermo et al., 2015). Based on available evidence, ICSI is not indicated for universal use in ART (Babayev et al., 2014; Bosch et al., 2020), and the observed increase in the use of ICSI over conventional IVF is likely to be largely due to concern over the perceived risk of fertilisation failure (Abbas et al., 2020). In a substantial RCT, ICSI was found to offer no advantage in non-male factor cases (Drakopoulos et al., 2019).

Risks for offspring

Early concerns regarding risks to the foetus (Wennerholm et al., 2000; Hansen et al., 2002) and the long-term health impact on children born after ICSI (te Velde et al., 1998; Van Steirteghem et al., 2002; Tournaye, 2002; Retzliff et al., 2003; Karpman et al., 2005) persist, and questions and controversies remain concerning its use (Fauser et al., 2014; Lacamara et al., 2017; Catford et al., 2018; Luke et al., 2021). These concerns do only reflect slight increases in risk above natural or IVF conception, but until such concerns are resolved fully, use of ICSI should remain restricted to patients where evidence indicates that they are outweighed by the benefit of this intervention.

Indications for ICSI

Male factor

Male factor subfertility is defined as abnormality in one or more semen parameters or in sperm function (Zegers-Hochschild et al., 2017). The minimum levels for sperm concentration,

motility and morphology in normal semen were last standardised in the 6th edition of the World Health Organization Laboratory Manual for Examination and Processing of Human Semen (WHO, 2021), using strict criteria for the evaluation of sperm morphology. Based on the 5th centile from data collected using standardised methodologies for semen analysis (Campbell et al., 2021), parameters have been established by the WHO 6th edition that define oligozoospermia (sperm concentration $<16 \times 10^6/\text{ml}$; total number of sperm in the ejaculate $<39 \times 10^6$), asthenozoospermia ($<42\%$ total motility; $<30\%$ progressive motility) and teratozoospermia ($<4\%$ normal forms). An international consensus meeting agreed that ICSI should not be used in normozoospermic men (Cairo Consensus Workshop Group, 2020).

Abnormal semen parameters

Although historically, evidence suggests that ICSI results in increased fertilisation rates compared with conventional IVF in the management of “mild” to “moderate” semen abnormality, these terms are often ill-defined, and there is a lack of contemporary evidence comparing outcomes of ICSI and conventional IVF in such cases. Moreover, early studies reported findings from small samples, and the semen parameters used to define abnormality were inconsistent and not easily comparable with those defined by the WHO (2021). Indeed, as pointed out by the Vienna Consensus group (2017), “...*the current visual evaluation of 200 or 400 spermatozoa used in the vast majority of laboratories to assess ‘% normal forms’ has such a large uncertainty of measurement that it cannot be considered a reliable predictor for IVF success/failure for individual men. Unless determined using a more robust methodology, sperm normal forms should not be used to direct ART treatment options.*”

A meta-analysis of 8 published studies concluded that the probability of fertilisation is improved significantly with the use of ICSI compared with IVF in couples suffering from “moderate” male factor subfertility, defined as any combination of oligozoospermia,

asthenozoospermia, and/or teratozoospermia (Tournaye et al., 2002). A later study reported that fertilisation rates are improved, and the risk of total failed fertilisation is decreased when ICSI is used rather than conventional IVF in the treatment of patients with “mild” oligoasthenoteratozoospermia, defined as a sperm concentration of $5\text{--}20 \times 10^6/\text{ml}$ and/or motility of 5%–15%, and/or 2%–8% morphologically normal forms according to Kruger strict criteria (Elizur et al., 2004).

Of the semen characteristics assessed during routine diagnostic semen analysis (WHO, 2021), studies have not tended to examine the effect of each parameter in isolation. However, a high percentage of abnormal spermatozoa has long been associated with reduced fertility, although even in cases of severe teratozoospermia, fertilisation *in vitro* with conventional IVF insemination may be achieved (reviewed by Ombelet et al., 1995). A number of early studies examined the outcome of conventional IVF in cases of isolated teratozoospermia in semen samples with normal sperm concentration and motility, and reported lower limits for the percentage normal forms that could be used to indicate the need to use ICSI rather than conventional IVF insemination ranging from <4% (Kruger et al., 1988; Robinson et al., 1992) to <8% (Zollner et al., 1996). In a retrospective analysis of 2144 cycles, 68.6% of those where semen analysis showed <4% normal forms achieved the normalised median fertilisation rate for the authors’ clinic, provided sperm concentration and motility were within the normal range (Robinson et al., 1992). A further study reported that fertilisation rates of >50% were achieved in 36% of cycles using conventional IVF insemination with semen samples where <5% normal forms were present (Lundin et al., 1997). In this later study, the pregnancy rate per embryo transfer in IVF cycles where the percentage of normal forms was <5% was at least equivalent to that in cycles using conventional IVF with normal semen, confirming that fertilising capacity not necessarily impaired in this poor prognosis group (Lundin et al., 1997). A later meta-analysis concluded that isolated teratozoospermia is not associated with decreased clinical

pregnancy rates with IVF with or without the use of ICSI (Hotaling et al., 2011). However, intra- and interlaboratory variation in sperm morphology assessment makes it difficult to prescribe a universal, definitive cut-off for percentage normal forms where ICSI is indicated rather than conventional IVF (Kohn et al., 2018).

For some cases of “moderate” or “mild” male factor subfertility, it may well be that a higher fertilisation rate is achieved using ICSI compared with conventional IVF, but in the absence of a clear consensus regarding the definitions of “mild”, “moderate” and “severe” male factor, centres electing to use ICSI rather than IVF should define and justify their criteria for doing so, such as through pre-treatment assessment of the quality of “trial semen preparations” (Vienna Consensus group, 2017).

Guidance and recommendations for the use of ICSI where semen parameters are abnormal

ART laboratories should define validated centre-specific criteria for semen parameters that determine when to use ICSI rather than conventional IVF to ensure the rate of total failed fertilisation rates remains below 5% per IVF cycle. GPP

In deriving centre-specific criteria for ICSI, pre-treatment assessment of the quality of semen preparations may be used, with defined parameters in prepared samples to indicate use of ICSI. B

ICSI should be used in cases of severe male factor subfertility. B

ICSI may be considered in cases of moderate male factor subfertility. B
--

Azoospermia

It is estimated that azoospermia accounts for up to 10% of male factor subfertility. ICSI is currently the only available treatment option for azoospermia other than the use of donor semen (Practice Committee of the ASRM in collaboration with the Society for Male Reproduction and Urology, 2019). The aetiology of obstructive azoospermia (OA) may be congenital, iatrogenic, the result of surgery (vasectomy), inflammation or injury, the presence of cysts in ejaculatory ducts and prostate, or Young's syndrome (Esteves & Agarwal, 2013; Nieschlag et al., 2010). Non-obstructive azoospermia (NOA) has its origins in spermatogenic failure without obstruction (Cerván-Martín et al., 2020).

For OA and NOA, both NICE Guidelines in the UK and best practice guidelines in the US include ICSI after testicular or epididymal sperm extraction by surgical sperm retrieval (SSR) for fertility treatment and management (NICE, 2013; Schlegel et al., 2021).

Round spermatids

Following the first report of fertilisation after ICSI using round spermatids in the management of azoospermia (Fishel et al., 1997), the ASRM stated that the technique should be considered experimental (ASRM, 2008) and in several countries, including the UK, treatment with ICSI using round spermatids is currently banned. However, using sophisticated microscopy and artificial oocyte activation (AOA) for a carefully selected patient group, 14 live births have been reported following ICSI using round spermatids (Tanaka et al., 2015). The same group have reported favourable findings concerning the health and cognitive development at 2 years of age of 90 babies born following ICSI using round spermatids, concluding that there were no significant differences between this small sample of ICSI infants conceived by round spermatid injection compared to those conceived naturally (Tanaka et al., 2018). From the limited number of cases reported to date, the success rate with ICSI and round spermatid injection appears to be low (3% live births from 2657 treatment cycles; Tanaka et al., 2018).

Guidance and recommendations for the use of ICSI in azoospermia
ICSI should be used as the method of insemination, rather than conventional IVF, in all ART treatment cycles where surgically retrieved sperm are used. A
The use of round spermatids for ICSI is considered experimental and not permitted in the UK. GPP

Globozoospermia

Globozoospermia is a rare cause of male subfertility characterised by the presence of round-headed, acrosomeless spermatozoa which are incapable of sperm-oocyte fusion or binding the human zona pellucida (Aitken et al., 1990). It accounts for fewer than 0.1% of cases of male subfertility (Dam et al., 2007). In men with partial globozoospermia, the fertilisation and live birth rates following ICSI do not differ from those of the general ICSI population (Dam et al., 2012).

In men where all the sperm in the ejaculate are globozoospermic, ICSI is necessary to achieve fertilisation *in vitro*. In the absence of large studies, it is difficult to evaluate the success rate of ICSI in cases of total globozoospermia, but it is known that while fertilisation can be achieved and successful live births can result, the rates of fertilisation and successful pregnancy outcome are variable and unpredictable. In general, fertilisation, pregnancy and live birth rates are lower with ICSI in globozoospermia than in other groups of subfertile men (reviewed by Dam et al., 2007), the reasons for which are not clear. The condition can present with a wide spectrum of effects (Coutton et al., 2015a,b), and the success rates following treatment may vary even between members of the same family (Kilani et al., 2004). While it is unclear which patients with total globozoospermia will succeed following ICSI, even with the limited data

available it is clear that ICSI should be offered to all globozoospermic patients. Currently, although there are known genetic causes for this disorder (DPY19L2, SPARA16, ZPBP, and PICK1; Fesahat et al., 2020), the prognostic value of genetic assessment with regard to outcome prior to treatment using ICSI has yet to be established (Coutton et al., 2015b).

The negative correlation between globozoospermia and ICSI outcomes highlights the need for appropriate management of these patients (Fesahat et al., 2020). A number of reports suggest artificial oocyte activation (AOA) following ICSI may improve fertilisation potential in men with total globozoospermia, bypassing the requirement of the acrosome and sperm function factors responsible for oocyte activation. Reduced expression of candidate genes in sperm responsible for oocyte activation, such as PLC ζ , has been reported in men with globozoospermia compared with fertile men and may explain reduced fertilisation rates with ICSI using globozoospermic sperm in the absence of AOA (Taylor et al, 2010; Kamali-Dolat Abadi et al., 2016; Tavalae & Nasr-Esfahani, 2016). Although live births following ICSI have been achieved in globozoospermia both with and without AOA (Kilani et al., 1998; Stone et al., 2000; Shang et al., 2019; Niu et al 2020), importantly, higher fertilisation rates have been reported following ICSI with AOA, than following conventional ICSI alone (Chansel-Debordeaux et al., 2015). Therefore, it has been proposed that AOA maybe used to enhance fertilisation in globozoospermic cases (Yanagida et al., 2008). ICSI with AOA should be considered in treatment of total globozoospermia (see *artificial oocyte activation (AOA)* below).

Guidance and recommendations for the use of ICSI in globozoospermia
ICSI should be used as the method of insemination, rather than conventional IVF, in all cases of total globozoospermia A

Genetic counselling should be offered to globozoospermic men prior to treatment. C

AOA may be considered in cases of total globozoospermia. C

Sperm DNA fragmentation

Tests of sperm DNA fragmentation use different techniques and caution should be exercised in interpreting and comparing results obtained from the use of different tests. Results must be interpreted according to validated data obtained from the specific test used (WHO 2021). There is increasing evidence to suggest that sperm DNA fragmentation (SDF) significantly affects reproductive outcomes, with reported associations between elevated sperm DNA fragmentation in semen and an increased incidence of miscarriage (Robinson et al., 2012), and reduced fertilisation rates, embryo quality, and pregnancy rates with ART (Simon et al., 2010; 2011), although a systematic review found significant limitations of the evidence due to weakness of methodologies and study designs (Cissen et al., 2016). As a primary intervention, lifestyle changes that reduce the generation of oxidative stress in spermatozoa have been suggested for patients with high sperm DNA fragmentation, with the aim of reducing SDF levels prior to ART (Esteves et al., 2021; Aitken & Bakos, 2021). While acknowledging that adherence to a healthy diet could improve semen quality and fecundity, the authors of a systematic review of observational studies concluded that such studies “*may prove associations but not causation*”, and that any observed associations must be confirmed with large prospective cohort studies and well-designed RCTs (Salas-Huetos et al., 2017).

In the management of patients where SDF levels remain high, it has been suggested that ICSI should be used, with the specific selection of progressively motile and morphologically normal spermatozoa for injection, since these characteristics may be

associated with lower levels of DNA fragmentation (Belloc et al., 2014). However, others have reported no such association (Le et al., 2019), and this remains controversial.

Several meta-analyses have reported conflicting results regarding the relationship between elevated SDF and ART outcomes including miscarriage and clinical pregnancy rates (Li et al., 2006; Zhao et al., 2014; Osman et al., 2015; Zhang et al., 2015; Deng et al., 2019; Ribas-Maynou et al., 2021). While findings remain inconclusive, there is a suggestion that the negative impact of DNA fragmentation on ART outcomes may be less with ICSI compared with IVF (Simon et al., 2017; Malic Voncina et al., 2021), although ICSI does not appear to result in a reduction in miscarriage rates compared with IVF in patients with elevated SDF (Zhao et al., 2014). There is a need for well-designed RCTs, where matched patients with similarly elevated SDF levels, as assessed using the same assay, are allocated to IVF and ICSI treatment groups, to provide conclusive evidence for any benefit from the use of ICSI in the management of elevated SDF.

Since DNA fragmentation may be associated with oxidative stress in the male reproductive tract, with levels of DNA fragmentation reported to be significantly higher in ejaculated compared with testicular spermatozoa (Moskovtsev et al., 2010; Bradley et al., 2016), it has been suggested that use of testicular sperm for ICSI may benefit patients with elevated SDF (Esteves et al., 2017). While the findings of a few small-scale studies support this practice, these have largely reported outcomes in couples with previous failed ICSI cycles using ejaculated spermatozoa (Pabuccu et al., 2017; Arafa et al., 2018).

<p>Guidance and recommendations for the use of ICSI in elevated sperm DNA fragmentation levels</p>

Further studies are needed to establish whether or not the use of ICSI in normospermic patients with elevated sperm DNA fragmentation levels leads to improved outcomes compared with IVF. **GPP**

Further studies are needed to establish whether or not the use of testicular sperm should be considered in patients with elevated sperm DNA fragmentation levels if previous ART cycle(s) with ejaculated sperm have been unsuccessful. **GPP**

Other indications

Unexplained infertility

Use of ICSI as first line treatment. Unexplained infertility, thought to represent 30% of subfertility, is defined as a failure to conceive despite normal genito-urinary anatomy, patent fallopian tubes, a healthy cervix and uterus, normal ovarian and testicular function with normal semen, and normal coital frequency (O'Flynn, 2014; Zegers-Hochschild et al., 2017). In patients with well-defined unexplained infertility, the rate of total failed fertilisation is approximately 5% per cycle with conventional IVF (Bhattacharya et al., 2001), and this has prompted investigations into the use of ICSI in such patients. However, many of the studies that have examined the efficacy of ICSI in non-male factor subfertility have included female factor such as tubal disease as well as unexplained infertility.

Initially, in the absence of evidence for improved pregnancy outcomes compared with conventional IVF, ICSI was not recommended for the management of patients with unexplained infertility (ASRM, 2012).

A small RCT comparing pregnancy rates in 60 couples with unexplained infertility following treatment either with IVF or ICSI found no significant differences in implantation, clinical pregnancy or live-birth rates between the two treatment groups (Foong et al., 2006).

One prospective cohort study where sibling oocytes were inseminated either using ICSI or conventional IVF found that for 42 couples with unexplained infertility and confirmed >4% normal sperm morphology, there was no statistical difference in fertilisation rate between ICSI and IVF (Younes et al., 2019).

The majority of studies that have examined the efficacy of ICSI in the treatment of non-male factor subfertility, including female factors and unexplained infertility, suggest that ICSI offers no advantage over conventional IVF in terms of either fertilisation or clinical pregnancy rates (reviewed by Abbas et al., 2020). An early Cochrane review (van Rumste et al., 2000), updated by the same authors in 2003, attempted to compare live birth rates following ICSI or IVF in couples with non-male factor subfertility (unexplained infertility or subfertility due to tubal disease). Two studies that reported live birth rates, but that used alternation rather than randomisation to allocate treatment, reported that for men with borderline semen parameters (10-20 million spermatozoa per ml, 30-50% motility, 4-14% normal forms), fertilisation rates were significantly higher per oocyte retrieved with IVF (477/736; 64.8%) than with ICSI (400/748; 53.5%), but there were no differences between the IVF and ICSI groups in terms of pregnancy, miscarriage or live birth rates (van Rumste et al., 2000, 2003). The authors were able to identify only one RCT (415 patients, 435 treatment cycles) that reported pregnancy rate, but not live birth or miscarriage rates or other adverse outcomes such as congenital malformations (Bhattacharya et al., 2001). They concluded that taking into consideration the additional cost, complexity, possible adverse effects and long term implications of ICSI compared with IVF, for couples with non-male factor subfertility IVF, and not ICSI, should be the treatment of choice. They acknowledged that this remained an open question at that time, in the absence of research focusing on live-birth rates and adverse events.

More recently, a retrospective population-based cohort analysis of 14,693 women who underwent their first cycle of IVF or ICSI between July 2009 and June 2014 in Victoria,

Australia found no difference in the cumulative live birth rate between ICSI and conventional IVF in couples with non-male factor (unexplained and female factor) subfertility (Li et al., 2018). Similarly, an analysis of clinical pregnancy and live birth rates in all fresh stimulated IVF and ICSI treatment cycles recorded on the UK HFEA registry database between 1998 and 2016 found no evidence that ICSI is more effective than conventional IVF in the treatment of couples with subfertility where semen parameters were normal (569,605 cycles; Supramaniam et al., 2020).

Evidence that in cases of unexplained infertility there is no difference between clinical pregnancy or live birth rates following either conventional IVF or ICSI (ASRM, 2020) is strongly supported by the findings of the only RCT to examine this directly (Dang et al., 2021). A further multi-centre RCT to investigate whether ICSI is more effective than conventional IVF in patients without severe male factor subfertility is currently being undertaken in Denmark, with patient enrolment between November 2019 and December 2023 (Berntsen et al., 2021).

Use of split IVF-ICSI as first line treatment. As a form of insurance against total failed fertilisation in patients with unexplained infertility, sibling oocytes may be “split” between IVF and ICSI insemination in the first ART treatment cycle. A systematic review of 11 studies and a total of 901 couples with well-defined unexplained infertility, where sibling oocytes were randomly allocated to insemination using conventional IVF or ICSI, found marginally higher fertilisation rates, and a significantly lower risk of total failed fertilisation with ICSI than with IVF (Johnson et al., 2013). The authors concluded that in the management of well-defined unexplained infertility, it may be appropriate to use ICSI, or split IVF-ICSI inseminations in order to avoid total fertilisation failure and potentially to increase cumulative pregnancy rates. However, variations between the data sets made it impossible to undertake a meta-analysis of pregnancy outcomes (Johnson et al., 2013).

Several retrospective studies have suggested that the parameters of prepared sperm samples may be indicative of fertilisation outcomes using conventional IVF (Rhemrev et al., 2001; Repping et al., 2002; Wiser et al., 2012). This has led to the suggestion that for couples with unexplained infertility where prepared sperm samples do not meet defined criteria for IVF, it is appropriate to use ICSI, or split IVF-ICSI insemination (Wiser et al., 2012). In a study of 259 cycles of split IVF-ICSI in couples with unexplained infertility, only 8 (3%) cycles failed to achieve fertilisation in sibling oocytes inseminated with conventional IVF (Lee et al., 2017). Thus, while split IVF-ICSI insemination for unexplained infertility may reduce the risk of total fertilisation failure, evidence suggests that this strategy will “rescue” only around 3% of cycles from total fertilisation failure.

Guidance and recommendations ART treatment in couples with unexplained infertility

ICSI should not be considered as first-line therapy for unexplained infertility. A

Split IVF-ICSI inseminations should not be used as first-line therapy for couples with unexplained infertility. A
--

Further research is needed to establish whether ICSI results in better clinical outcomes than IVF in couples with unexplained infertility. GPP

Use of ICSI following total failed fertilisation with IVF

Rescue ICSI in the same ART cycle. Rescue ICSI is the process where metaphase II oocytes that have failed to fertilise following IVF insemination are injected with sperm using ICSI. One study reported a fertilisation rate of 74.6 % with rescue ICSI of 1831 unfertilised oocytes from 313 cycles in patients with complete failed fertilisation following IVF (Cao et al., 2016).

Historically, concerns that some oocytes that fail to develop pronuclei following conventional IVF insemination may have been penetrated by spermatozoa, and that rescue ICSI using these oocytes would increase the likelihood of polyploid embryos developing led to the antecedent of ARCS (ACE) and subsequently the HFEA in the UK to stipulate that oocytes which have failed to show signs of normal fertilisation after IVF or ICSI insemination must not be re-inseminated, whether by IVF or ICSI (21.4(c); HFEA Code of Practice, 9th Edition). There is a paucity of data either to support or refute this concern, although a single report found 22.6% of 509 rescue ICSI cycles undertaken between 2014 and 2019 resulted in one or more embryos with 3 pronuclei (Chen et al., 2021).

A systematic review of rescue ICSI which included 1,863 patients found an overall pregnancy rate of 14.4%, but contained insufficient data to enable an evaluation of any effect on malformation rates (Beck-Fruchter et al., 2014). A further systematic review which included 22 original studies found that implantation and clinical pregnancy rates following fresh embryo transfer after rescue ICSI were relatively low (5% and 10% respectively; Paffoni et al., 2021). However, the transfer of cryopreserved rescue ICSI embryos yielded significantly higher implantation and pregnancy rates (18% and 36% respectively), leading the authors to conclude that rescue ICSI in combination with frozen embryo transfer to overcome technical and biological issues associated with delayed fresh transfer after rescue ICSI may be beneficial (Paffoni et al., 2021).

With regard to risks to offspring, a single study which compared 233 children born after rescue ICSI with 906 children born following conventional ICSI found no differences in neonatal outcomes (Chen et al., 2014). Among the studies examined for their systematic review (Paffoni et al., 2021), fewer than 180 births deriving from fresh and frozen cycles using rescue ICSI were described, and other than 2 miscarriages due to malformations and 1 case of microtia, no relevant health problems in newborns were reported. Therefore, information to

date from the small number of births that have resulted from rescue ICSI does not suggest an increase in adverse outcomes, including malformation rates, following its application.

ICSI in the subsequent ART cycle. Total fertilisation failure after conventional IVF insemination is considered a reliable predictor of failed fertilisation in subsequent IVF cycles, with a reported recurrence rate ranging from 29% (Barlow et al., 1990) to between 45% and 70% (Roest et al., 1998). Numerous studies suggest that ICSI is beneficial in overcoming previous fertilisation failure using conventional IVF (Palermo et al., 1996; Benadiva et al., 1999), and this is reflected in the NICE guidelines which state that ICSI should be considered for couples in whom a previous IVF treatment cycle has resulted in failed or very poor fertilisation (NICE, 2004). However, it has been reported that fertilisation may be achieved in between 94% and 97% of subsequent repeat IVF insemination in such patients (Lipitz et al., 1994; Kinzer et al., 2008). Moreover, conventional IVF insemination after a cycle of fertilisation failure cycle has been shown to result in pregnancy and live birth rates equivalent to those achieved in other patients following a routine second cycle of IVF (22% compared with 24%) and with patients who had ICSI in their second cycle (24%; Kinzer et al., 2008). In many cases, total fertilisation failure is related more to a suboptimal response to ovarian stimulation than to semen parameters, and improved stimulation in a subsequent cycle may increase the chance of fertilisation with conventional IVF (Kinzer et al., 2008).

Split IVF-ICSI in the subsequent ART cycle. Following a cycle of unexplained total failed fertilisation with conventional IVF, a number of small studies have reported outcomes after splitting mature sibling oocytes between ICSI and conventional IVF insemination in the subsequent cycle (Elizur et al., 2004; van der Westerlaken et al., 2005). The findings suggest that performing ICSI on at least some of the oocytes will avoid repeated total fertilisation failure. However, the possibility should be considered that, in some instances, failed fertilisation reflects poor oocyte quality, or other sub-optimal clinical or laboratory factors,

rather than poor sperm function. Each case of unexplained total failed fertilisation following conventional IVF should be assessed as to whether or not alternative management, such as modification of ovarian stimulation in a subsequent cycle, may be more appropriate than changing the method of insemination from IVF to ICSI (Kinzer et al., 2008).

Use of ICSI following poor fertilisation with IVF. There is no consistent definition of a “poor” fertilisation rate in the literature. The Vienna Consensus on KPIs in the ART laboratory established a competency reference value of $\geq 60\%$ normal fertilisation as the accepted minimum rate after conventional IVF, with poor fertilisation defined as $< 25\%$ normal fertilisation of inseminated oocytes (ESHRE SIG/Alpha, 2017).

ICSI in the subsequent ART cycle. There is evidence from a small randomised study to support the use of ICSI as a secondary measure to avoid a repetition of a poor ($< 25\%$) fertilisation rate (Van der Westerlaken et al., 2005), but there are no data available comparing live birth outcomes after conventional IVF or ICSI treatment specifically in patients with previous poor fertilisation following IVF.

In the UK, the use of ICSI for patients with a history of poor fertilisation with IVF is endorsed by NICE guideline CG156 (NICE 2013) and by the HFEA (2019), although ‘poor’ is not defined.

Split IVF-ICSI in the subsequent cycle. An alternative strategy in cycles following poor fertilisation with IVF is to split oocytes between insemination using conventional IVF and ICSI (Elizur et al., 2004; Van der Westerlaken et al., 2005; Kinzer et al., 2008). In one retrospective study, ICSI did not significantly improve fertilisation rates in 27 couples with normal semen characteristics and low fertilisation rates ($\leq 30\%$) in previous IVF treatments, nor were differences observed in embryo cleavage rates after ICSI or conventional IVF (Elizur et al.,

2004). It can be concluded that there may be no benefit in performing split IVF-ICSI in couples with normal sperm characteristics following an IVF cycle with poor fertilisation.

Guidance and recommendations for the ART cycle following an IVF treatment cycle with total failed fertilisation or poor fertilisation

ICSI can be used as the method of insemination for patients with unexplained infertility where a previous IVF treatment cycle has resulted in total failed fertilisation. **B**

ICSI may be used as the method of insemination when a previous IVF treatment cycle has resulted in poor fertilisation, with “poor” defined as <25% of mature (MII) oocytes inseminated oocytes achieving normal fertilisation. **B**

Despite its prohibition in the UK, evidence to date does not support concerns that rescue ICSI may increase the incidence of abnormalities. Where permitted by law, rescue ICSI may be considered. **B**

Cryopreserved oocytes

Oocyte vitrification currently requires the removal of cumulus cells prior to cryopreservation which, together with vitrification itself, may alter the structure of the zona pellucida, possibly through premature cortical granule discharge, potentially resulting in inhibition of sperm penetration after conventional IVF insemination. Although there is a paucity of direct data to indicate that ICSI is required to fertilise human cryopreserved oocytes, it is known that cumulus cells serve multiple functions including mechanical entrapment of sperm and sperm capacitation (Van Soom et al., 2002), release of chemotactic factors to aid fertilisation (Oren-Benaroya et al., 2008) and mediation or promotion of fertilisation (Tanii et al., 2011). Therefore, ICSI has been adopted as the method of insemination for vitrified-warmed oocytes

(reviewed by Gook & Edgar, 2007). The ASRM have advised that ICSI without male factor subfertility may be of benefit for select patients undergoing ART with previously cryopreserved oocytes (ASRM, 2020).

Guidance and recommendations for the use of ICSI with cryopreserved oocytes
--

ICSI should be used as the method of insemination of thawed/warmed cryopreserved oocytes. C
--

In vitro matured oocytes

ICSI is sometimes used for insemination of *in vitro* matured oocytes, on the assumption that structural alterations to the zona pellucida may occur during *in vitro* maturation (IVM) thereby preventing fertilisation using conventional IVF. It has been reported that the fertilisation rate is higher using ICSI compared with IVF, for both cumulus-intact (84.1% and 56.3% respectively; n=289; p<0.01) and denuded IVM oocytes (84.5% and 39.5% respectively; n=152; p<0.01; Hwang et al., 2000). The cleavage rates for both sets of fertilised oocytes were similar.

These findings contrast with those of a second RCT carried out in patients with polycystic ovary syndrome (PCOS), where 113 sibling IVM oocytes were assigned randomly for insemination either using ICSI or IVF. No significant difference was found between the IVF or ICSI groups of IVM oocytes in terms of fertilisation, blastocyst development, or clinical pregnancy rates for fresh and cryopreserved embryo transfers between the two groups (Walls et al., 2012).

Further studies are needed to establish the recommended method for insemination of IVM oocytes.

Guidance and recommendations for the use of ICSI after <i>in vitro</i> maturation
Conventional IVF may be used as the method of insemination for cumulus intact <i>in vitro</i> matured oocytes, unless ICSI is indicated by poor semen parameters. C
Further studies are required comparing outcomes following IVF and ICSI with <i>in vitro</i> matured oocytes. GPP

Preimplantation genetic testing (PGT)

The ASRM have advised that ICSI without male factor subfertility may be of benefit for select patients undergoing ART with PGT for monogenic disease (PGT-M; ASRM, 2020). Historically, ICSI has been used for PGT-M cycles using fresh oocytes in order to ensure monospermic fertilisation and to eliminate the risk of introducing contaminating DNA from sperm that may remain attached to the zona pellucida following conventional IVF insemination. However, a recent cohort study looking at 927 patients undergoing PGT-M where IVF or ICSI was used to achieve fertilisation found no difference between the two groups in the incidence of PCR failure and/or incomplete diagnosis, and both groups showed negligible contamination with paternal DNA (Feldman et al., 2017).

In PGT for aneuploidy (PGT-A), conventional insemination (IVF) may be used where semen parameters are normal. However, in a small retrospective study to evaluate rates of euploidy, aneuploidy, and mosaicism in trophoctoderm biopsy samples following IVF (n=75) or ICSI (n=227) in PGT-A cycles using next generation sequencing (NGS), although similar rates of euploid, aneuploid, and “no result embryos” were found in both groups, higher rates of mosaicism were found in IVF embryos The authors concluded that ICSI may be preferable

to conventional IVF insemination to minimise the rate of mosaic results in NGS PGT-A cycles (Palmerola et al., 2019).

Guidance and recommendations for the use of ICSI prior to genetic testing of embryos

Conventional IVF may be used as the method of insemination for PGT-M cycles, unless ICSI is indicated by poor semen parameters. B
--

Further studies are necessary to establish whether or not ICSI is necessary to minimise the rate of mosaic results in NGS PGT-A cycles. GPP
--

Advanced maternal age

There is currently no conclusive evidence that ICSI offers any advantage over conventional IVF in women of advanced age, with the majority of studies failing to identify a difference in fertilisation or pregnancy rates in patients with normal semen parameters (Luna et al., 1998; Tannus et al., 2017; ASRM, 2012).

A retrospective study of 54 couples with non-male factor subfertility, where sibling oocytes were inseminated randomly using either ICSI (n=245) or IVF (n=259), reported an increase in the fertilisation rate and percentage of good quality embryos following ICSI compared with IVF in women aged 35-39 years; the same increases were not seen in women aged 40-45 years (Farhi et al., 2019). In a separate retrospective study of IVF and ICSI outcomes in 685 women aged ≥ 40 years with unexplained infertility, the pregnancy and live birth rates were the same in both groups, irrespective of the method of insemination (Gennarelli et al., 2019).

Guidance and recommendations for the use of ICSI for advanced maternal age
Conventional IVF should be used rather than ICSI as the method of insemination for oocytes from women of all ages, unless ICSI is indicated by poor semen parameters. A

Low oocyte yield

Current evidence does not support the use of ICSI rather than conventional IVF for low oocyte yield (ASRM, 2012). An RCT of 104 treatment cycles in 96 couples with non-male factor subfertility where ≤ 6 oocytes were retrieved reported no significant difference in fertilisation rate, embryo number or pregnancy rates whether IVF or ICSI was used for insemination (Moreno et al., 1998). These findings are supported by a recent large European multicentre retrospective analysis of outcomes in 4891 patients with non-male factor subfertility following treatment using either ICSI (n=4227) or conventional IVF (n=664) (Drakopoulos et al., 2019). In this series, fresh and cumulative live birth rates did not differ significantly for IVF and ICSI in women showing poor (1-3 oocytes), suboptimal (4-9 oocytes), normal (10-15 oocytes), and high (>15 oocytes) response to ovarian stimulation.

Guidance and recommendations for the use of ICSI for low oocyte yield
Conventional IVF should be used rather than ICSI as the method of insemination for oocytes from women with low oocyte yield, unless ICSI is indicated by poor semen parameters. A

HIV/Hepatitis B/Hepatitis C positive patients

In the treatment of patients with blood-borne viruses (BBV), perceived risks are seroconversion after treatment of the uninfected female partner where the male partner is BBV-positive,

transmission of different strains of HIV to the female partner in sero-concordant couples, vertical transmission to the baby, and infection of clinical staff (Devaux et al., 2003; Ethics Committee of the ASRM, 2015).

Although there is currently insufficient evidence to support the use of ICSI rather than conventional IVF in the treatment of patients who test positive for HIV, Hepatitis B or Hepatitis C, it has been proposed that ICSI, where each oocyte is exposed to a single spermatozoon, may pose less risk of viral transmission than conventional IVF (Wu et al., 2015). Semen washing and preparation procedures to reduce or eradicate viral load are common practice in treating sero-discordant couples where the male partner is BBV-positive (Garrido et al., 2004, 2009; Inoue et al., 2017).

A study of 741 sero-discordant couples where the male partner was HIV-positive reported no seroconversion in female partners after intra-uterine insemination (IUI; 2400 cycles), or IVF/ICSI (total 283 cycles; no details were provided concerning the number of IVF or ICSI cycles) when semen was washed prior to use in treatment. Moreover, there was no vertical transmission in the newborns in any of the treatment groups (Savasi et al., 2007). A similar, larger multi-centre retrospective analysis of outcomes in 1036 sero-discordant couples was carried out by the European CREAThE Network, which found no seroconversion in the female partners when washed semen was used for IUI (2840 cycles), IVF (107 cycles) or ICSI (394 cycles), although approximately 7% of cases were lost to follow up and no results were available for evaluation of potential vertical transmission (Bujan et al., 2007). The most recent systematic review and meta-analysis concluded that semen washing provides a safe and effective treatment prior to ART for HIV sero-discordant couples. In the studies reviewed, with a total of 11,585 ART cycles in 3994 women, there were no reported instances of seroconversion in HIV-negative women after IUI, IVF or ICSI treatment with washed sperm

from their HIV-positive partners. Among those studies that measured HIV transmission to infants, there were no reports of cases of vertical transmission (Zafer et al., 2016).

The 2021 ESHRE guideline on medically assisted reproduction (MAR) in patients with a viral infection/disease supports the use of IUI, IVF or ICSI in sero-discordant couples and when both partners test positive for hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV), and recommends specific semen preparation procedures to reduce or eradicate viral load (ESHRE, 2021).

Guidance and recommendations for treating viral positive patients
There is insufficient evidence to indicate that ICSI is necessary rather than conventional IVF in the treatment of BBV-positive patients and sero-discordant couples, unless ICSI is indicated by poor semen parameters. B

Prevention of polyspermy

The reported incidence of zygotes with three pronuclei ranges between 4% and 8% following conventional IVF (Staessen & Van Steirteghem, 1997; Macas et al., 1996; Plachot et al., 1992; Porter et al., 2003; cited in Joergensen et al., 2015), and of 6% following ICSI (Staessen & Van Steirteghem, 1997). Triploidy may arise either through polyspermic fertilisation of IVF-inseminated oocytes, or through oocyte-derived meiotic failure, which will manifest as triploidy in both IVF- and ICSI-inseminated oocytes (Porter et al., 2003).

It is not possible to establish the origin of triploidy without further investigations that are not routinely available in the clinical setting, so for couples in whom conventional IVF results in a high proportion of triploid zygotes, the use of ICSI in subsequent cycles is likely to minimise the risk of a recurrence if the origin of triploidy is polyspermy (Egozcue et al., 2002;

Jun et al., 2006). However, if triploidy following IVF originates through oocyte-derived meiotic failure, ICSI will not resolve the problem, which may recur in subsequent cycles.

Guidance and recommendations for the prevention of polyspermy
ICSI may be considered when a previous IVF cycle resulted in a high percentage of zygotes developed 3 or more pronuclei in a previous IVF cycle. GPP
Reduction in sperm insemination concentration at IVF may be considered when a previous IVF cycle resulted in a high percentage of zygotes developed 3 or more pronuclei in a previous IVF cycle. GPP

Use of frozen semen

In mouse models it has been shown that when cryopreserved sperm are used for insemination, higher fertilisation rates are achieved with ICSI than with conventional IVF (Szczygiel et al., 2002). However, there have been several retrospective studies in humans comparing outcomes following conventional IVF using cryopreserved donor sperm with those using fresh semen samples showing no significant difference in pregnancy rates (Cohen et al., 1985; Robinson et al., 1993; Le Lannou et al., 1995; Clarke et al., 1997). A retrospective study of 69 cycles of conventional IVF using cryopreserved donor sperm reported an association between poor outcomes and progressive motility of <88% in the sperm preparation used for treatment (Wang et al., 2009). Further studies are needed to establish the recommended method of fertilisation when using frozen sperm, and to define a reference value for percentage sperm motility in the prepared sample that indicates the use of ICSI rather than IVF when using cryopreserved semen.

Guidance and recommendations for the use of frozen semen

ICSI should be considered if the percentage motility of cryopreserved sperm after preparation does not meet the laboratory's criteria for IVF. **B**

ART laboratories should define validated centre-specific criteria for prepared cryopreserved sperm to determine when to use ICSI rather than conventional IVF to minimise the risk of poor or failed fertilisation. **GPP**

Further studies are required comparing outcomes of IVF and ICSI using frozen ejaculated sperm. **GPP**

ICSI-associated techniques

A number of techniques have been developed, with the aim of improving ICSI outcomes by selecting sperm with specific morphological or physiological characteristics, by visualising the meiotic spindle in the oocyte at the time of ICSI, or by attempting to activate the oocyte after sperm injection.

Intracytoplasmic Morphologically Selected Sperm Injection (IMSI)

In routine ICSI, sperm are selected for injection using microscopy with 200x – 400x magnification. For IMSI, higher magnification (>6000x) is used, enabling the selection of motile sperm according to detailed morphological assessment (Bartoov et al., 2002).

Two Cochrane reviews have concluded that current evidence from RCTs neither supports nor refutes the clinical use of IMSI rather than routine ICSI, when comparing outcomes in terms of live birth, risk of miscarriage and clinical pregnancy rate (He et al., 2018; Teixeira et al., 2020).

The significant increase in time required for IMSI procedures in comparison to routine ICSI as well as the additional expense in terms of both practitioners' time and the cost of essential microscopic equipment should be considered before selecting IMSI for routine use (Berkovitz et al., 2005).

Guidance and recommendations for the use of IMSI
There is no evidence that the use of IMSI as a method of sperm selection and insemination results in an increase in clinical pregnancy or live birth rates compared with routine ICSI. A

Physiological Intra-Cytoplasmic Sperm injection (PICSI)

PICSI enables the selection of spermatozoa according to their ability to bind to hyaluronic acid or hyaluronan, a major component of the cumulus complex surrounding the oocyte (Jakab et al., 2005). The head of a mature spermatozoon has a specific receptor that allows it to bind to hyaluronan, while immature sperm lack this hyaluronan-binding capacity (Huszar et al., 2006). Hyaluronan-selected sperm are reported to have reduced levels of DNA damage and aneuploidy (Pregl Breznik et al., 2013).

A Cochrane review comparing outcomes following conventional ICSI and PICSI found insufficient evidence to determine whether the use of sperm selected by hyaluronan binding improves live birth or pregnancy outcomes compared with the use of conventionally selected sperm (McDowell et al., 2014). A later review found that the rate of live birth, clinical pregnancy, implantation, embryo quality, fertilisation and miscarriage were similar for both ICSI and PICSI (Avalos-Duran, 2018).

A large multi-centre RCT comparing outcomes following conventional ICSI and PICSI found no significant difference in terms of live birth rates (Miller et al., 2019), although a

reduced risk of miscarriage was identified (Kirkman-Brown et al., 2019), and the age-related increase in miscarriage rate (after clinical pregnancy) was mitigated by PICSI (West et al., 2022).

As with IMSI, PICSI takes longer than routine ICSI, together with the need to use relatively expensive, commercially prepared PICSI dishes for sperm selection means that the cost of the procedure is significantly higher than that of routine ICSI.

Guidance and recommendations for the use of PICSI
There is no evidence that PICSI results in an increase in live birth rates compared with routine ICSI. A
Further studies are required to establish whether PICSI could be recommended where there is an increased miscarriage risk. C

Computer-assisted polarised light microscopy

The integrity of the oocyte's spindle can be assessed according to its birefringence using computer-assisted polarisation microscopy (Rienzi et al., 2005). In a study where sibling oocytes were randomly allocated to selection for ICSI according to the alignment of the meiotic spindle or to ICSI without selection, fertilisation and embryo morphology was improved in the spindle-aligned group (Asa et al., 2017), but a similar study found no significant difference between the selected and unselected oocyte groups in terms of clinical pregnancy rates (Swiatecka et al., 2014). Other groups have reported increased fertilisation potential, embryo development and implantation and clinical pregnancy rates in embryos derived from selected oocytes where the meiotic spindle had been visualized at the time of ICSI (Wang et al., 2001; Madaschi et al., 2008, 2009).

A meta-analysis which included 10 controlled trials concluded that while fertilisation rates were higher when oocytes were selected for injection using meiotic spindle visualisation than with routine ICSI using unselected oocytes, the clinical pregnancy and implantation rates were not statistically different (Petersen et al., 2009).

As with PICSI and IMSI, there are considerable additional risks associated with meiotic spindle visualisation and additional costs in terms of equipment and practitioners' time.

Guidance and recommendations for the use of ICSI with spindle visualisation
There is no evidence to indicate that ICSI with oocyte selection by meiotic spindle visualisation yields an increase in clinical pregnancy and implantation rates compared with routine ICSI using unselected oocytes. A

Artificial oocyte activation (AOA)

The failure of some mature oocytes to fertilise following ICSI may be associated with sperm-associated oocyte activation deficiency. On penetration of the oocyte, phospholipase C-zeta (PLC ζ) from functional sperm triggers the periodic release of Ca²⁺ from intracellular reserves resulting in the cytosolic calcium (Ca²⁺) oscillations associated with oocyte activation. PLC ζ , and other stimulants such as calcium ionophore A23187, ionomycin, strontium chloride or even electric pulses have been used to produce cytosolic Ca²⁺ influx and activate oocytes artificially. There are concerns that AOA may lead to an increased risk of malformations, and further studies are required to establish whether or not AOA is an effective and safe method to use in attempts to overcome ICSI failure (Anifandis et al., 2019).

A meta-analysis of published reports of the use of calcium ionophore in AOA concluded that rates of fertilisation, cleavage, blastulation, and implantation, as well as overall pregnancy and live-birth rates, were significantly higher with the use of calcium ionophore for AOA after ICSI compared with no activation (Murugesu et al., 2017). However, until studies have been carried out that examine any potential epigenetic consequences or differences in gene expression associated with AOA, its use remains experimental (Ebner & Montag, 2016).

An RCT has compared outcomes following ICSI with or without AOA in 343 couples with primary infertility and 2 previous ICSI treatment cycles with low (<30%) or no fertilisation and/or with male factor subfertility. The findings indicated that AOA using either SrCl₂ (n=115) or calcimycin (n=113) resulted in improved clinical pregnancy and live birth rates compared with ICSI without AOA (n=115; Fawzy et al., 2018).

Guidance and recommendations for the use of ICSI with artificial oocyte activation

AOA should not be used routinely with ICSI as its safety, in terms of the potential developmental consequences and birth outcomes, has yet to be established. A
--

ICSI with AOA may be used where two previous routine ICSI cycle(s) have resulted in <30% or no fertilisation. A
--

Where AOA is used, patients should be advised that safety, in terms of the potential developmental consequences and birth outcomes, has not been established. GPP
--

Patients should be provided with safety data relating to the specific AOA technique used. GPP
--

Methods for selecting viable sperm from immotile samples

For samples where all the spermatozoa are immotile, whether ejaculated (total asthenozoospermia), testicular or epididymal, identifying live sperm for use in ICSI is

challenging. Reviews of the functional tests that are available and may be used for the identification and selection of viable sperm for ICSI from an immotile population agree that for most, larger series are needed to evaluate fully each method and their indications before considering their wider application (Herbement & Sifer, 2015; Simopoulou et al., 2016; Vaughan & Sakkas, 2019).

Modified Hypo Osmotic Swelling Test (HOST)

The hypo osmotic sperm swelling test (HOST) was introduced as a functional assay for infertility diagnosis (Jeyendran et al., 1984). It distinguishes between live and dead immotile spermatozoa through exposure to hypo-osmotic conditions. Water influx into the cytoplasm of live sperm through the functioning plasma membrane results in differing tail swelling patterns, distinguishing them from dead sperm with no tail swelling. HOST may be used for the identification and selection of live sperm for use in ICSI from a population of immotile forms.

In an RCT comparing fertilisation and clinical pregnancy rates when immotile testicular sperm were selected for use in ICSI according to either HOST or morphology showed that rates of both were significantly higher using the HOST compared with morphological assessment for sperm selection (Sallam et al., 2005). According to the WHO (2021), HOST can be used in cases of total asthenozoospermia in order to select viable spermatozoa for ICSI. To date, no studies have evaluated the effect on the children born following ICSI and sperm selection using HOST.

Motility stimulants

Early comparisons of different chemicals for their ability to enhance sperm motility (Hammit et al., 1989) led to the first clinical use of pentoxifylline (PF) as an initiator of motility in severe male factor subfertility (Yovich et al., 1988) and in immotile testicular sperm prior to ICSI (Tasdemir et al., 1998). PF and its fellow methylxanthine derivative theophylline are

phosphodiesterase inhibitors and increase levels of intracellular cyclic AMP which plays a role in sperm motility. Both are now commonly used with sperm samples with no or only occasional motile sperm to reduce significantly the length of time spent performing ICSI with such samples. A retrospective comparison of outcomes following ICSI in 77 cases where all the testicular sperm retrieved after SSR were immotile found that for the 47 cycles where supplementation with PF was used, some sperm became motile allowing easier identification of vital sperm, shortening the length of time taken for ICSI, yielding improved fertilisation rates and an increase in the number of embryos available for use in treatment compared with those without use of PF (Kovacic et al., 2006).

Despite concerns regarding the safety of use of these chemical compounds for sperm motility enhancement and ICSI there is, as yet, no evidence of an increase in adverse outcomes for offspring. No anomalies were found in the offspring following PF treatment in two case reports where live births were reported (Hattori et al., 2011; Ebner et al., 2014). A review of 122 babies born after ICSI with sperm selection following motility enhancement with PF found no evidence of an increase in adverse outcomes, including malformation rates, following the use of PF (Navas et al., 2017). However, to date there have been no large follow-up studies of pregnancy and neonatal outcomes after use of methylxanthine motility enhancers reported.

Laser assisted immotile sperm selection (LAISS)

Laser assisted immotile sperm selection (LAISS) targets the tips of immotile sperm with a laser beam of approximately 200 μ J and an irradiation time of about 2 ms (Aktan et al., 2004). The thermal effect of the laser causes a localised increase in temperature resulting in changes to the sperm cell membrane proteins, and spermatozoa whose tails curl after the laser shot are regarded as viable and can be selected for use in ICSI. LAISS has the advantages that it can be performed with normal culture media, does not require the use of chemical reagents, is easy to

perform, the selected spermatozoa can be used directly for ICSI, and the laser energy level can be adjusted as needed. The percentage of immotile spermatozoa classified as viable by the laser test has been shown to be similar to that detected by the HOST (Aktan et al, 2004).

A small number of studies have reported the use of LAISS for the selection of viable but immotile spermatozoa for ICSI (Nordhoff, 2015; Simopoulou et al., 2016; Chen et al., 2019), and it appears that similar fertilisation and embryo cleavage rates can be achieved using LAISS to that with use of motile testicular spermatozoa (Chen et al., 2016). A single study of ICSI using testicular sperm found no negative effect on pregnancy rates, perinatal or neonatal outcomes after using LAISS for selection of viable immotile sperm in 33 cycles compared with 99 cycles where motile sperm were used (Chen et al., 2021).

While LAISS is an effective and rapid method of identifying viable sperm for ICSI, the requirement for expensive, specialised equipment and the lack of studies comparing its effectiveness with alternative methods for selection of viable immotile sperm mean its use has not been adopted widely.

Guidance and recommendations for the use of methods for the selection of viable sperm from a population of immotile forms
--

HOST, pentoxifylline and LAISS can be used in the absence of motile sperm for the selection of viable immotile sperm for use in ICSI. B
--

Further research is required to investigate the long-term effects on children born as a result of the use of HOST, pentoxifylline or LAISS for viable immotile sperm selection. GPP
--

Risks of ICSI

Damage to oocytes

Degeneration of oocytes after injection is a recognised risk associated with ICSI (Rubino et al., 2016), and it has been suggested that the rate of oocyte damage following ICSI should be no greater than 10% (ESHRE SIG/Alpha, 2017; Hughes, 2012). While it is possible that oocyte damage may be a consequence of the procedure itself, equally it may be a reflection of poor oocyte quality, where non-viable oocytes are more vulnerable than good quality oocytes to microinjection (Rosen et al., 2006).

Inherited male subfertility

For the 20-25% of couples where the male partner has severe male factor subfertility, there is a risk that associated genetic factors will be inherited by offspring that are conceived after ICSI (reviewed by Krausz & Riera-Escamilla, 2018). Genetic defects detected in the sperm of men with severe male factor subfertility include deletion of heterochromatin, and an increase in DNA fragmentation, telomere length and apoptosis (Loke & Craig, 2016), and genetic testing is recommended in the evaluation of the severely oligozoospermic (total sperm count $<5 \times 10^6$) or azoospermic male patient (Krausz & Riera-Escamilla, 2018). Given the increased risk of chromosomal abnormalities in these men, it is recommended that karyotyping and a Y chromosome microdeletion assay is performed (ASRM, 2015).

Couples who are contemplating ICSI treatment where Y chromosome microdeletion(s) have been identified in the male partner (Colaco & Modi, 2018) should receive appropriate genetic counselling, since a male child has 100% risk of inheriting this disorder and will require fertility treatment with ICSI if he wishes to father children in later life.

Preliminary data suggest that young men conceived following ICSI may have impaired spermatogenesis (Rumbold et al., 2019), although the mechanisms underlying this remain unclear and require confirmation through larger studies. Semen analyses in 54 young male adults conceived following ICSI found that median sperm concentration, total sperm count and

total motile sperm count were significantly lower compared to the same parameters in semen from 57 spontaneously-conceived peers (Belva et al., 2016). In a later study, the group reported that in the same ICSI 54 male offspring, the risk of sperm concentration and total sperm count below the reference values was increased, and semen parameters did not correlate in paired father-son semen analyses (Belva et al., 2019). There is a need for large studies that compare spermatogenesis and sperm quality between offspring conceived after ICSI for the treatment of male factor and non-male factor subfertility in order to clarify the nature and extent of any intergenerational impact.

Guidance and recommendations for the management of
Genetic counselling should be offered to couples who are contemplating ICSI treatment where Y chromosome microdeletion(s) have been identified in the male partner. C

Birth defects and child development

While some of the adverse outcomes that have been reported in ART offspring may be due to the increased risk of health problems in the offspring of couples pursuing ART, rather than a consequence of the ART itself (Sanchez-Calabuig, 2014), it remains difficult to distinguish between the two. Early follow-up studies of ICSI children provided preliminary reassurance that the procedure is safe, in that any slight increase identified in the incidence of neonatal malformations or de novo abnormalities observed in ICSI offspring may be related to the genetics of the subfertile couples rather than to ICSI itself. A comparison of 3372 children conceived by ICSI and 9016 children conceived naturally found a relative risk for congenital malformation of 1.44 (95% CI: 1.25–1.6) for ICSI (Loke & Craig, 2016). An analysis of 6163 IVF and ICSI births in South Australia among a total of 308,974 births in the same period concluded that the risk of birth defects associated with ICSI remained increased, although even

after multivariate adjustment the possibility of further confounding variables could not be ruled out (Davies et al., 2012).

A population-based cohort study compared ART cycles undertaken between 2004 and 2015 reported to the US Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) with a 10:1 sample of non-ART births within the same study period and concluded that ART in general is associated with increased risks of birth defects compared with natural conception (Luke et al., 2021). Risks of a major non-chromosomal birth defect, cardiovascular defect and any defect were increased in singleton children, and of chromosomal defects were increased in twins, and the use of ICSI was found to increase this risk further, with the greatest increase where a male factor was identified. Thus, compared to naturally-conceived singletons the study reported a 30% increase in the risk of a major non-chromosomal birth defect with the use of ICSI in the absence of male factor, increasing to 42% in the presence of a male factor diagnosis.

In contrast, a recent systematic review and meta-analysis found no support for any robust conclusions concerning the relative risk of chromosomal abnormalities in pregnancies and children following conventional IVF, ICSI or natural conception (Berntsen et al., 2021). No RCTs examining the risk of chromosome abnormalities in ICSI offspring were identified, all studies had a critical risk of bias, and only five studies were eligible for pooled analyses on adjusted data. These showed no evidence of an increased risk of overall chromosome abnormalities when comparing ICSI to either conventional IVF or natural conception. In contrast, meta-analyses on unadjusted data did show an increased risk of overall chromosome abnormalities in ICSI compared to both conventional IVF and natural conception, and an increased risk of *de novo* abnormalities in ICSI compared to natural conception. Despite this, based on a very low certainty of evidence, the authors concluded that no indication of an

increased risk of chromosome abnormalities in ICSI offspring could be found, and that if it does exist, the absolute risk is small (Berntsen et al., 2021).

Regarding neurodevelopmental outcomes, a national cohort study of 18-month-old naturally-conceived infants of fertile and subfertile couples found no differences in their psychomotor development, although slight delays in some cognitive/language milestones were noted in the subfertile group (Zhu et al., 2009). Among children born to subfertile couples, either naturally or following fertility treatment, those conceived following ICSI displayed the highest relative risk of delay for most milestones investigated. An earlier, smaller study of 97 ICSI, 80 IVF and 110 naturally-conceived children at 5 years of age had found no significant differences in IQ between ICSI offspring and other groups, concluding that any differences observed were more likely due to parental cognitive ability than to the mode of conception (Leslie et al., 2003). Psychological evaluation of 300 singletons conceived after ICSI found that although a higher proportion of ICSI children obtained scores below the cut-off on some of the visual-spatial tests that merited further follow-up, ICSI did not appear to be associated with effects on psychological well-being or cognitive development at age 5 (Ponjaert-Kristoffersen et al., 2004). These findings were supported tentatively by a later systematic review of 80 studies published worldwide (Bay et al., 2013), and a more recent systematic review of 24 studies concluded that cognitive and motor performance are comparable between ICSI and naturally-conceived offspring, explaining any differences that had been reported as being due to limitations in methodology (Catford et al., 2018). Although an association has been reported between ICSI and autism (Sandin et al., 2013; Kissin et al., 2015), causation has not been established, and when confounding factors such as multiple gestation are taken into account the reported association is not supported (Barad et al., 2015).

Many of the reports to date have attempted to draw comparisons between ICSI outcomes with those of spontaneous conceptions in fertile women, rather than couples who

have experienced subfertility and either conceived spontaneously or following ART treatment without the use of ICSI (Esteves et al., 2018).

Imprinting disorders. Some male subfertility may be associated with epigenetic defects arising during of spermatogenesis, and include failure to complete de-methylation of maternal imprints, failure to establish fully paternal methylation imprints, variation in the protamine levels and increased histone retention in sperm (reviewed by McSwiggin & O'Doherty, 2018). A comparison of DNA methylation between 174 naturally-conceived and 20 ICSI-conceived twins revealed significant differences in DNA methylation at the H19/IGF2 ICR (Loke et al., 2015). In contrast, a study comparing X-inactivation in newborn placentas from 60 ICSI-conceived, 72 IVF-conceived and 52 naturally-conceived females, found no difference in DNA methylation patterns between the three groups (Wu et al., 2014).

Despite the undoubted links between some male subfertility and abnormal DNA methylation, and the limited evidence suggesting that epigenetic defects may be more severe in ICSI offspring than in those conceived after conventional IVF, whether the differences in DNA methylations seen in ICSI offspring are driven specifically by ICSI, or are simply associated with elements of male subfertility remains unclear (Loke & Craig, 2016).

Long term health outcomes

Few studies have investigated the long-term health outcomes of ICSI offspring and a careful consideration of confounding variables is needed. Significant confounders include parental age, socio-economic status, and educational level. A contributing factor to early reports of increased pregnancy complications is likely to have been the transfer of multiple embryos resulting in multiple pregnancies (Fauser et al., 2014). Attempts to assess the general physical health of children born from ICSI have reported issues as diverse as significantly increased

incidences of surgical interventions (Bonduelle et al., 2004), cryptorchidism, hospital admissions and urogenital surgeries (Ludwig et al., 2009; Bonduelle et al., 2005) and in blood pressure (Belva et al., 2007) compared with spontaneously conceived children. Conversely, another study found no difference (Knoester et al., 2007). The current published expert consensus suggests no difference in the medical health of ICSI children when matched to age and gender matched controls in the fertile population (Fauser et al., 2014).

The suggestions that, compared to spontaneously conceived children, ICSI-conceived children may be prone to adiposity (an investigation of 217 ICSI-conceived singletons; Belva et al, 2012), and exhibit altered insulin resistance and differences in cortisol levels (an investigation of 42 ICSI-conceived children; Gkourogianni et al, 2014) require substantiation.

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.

Provision of information and informed consent

It is the responsibility of ART clinics to obtain informed consent from patients prior to the commencement of treatment to ensure that patients fully understand its nature, purpose and implications. Clinic staff should establish that patients understand the choices available for their treatment, including the choice of method of insemination of the eggs collected, as well as their consequences, risks and, where applicable costs (HFEA Code of Practice). The laboratory criteria for the insemination method recommended should be discussed with patients prior to consent.

The recommendation to undertake ICSI rather than conventional IVF during ART treatment rests primarily with Clinical Scientist specialists in clinical embryology, and should be based on semen parameters assessed according to methods described in the most recent World Health Organisation laboratory manual (WHO, 2021). Each ART clinic should establish

its own criteria, set out in the clinic's protocols, to stipulate when IVF or ICSI insemination should be used, and the rationale for using ICSI in individual cases should be detailed in the patients' medical records. Any decision to change the proposed and consented insemination method at any point during treatment should be made with patients' informed consent following discussion with appropriately qualified and trained personnel.

<p>Guidance and recommendations for counselling and taking consent for patients embarking on ICSI treatment</p>
--

<p>Clinics should provide patients with appropriate information to enable them to provide informed consent regarding the use of ICSI in their fertility treatment. GPP</p>

<p>Appropriate patient information should include any cost implications of the use of ICSI. GPP</p>
--

Guidance and recommendations

*ART laboratories should define validated centre-specific criteria for semen parameters that determine when to use ICSI rather than conventional IVF to ensure the rate of total failed fertilisation rates remains below 5% per IVF cycle. **GPP***

*In deriving centre-specific criteria for ICSI, pre-treatment assessment of the quality of semen preparations may be used, with <90% progressive motility in prepared samples as a threshold for considering use of ICSI. **B***

*ICSI should be used in cases of severe male factor subfertility. **A***

*ICSI may be considered in cases of moderate male factor subfertility. **B***

ICSI should be used as the method of insemination, rather than conventional IVF, in all ART treatment cycles where surgically retrieved sperm are used. A

*The use of round spermatids for ICSI is considered experimental and not permitted in the UK. **GPP***

ICSI should be used as the method of insemination, rather than conventional IVF, in all cases of total globozoospermia. A

Genetic counselling should be offered to globozoospermic men prior to treatment. C

AOA may be considered in cases of total globozoospermia. C

*Further studies are needed to establish whether or not the use of ICSI in normospermic patients with elevated sperm DNA fragmentation levels leads to improved outcomes compared with IVF. **GPP***

*Further studies are needed to establish whether or not the use of testicular sperm should be considered in patients with elevated sperm DNA fragmentation levels if previous ART cycle(s) with ejaculated sperm have been unsuccessful. **GPP***

ICSI should not be considered as first-line therapy for unexplained infertility. A

Split IVF-ICSI inseminations should not be used as first-line therapy for couples with unexplained infertility. A

*Further research is needed to establish whether ICSI results in better clinical outcomes than IVF in couples with unexplained infertility. **GPP***

ICSI can be used as the method of insemination for patients with unexplained infertility where a previous IVF treatment cycle has resulted in total failed fertilisation. B

*ICSI may be used as the method of insemination when a previous IVF treatment cycle has resulted in poor fertilisation, with “poor” defined as <25% of inseminated oocytes achieving normal fertilisation. **B***

*Despite its prohibition in the UK, evidence to date does not support concerns that rescue ICSI may increase the incidence of abnormalities. Where permitted by law, rescue ICSI may be considered. **B***

*ICSI should be used as the method of insemination of thawed/warmed cryopreserved oocytes. **C***

*Conventional IVF may be used as the method of insemination for cumulus intact in vitro matured oocytes, unless ICSI is indicated by poor semen parameters. **C***

*Further studies are required comparing outcomes following IVF and ICSI with in vitro matured oocytes. **GPP***

*Conventional IVF may be used as the method of insemination for PGT-M cycles, unless ICSI is indicated by poor semen parameters. **B***

*Further studies are necessary to establish whether or not ICSI is necessary to minimise the rate of mosaic results in NGS PGT-A cycles. **GPP***

*Conventional IVF should be used rather than ICSI as the method of insemination for oocytes from women of all ages, unless ICSI is indicated by poor semen parameters. **A***

*Conventional IVF should be used rather than ICSI as the method of insemination for oocytes from women with low oocyte yield, unless ICSI is indicated by poor semen parameters. **A***

*There is insufficient evidence to indicate that ICSI is necessary rather than conventional IVF in the treatment of BBV-positive patients and sero-discordant couples, unless ICSI is indicated by poor semen parameters. **B***

*ICSI may be considered when a previous IVF cycle resulted in a high percentage of zygotes developed 3 or more pronuclei in a previous IVF cycle. **GPP***

*Reduction in sperm insemination concentration at IVF may be considered when a previous IVF cycle resulted in a high percentage of zygotes developed 3 or more pronuclei in a previous IVF cycle. **GPP***

*ICSI should be considered if the percentage motility of cryopreserved sperm after preparation does not meet the laboratory's criteria for IVF. **B***

*ART laboratories should define validated centre-specific criteria for prepared cryopreserved sperm to determine when to use ICSI rather than conventional IVF to minimise the risk of poor or failed fertilisation. **GPP***

*Further studies are required comparing outcomes of IVF and ICSI using frozen ejaculated sperm. **GPP***

*There is no evidence that the use of IMSI as a method of sperm selection and insemination results in an increase in clinical pregnancy or live birth rates compared with routine ICSI. **A***

*There is no evidence that PICSi results in an increase in live birth rates compared with routine ICSI. **A***

*Further studies are required to establish whether PICSi could be recommended where there is an increased miscarriage risk. **C***

*There is no evidence to indicate that ICSI with oocyte selection by meiotic spindle visualisation yields an increase in clinical pregnancy and implantation rates compared with routine ICSI using unselected oocytes. **A***

*AOA should not be used routinely with ICSI as its safety, in terms of the potential developmental consequences and birth outcomes, has yet to be established. **A***

ICSI with AOA may be used where two previous routine ICSI cycle(s) have resulted in <30% or no fertilisation. A

*Where AOA is used, patients should be advised that safety, in terms of the potential developmental consequences and birth outcomes, has not been established. **GPP***

Patients should be provided with safety data relating to the specific AOA technique used.

GPP

*HOST, pentoxifylline and LAISS can be used in the absence of motile sperm for the selection of viable immotile sperm selection for use in ICSI. **B***

*Further research is required to investigate the long-term effects on children born as a result of the use of HOST, pentoxifylline or LAISS for viable immotile sperm selection. **GPP***

*Genetic counselling should be offered to couples who are contemplating ICSI treatment where Y chromosome microdeletion(s) have been identified in the male partner. **C***

*Clinics should provide patients with appropriate information to enable them to provide informed consent regarding the use of ICSI in their fertility treatment. **GPP***

*Appropriate patient information should include any cost implications of the use of ICSI. **GPP***

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article. The scope of the article was prepared and authors identified by MJP and GH. Evidence was critically appraised and graded by authors for each section. The overall paper was compiled by VNB and MJP with GH and JK providing critical input. The final paper including the evidence grading was approved by all the authors with any disagreements resolved through discussion with final decision made by VNB, MJP, GH and JK.

References

- Abbas, A. M., Hussein, R. S., Elsenity, M. A., Samaha, I. I., El Etriby, K. A., Abd El-Ghany, M. F., Khalifa, M. A., Abdelrheem, S. S., Ahmed, A. A., & Khodry, M. M. (2020). Higher clinical pregnancy rate with in-vitro fertilization versus intracytoplasmic sperm injection in treatment of non-male factor infertility: Systematic review and meta-analysis. *Journal of Gynecology Obstetrics and Human Reproduction* 49(6), 101706.
<https://doi.org/10.1016/j.jogoh.2020.10170>
- Adamson, G. D., de Mouzon, J., Chambers, G. M., Zegers-Hochschild, F., Mansour, R., Ishihara, O., Banker, M., & Dyer, S. (2018). International Committee for Monitoring Assisted Reproductive Technology: world report on assisted reproductive technology, 2011. *Fertility and Sterility* 110(6), 1067-1080.
<https://doi.org/10.1016/j.fertnstert.2018.06.039>
- Agarwal, A., Mulgund, A., Hamada, A., & Chyatte, M. R. (2015). A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology* 13(37).
<https://doi.org/10.1186/s12958-015-0032-1>
- Aitken, R.J., Kerr, L., Bolton, V., & Hargreave, T. (1990). Analysis of sperm function in globozoospermia: implications for the mechanism of sperm-zona interaction. *Fertility and Sterility* 54(4), 701-707. [https://doi.org/10.1016/s0015-0282\(16\)53833-3](https://doi.org/10.1016/s0015-0282(16)53833-3)
- Aitken, R. J., & Bakos, H. W. (2021). Should we be measuring DNA damage in human spermatozoa? New light on an old question. *Human Reproduction* 36(5), 1175-1185.
<https://doi.org/10.1093/humrep/deab004>
- Aktan, T. M., Montag, M., Duman, S., Gorkemli, H., & Yurdakul, T. (2004). Use of a laser to detect viable but immotile spermatozoa. *Andrologia* 36(6), 366–369.

<https://doi.org/10.1111/j.1439-0272.2004.00636.x>

American Society for Reproductive Medicine (2008). Intracytoplasmic sperm injection (ICSI). *Fertil Steril* 90 (5 Suppl), S187. <https://doi.org/10.1016/j.fertnstert.2008.08.045>

American Society of Reproductive Medicine and Society of Assisted Reproductive Technology (2012). Intracytoplasmic sperm injection (ICSI) for non male factor infertility: a committee opinion. *Fertility and Sterility* 98(6), 1395-1399.

<https://doi.org/10.1016/j.fertnstert.2012.08.026>

American Society of Reproductive Medicine and Society of Assisted Reproductive Technology (2020). Intracytoplasmic sperm injection (ICSI) for non-male factor infertility: a committee opinion. *Fertility and Sterility* 114(2), 239-245.

<https://doi.org/10.1016/j.fertnstert.2020.05.032>

American Society for Reproductive Medicine (2015) Diagnostic evaluation of the infertile male: a committee opinion. *Fertility and Sterility* 103(3), e18-25.

<https://doi.org/10.1016/j.fertnstert.2014.12.103>

American Society for Reproductive Medicine in collaboration with the Society for Male Reproduction and Urology. (2019). The management of obstructive azoospermia: a committee opinion. *Fertility and Sterility* 111(5), 873-880.

<https://doi.org/10.1016/j.fertnstert.2019.02.013>

American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. *Fertility and Sterility* 99(1), 37-43. <https://doi.org/10.1016/j.fertnstert.2012.09.028>

Andersen, A. N., Carlsen, E., & Loft, A. (2008). Trends in the use of intracytoplasmic sperm injection marked variability between countries. *Human Reproduction Update* 14(6), 593-604. <https://doi.org/10.1093/humupd/dmn032>

Anifandis, G., Michopoulos, A., Daponte, A., Chatzimeletiou, K., Simopoulou, M., Messin,i

- C. I., Polyzos, N. P., Vassiou, K., Dafopoulos, K., & Goulis, D. G. (2019). Artificial oocyte activation: physiological, pathophysiological and ethical aspects. *Systems Biology in Reproductive Medicine* 65(1), 3-22. <https://doi.org/10.1080/19396368.2018.1516000>
- Arafa. M., AlMalki, A. AlBadr, M., Burjaq, H., Majzoub, A., AlSaid, S., & Elbardisi, H. (2018). ICSI outcome in patients with high DNA fragmentation: Testicular versus ejaculated spermatozoa. *Andrologia* 50(1). <https://doi.org/10.1111/and.12835>
- Asa, I., Tabatabaee, R., Farrokhi, A., & Nejatbakhsh, R. (2017). Relationship between meiotic spindles visualization and intracytoplasmic sperm injection outcomes in human oocytes. *Anatomy and Cell Biology* 50(1), 26–32. <https://doi.org/10.5115/acb.2017.50.1.26>
- Avalos-Durán, G., Cañedo-Del Ángel, A. M. E., Rivero-Murillo, J., Zambrano-Guerrero, J. E., Carballo-Mondragón, E., & Checa-Vizcaíno, M. Á. (2018). Physiological ICSI (PICSI) vs. Conventional ICSI in Couples with Male Factor: A Systematic Review. *JBRA Assisted Reproduction* 22(2), 139-147. <https://doi.org/10.5935/1518-0557.20180027>
- Aydin, T., Yücel, B., Sofikerim, M., Karadag, M., & Tokat, F. (2016). Azoospermia Type Does Not Affect Intracytoplasmic Sperm Injection Results When Microdissection Testicular Sperm Extraction Is Performed. *Journal of Reproductive Medicine* 61(11-12), 581–584.
- Babayev, S. N., Park, C. W., & Bukulmez, O. (2014). Intracytoplasmic sperm injection indications: how rigorous? *Seminars in Reproductive Medicine* 32(4), 283-290. <https://doi.org/10.1055/s-0034-1375180>
- Barad, D. H., Kushnir, V. A., Albertini, D., & Gleicher, N. (2015). CDC analysis of ICSI/autism: association is not causation. *Human Reproduction* 30(7), 1745-1746. <https://doi.org/10.1093/humrep/dev104>
- Barlow, P., Englert, Y., Puissant, F., Lejeune, B., Delvigne, A., Van Rysselberge, M., & Leroy, F. (1990). Fertilization failure in IVF: why and what next? *Human Reproduction*

- 5(4), 451-456. <https://doi.org/10.1093/oxfordjournals.humrep.a137122>
- Barratt, C. L. R., Björndahl, L., De Jonge, C. J., Lamb, D. J., Martini, F. O., McLachlan, R., Oates, R. D., van der Poel, S., John, B. S., Sigman, M., Sokol, R., & Tournaye, H. (2017). The diagnosis of male infertility: An analysis of the evidence to support the development of global WHO guidance-challenges and future research opportunities. *Human Reproduction Update* 23(6), 660–680. <https://doi.org/10.1093/humupd/dmx021>
- Bartoov, B., Berkovitz, A., Eltes, F., Kogosowski, A., Menezo, Y., & Barak, Y. (2002). Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. *Journal of Andrology* 23(1), 1-8. <https://doi.org/10.1002/j.1939-4640.2002.tb02595.x>
- Bay, B., Mortensen, E. L., & Kesmodel, U. S. (2013). Assisted reproduction and child neurodevelopmental outcomes: a systematic review. *Fertility and Sterility* 100(3), 844-853. <https://doi.org/10.1016/j.fertnstert.2013.05.034>
- Beck-Fruchter, R., Lavee, M., Weiss, A., Geslevich, Y., & Shalev, E. (2014). Rescue intracytoplasmic sperm injection: a systematic review. *Fertility and Sterility* 101(3), 690-698. <https://doi.org/10.1016/j.fertnstert.2013.12.004>
- Belloc, S., Benkhalifa, M., Cohen-Bacrie, M., Dalleac, A., Chahine, H., Amar, E., & Zini, A. (2014). Which isolated sperm abnormality is most related to sperm DNA damage in men presenting for infertility evaluation. *Journal of Assisted Reproduction and Genetics* 31(5), 527-532. <https://doi.org/10.1007/s10815-014-0194-3>
- Belva, F., Bonduelle, M., Roelants, M., Michielsen, D., Van Steirteghem, A., Verheyen, G., & Tournaye, H. (2016). Semen quality of young adult ICSI offspring: the first results. *Human Reproduction* 31(12), 2811-2820. <https://doi.org/10.1093/humrep/dew245>. [Epub 2016 Oct 5](#)
- Belva, F., Bonduelle, M., & Tournaye, H. (2019). Endocrine and reproductive profile of boys and young adults conceived after ICSI. *Current Opinions in Obstetrics and Gynecology*

31(3), 163-169. <https://doi.org/10.1097/GCO.0000000000000538>

Belva, F., Henriët, S., Liebaers, I., Van Steirteghem, A., Celestin-Westreich, S., & Bonduelle, M. (2007). Medical outcome of 8-year-old singleton ICSI children (born ≥ 32 weeks' gestation) and a spontaneously conceived comparison group. *Human Reproduction* 22(2), 506–515. <https://doi.org/10.1093/humrep/del372>

Belva, F., Painter, R.C., Schiettecatte, J., Bonduelle, M., Roelants, M., Roseboom, T. J., Tournaye, H., & De Schepper, J. (2013). Gender-specific alterations in salivary cortisol levels in pubertal intracytoplasmic sperm injection offspring. *Hormone Research in Paediatrics* 80(5), 350-355. <https://doi.org/10.1159/000355515>

Benadiva, C. A., Nulsen, J., Siano, L., Jennings, J., Bet Givargis, H., & Maier D. (1999). Intracytoplasmic sperm injection overcomes previous fertilization failure with conventional in vitro fertilization. *Fertility and Sterility* 72(6), 1041-1044. [https://doi.org/10.1016/s0015-0282\(99\)00403-3](https://doi.org/10.1016/s0015-0282(99)00403-3)

Berkovitz, A., Eltes, F., Yaari, S., Katz, N., Barr, I., Fishman, A., & Bartoov, B. (2005). The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm. *Human Reproduction* 20(1), 185-190. <https://doi.org/10.1093/humrep/deh545>

Berntsen, S., Nøhr, B., Grøndahl, M. L., Petersen, M. R., Andersen, L. F., Englund, A. L., Knudsen, U. B., Prætorius, L., Zedeler, A., Nielsen, H. S., Pinborg, A., & Freiesleben, N. C. (2021). In vitro fertilisation (IVF) versus intracytoplasmic sperm injection (ICSI) in patients without severe male factor infertility: study protocol for the randomised, controlled, multicentre trial INVICSI. *British Medical Journal Open* 11(6), e051058. <https://doi.org/10.1136/bmjopen-2021-051058>

Berntsen, S., Laivuori, H., la Cour Freiesleben, N., Loft, A., Söderström-Anttila, V., Oldereid N. B., Romundstad, L. B., Magnusson, Å., Petzold, M., Bergh, C., & Pinborg, A. (2021).

A systematic review and meta-analysis on the association between ICSI and chromosome abnormalities. *Human Reproduction Update* 27(5), 801-847.

<https://doi.org/10.1093/humupd/dmab005>

Bhattacharya, S., Hamilton, M., Shaaban, M., Khalaf, Y., Seddler, M., Ghobara, T., Braude, P., Kennedy, R., Rutherford, A., Hartshorne, G., & Templeton, A. (2001). Conventional invitro fertilization versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomized controlled trial. *Lancet* 357(9274), 2075-2079.

[https://doi.org/10.1016/s0140-6736\(00\)05179-5B](https://doi.org/10.1016/s0140-6736(00)05179-5B)

Bodri, D., Sugimoto, T., Yao Serna, J., Kondo, M., Kato, R., Kawachiva, S., & Matsumoto, T. (2015). Influence of different oocyte insemination techniques on early and late morphokinetic parameters: retrospective analysis of 500 time-lapse monitored blastocysts. *Fertility and Sterility* 104(5), 1175-1181.e1-2.

<https://doi.org/10.1016/j.fertnstert.2015.07.1164>

Bonduelle, M., Bergh, C., Niklasson, A., Palermo, G.D., & Wennerholm, U. B., Collaborative Study Group of Brussels, Gothenburg and New York (2004). Medical follow-up study of 5-year-old ICSI children. *Reproductive BioMedicine Online* 9(1), 91–101. [https://doi.org/10.1016/s1472-6483\(10\)62116-5](https://doi.org/10.1016/s1472-6483(10)62116-5)

Bonduelle, M., Wennerholm, U. B., Loft, A., Tarlatzis, B. C., Peters, C., Henriët, S., Mau, C., Victorin-Cederquist, A., Van Steirteghem, A., Balaska, A., Emberson, J. R., & Sutcliffe, A. G (2005). A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Human Reproduction* 20(2), 413–419. <https://doi.org/10.1093/humrep/deh592>

Bosch, E., Espinós, J. J., Fabregues, F., Fontes, J., García-Velasco, J., Llácer, J., Requena, A., Checa, M. A., & Bellver, J., Spanish Infertility SWOT Group (SISG) (2020). ALWAYS ICSI? A SWOT analysis. *Journal of Assisted Reproduction and Genetics* 37(9), 2081-

2092. <https://doi.org/10.1007/s10815-020-01836-0>
- Boulet, S. L., Mehta, A., Kissin, D. M., Warner, L., Kawwass, J. F., & Jamieson, D. J. (2015). Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. *JAMA* 313(3), 255-263. <https://doi.org/10.1001/jama.2014.17985>
- Bouwman, C. A., Lintsen, B. M., Eijkemans, M. J., Habbema, J. D., Braat, D. D., & Hakkaart, L. (2008). A detailed cost analysis of in vitro fertilization and intracytoplasmic sperm injection treatment. *Fertility and Sterility* 89(2), 331-341. <https://doi.org/10.1016/j.fertnstert.2007.03.003>
- Bradley, C. K., McArthur, S. J., Gee, A. J., Weiss, K. A., Schmidt, U., & Toogood, L. (2016). Intervention improves assisted conception intracytoplasmic sperm injection outcomes for patients with high levels of sperm DNA fragmentation: a retrospective analysis. *Andrology* 4(5), 903-910. <https://doi.org/10.1111/andr.12215>
- Bujan, L., Hollander, L., Coudert, M., Gilling-Smith, C., Vucetich, A., Guibert, J., Vernazza, P., Ohl, J., Weigel, M., Englert, Y., & Semprini, A.E. (2007). Safety and efficacy of sperm washing in HIV-1-serodiscordant couples where the male is infected: Results from the European CREAThE network. *AIDS* 21(14), 1909–1914. <https://doi.org/10.1097/QAD.0b013e3282703879>
- Bungum, L., Bungum, M., Humaidan, P., & Andersen, C. (2004). A strategy for treatment of couples with unexplained infertility who failed to conceive after intrauterine insemination. *Reprod Biomed Online* 8: 584–589. [https://doi.org/10.1016/s1472-6483\(10\)61107-8](https://doi.org/10.1016/s1472-6483(10)61107-8)
- Cairo Consensus and Workshop Group (2020). The current status and future of andrology: A consensus report from the Cairo workshop group. *Andrology* 8(5), 27-52. <https://doi.org/10.1111/andr.12720>
- Campbell, M. J., Lotti, F., Baldi, E., Schlatt, S., Festin, M. P., Bjorndahl, L., Toskin, I., & Barratt C. L. R. (2021). Distribution of Semen Examination Results 2020 - a follow up of

- data collated for the WHO semen analysis manual 2010. *Andrology* 9(3), 817-822.
<https://doi.org/10.1111/andr.12983>
- Cao, S., Wu, X., Zhao, C., Zhou, L., Zhang, J., & Ling, X. (2016). Determining the need for rescue intracytoplasmic sperm injection in partial fertilisation failure during a conventional IVF cycle. *Andrologia* 48(10), 1138-1144. <https://doi.org/10.1111/and.12551>
- Carlsen, E., Giwercman, A., Keiding, N., & Skakkebaek, N. E. (1992). Evidence for decreasing quality of semen during past 50 years. *British Medical Journal* 305(6854), 609–613. <https://doi.org/10.1097/00006254-199303000-00023>
- Catford, S. R., McLachlan, R. I., O'Bryan, M. K., & Halliday, J. L. (2018). Long-term follow-up of ICSI-conceived offspring compared with spontaneously conceived offspring: a systematic review of health outcomes beyond the neonatal period. *Andrology* 6(5), 635-653. <https://doi.org/10.1111/andr.12526>
- Cerván-Martín, M., Castilla, J. A., Palomino-Morales, R. J., & Carmona, F. D. (2020). Genetic Landscape of Nonobstructive Azoospermia and New Perspectives for the Clinic. *Journal of Clinical Medicine* 9(2), 300. <https://doi.org/10.3390/jcm9020300>
- Chansel-Debordeaux, L., Dandieu, S., Bechoua, S., & Jimenez, C. (2015). Reproductive outcome in globozoospermic men: update and prospects. *Andrology* 3(6), 1022-1034. <https://doi.org/10.1111/andr.12081>
- Chen, H. H., Feng, G. X., Zhang, B., Zhou, H., Wang, C. Z., Shu, J. H., Gan, X., Lin, R., Huang, D., & Huang, Y. (2017). A new insight into male fertility preservation for patients with completely immotile spermatozoa. *Reproductive Biology and Endocrinology* 15(1), 74. <https://doi.org/10.1186/s12958-017-0294-x>
- Chen, H. H., Zhou, H., Shu, J. H., Gan, X. Y., Wang, C. Z., Lin, R. Y. (2019). A point of confusion for embryologists in the identification of viable spermatozoa by the eosin-nigrosin test. *Clinical and Experimental Reproductive Medicine* 46(1), 36–40.

<https://doi.org/10.5653/cerm.2019.46.1.36>

- Chen, H. H., Wang, C., Zhou, H., Shu, J., Gan, X., Xu, K., Wu, Z., Deng, X., Huang, G., & Lin, R. (2021). Laser-assisted selection of immotile spermatozoa has no effect on obstetric and neonatal outcomes of TESA-ICSI pregnancies. *Reproductive Biology and Endocrinology* 19(1), 159. <https://doi.org/10.1186/s12958-021-00835-9>
- Chen, L., Xu, Z., Zhang, N., Wang, B., Chen, H., Wang, S., & Sun, H. (2014). Neonatal outcome of early rescue ICSI and ICSI with ejaculated sperm. *Journal of Assisted Reproduction and Genetics* 31(7), 823-828. <https://doi.org/10.1007/s10815-014-0245-9>
- Chen, W., Bai, H., Li, M., Xue, X., & Shi, J. (2021). Effects of three pro-nuclei (3PN) incidence on laboratory and clinical outcomes after early rescue intracytoplasmic sperm injection (rescue-ICSI): an analysis of a 5-year period. *Gynecological Endocrinology* 37(2), 137-140. <https://doi.org/10.1080/09513590.2020.1757640>
- Cissen, M., van Wely, M., Scholten, I., Mansell, S., de Bruin, J. P., Mol, B. W., Braat, D., Repping, S., & Hamer, G. (2016). Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: a systematic review and meta-analysis. *PLoS One* 11(11), e0165125. <https://doi.org/10.1371/journal.pone.0165125>
- Clarke, G. N., Bourne, H., Hill, P., Johnston, W. I., Speirs, A., McBain, J. C. & Baker, H. W. (1997). Artificial insemination and in-vitro fertilization using donor spermatozoa: a report on 15 years of experience. *Human Reproduction* 12(4), 722-726. <https://doi.org/10.1093/humrep/12.4.722>
- Cohen, J., Edwards, R. G., Fehilly, C. B., Fishel, S. B., Hewitt, J., Rowland, G. F., Steptoe, P. C., Walters, D. E., & Webster, J. (1985). In vitro fertilization using cryopreserved donor semen in cases where both partners are infertile. *Fertility and Sterility* 43(4), 570-574. [https://doi.org/10.1016/s0015-0282\(16\)48499-2](https://doi.org/10.1016/s0015-0282(16)48499-2)
- Colaco, S., & Modi, D. (2018). Genetics of the human Y chromosome and its association

- with male infertility. *Reproductive Biology and Endocrinology* 16(1), 14.
<https://doi.org/10.1186/s12958-018-0330-5>
- Coutton, C., Arnoult, C., & Ray, P. F. (2015). Comprehensive investigation in patients affected by globozoospermia. *Andrology* 3(6), 1183. <https://doi.org/10.1111/andr.12096>
- Coutton, C., Escoffier, J., Martinez, G., Arnoult, C., & Ray, P. F. (2015). Teratozoospermia: spotlight on the main genetic actors in the human. *Human Reproduction Update* 21(4), 455-485. <https://doi.org/10.1093/humupd/dmv020>
- Dam, A. H., Feenstra, I., Westphal, J. R., Ramos, L., van Golde, R. J., & Kremer, J. A. (2007). Globozoospermia revisited. *Human Reproduction Update* 13(1), 63-75.
<https://doi.org/10.1093/humupd/dml047>
- Dam, A. H. D. M., Pijnenburg, A. J. E., Hendriks, J. C. M., Westphal, H., Ramos, L., & Kremer, J. A. M. (2012). Intracytoplasmic sperm injection in partial globozoospermia. *Fertility and Sterility* 97(1), 60-66. <https://doi.org/10.1016/j.fertnstert.2011.10.003>
- Dang, V. Q., Vuong, L. N., Luu, T. M., Pham, T., Ho, T. M., Ha, A. N., Truong, B. T., Phan, A. K., Nguven, D. P., Pham, T. N., Pham, Q. T., Wang, R., Norman, R. J., & Mol, B. W. (2021). Intracytoplasmic sperm injection versus conventional in-vitro fertilisation in couples with infertility in whom the male partner has normal total sperm count and motility: an open-label, randomised controlled trial. *Lancet* 397(10284), 1554-1563.
[https://doi.org/10.1016/s0140-6736\(21\)00535-3](https://doi.org/10.1016/s0140-6736(21)00535-3)
- Davies, M. J., Moore, V. M., Wilson, K. J., Van Essen, P., Priest, K., Scott, H., Haan, E. A., & Chan, A. (2012). Reproductive technologies and the risk of birth defects. *New England Journal of Medicine* 366(19), 1803-1813. <https://doi.org/10.1056/NEJMoa1008095>
- Deng, C., Li, T., Xie, Y., Guo, Y., Yang, Q. Y., Liang, X., Deng, C. H., & Liu, G. H. (2019). Sperm DNA fragmentation index influences assisted reproductive technology outcome: A systematic review and meta-analysis combined with a retrospective cohort study.

Andrologia 51(6), e13263. <https://doi.org/10.1111/and.13263>

Drakopoulos, P., Garcia-Velasco, J., Bosch, E., Blockeel, C., de Vos, M., Santos-Ribeiro, S., Makrigiannakis, A., Tournaye, H., & Polyzos, N. P. (2019). ICSI does not offer any benefit over conventional IVF across different ovarian response categories in non-male factor infertility: a European multicenter analysis. *Journal of Assisted Reproduction and Genetics* 36(10), 2067-2076. <https://doi.org/10.1007/s10815-019-01563-1>

Dyer, S., Chambers, G. M., de Mouzon, J., Nygren, K. G., Zegers-Hochschild, F., Mansour, R., Ishihara, O., Banker, M., & Adamson, G.D. (2016). International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010. *Human Reproduction* 31(7), 1588-1609. <https://doi.org/10.1093/humrep/dew082>

Ebner, T., Shebl, O., Mayer, R. B., Moser, M., Costamoling, W., & Oppelt, P. (2014). Healthy live birth using theophylline in a case of retrograde ejaculation and absolute asthenozoospermia. *Fertility and Sterility* 101(2), 340–3. <https://doi.org/10.1016/j.fertnstert.2013.10.006>

Ebner, T., & Montag, M. (2016). Artificial oocyte activation: evidence for clinical readiness. *Reproductive BioMedicine Online* 32(3), 271-273. <https://doi.org/10.1016/j.rbmo.2015.12.004>

Egozcue, S., Blanco, J., Vidal, F., & Egozcue, J. (2002). Diploid sperm and the origin of triploidy. *Human Reproduction* 17(1), 5-7. <https://doi.org/10.1093/humrep/17.1.5>

Elizur, S. E., Levron, J., Seidman, D. S., Kees, S., Levran, D., & Dor, J. (2004). Conventional in vitro fertilization versus intracytoplasmic sperm injection for sibling oocytes in couples with mild oligoteratoasthenozoospermia and couples with normal sperm. *Fertility and Sterility* 82(1), 241-243. <https://doi.org/10.1016/j.fertnstert.2003.11.053>

ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive

- Medicine (2017). The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators. *Reproductive BioMedicine Online* 35(5), 494-510. <https://doi.org/10.1016/j.rbmo.2017.06.015>
- ESHRE (2021). Guideline for Medically assisted reproduction in patients with a viral infection or disease. *Human Reproduction Open*. <https://doi.org/10.1093/hropen/hoab037>
- Esteves, S. C., & Agarwal, A. (2013). Reproductive outcomes, including neonatal data, following sperm injection in men with obstructive and nonobstructive azoospermia: case series and systematic review. *Clinics (Sao Paulo)* 68(Suppl 1), 141–150. [https://doi.org/10.6061/clinics/2013\(sup01\)16](https://doi.org/10.6061/clinics/2013(sup01)16)
- Esteves, S. C., Roque, M., & Agarwal, A. (2016). Outcome of assisted reproductive technology in men with treated and untreated varicocele: Systematic review and meta-analysis. *Asian Journal of Andrology* 18(2), 254–258. <https://doi.org/10.4103/1008-682X.163269>
- Esteves, S. C., Roque, M., Bradley, C. K., & Garrido, N. (2017). Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis. *Fertility and Sterility* 108(3), 456-467. <https://doi.org/10.1016/j.fertnstert.2017.06.018>
- Esteves, S. C., Roque, M., Bedoschi, G., Haahr, T., & Humaidan, P. (2018). Intracytoplasmic sperm injection for male infertility and consequences for offspring. *Nature Reviews Urology* 15(9), 535-562. <https://doi.org/10.1038/s41585-018-0051-8>
- Esteves, S. C., Zini, A., Coward, R. M., Evenson, D. P., Gosálvez, J., Lewis, S. E. M., Sharma, R., & Humaidan, P. (2021). Sperm DNA fragmentation testing: Summary evidence and clinical practice recommendations. *Andrologia* 53(2), e13874. <https://doi.org/10.1111/and.13874>
- Ethics committee of the American Society for Reproductive Medicine (2015). Human

- immunodeficiency virus (HIV) and infertility treatment: A committee opinion. *Fertility and Sterility* 104(1), e1–e8. <https://doi.org/10.1016/j.fertnstert.2015.04.004>
- Fesahat, F., Henkel, R., & Agarwal, A. (2020). Globozoospermia syndrome: An update. *Andrologia*. 52(2), e13459. <https://doi.org/10.1111/and.13459>
- Farhi, J., Cohen, K., Mizrachi, Y., Weissman, A., Raziel, A., & Orvieto, R. (2019). Should ICSI be implemented during IVF to all advanced-age patients with non-male factor subfertility? *Reproductive Biology and Endocrinology* 17(1), 30. <https://doi.org/10.1186/s12958-019-0474-y>
- Fausser, B. C., Devroey, P., Diedrich, K., Balaban, B., Bonduelle, M., Delemarre-van de Waal, H. A., Estella, C., Ezcurra, D., Geraedts, J. P., Howles, C. M., Lerner-Geva, L., Serna, J., & Wells, D., Evian Annual Reproduction (EVAR) Workshop Group 2011. (2014). Health outcomes of children born out after IVF/ICSI: A review of current expert opinion and literature. *Reproductive BioMedicine Online* 28(2), 162-182. <https://doi.org/10.1016/j.rbmo.2013.10.013>
- Fawzy, M., Emad, M., Mahran, A., Sabry, M., Fetih, A. N., Abdelghafar, H., & Rasheed, S. (2018). Artificial oocyte activation with SrCl₂ or calcimycin after ICSI improves clinical and embryological outcomes compared with ICSI alone: results of a randomized clinical trial. *Human Reproduction* 33(9), 1636-1644. <https://doi.org/10.1093/humrep/dey258>
- Feldman, B., Aizer, A., Brengauze, M., Dotan, K., Levron, J., Schiff, E., & Orvieto, R. (2017). Pre-implantation genetic diagnosis- should we use ICSI for all? *Journal of Assisted Reproduction and Genetics* 34(9), 1179-1183. <https://doi.org/10.1007/s10815-017-0966-7>
- Fesahat, F., Henkel, R., & Agarwal, A. (2020). Globozoospermia syndrome: An update. *Andrologia* 52(2): e13459. <https://doi.org/10.1111/and.13459>
- Fishel, S., Green, S., Hunter, A., Lisi, F., Rinaldi, L., Lisi, R., & McDermott, H. (1997).

- Human fertilization with round and elongated spermatids. *Human Reproduction* 12(2), 336-340. <https://doi.org/10.1093/humrep/12.2.336>
- Foong, S. C., Fleetham, J. A., O'Keane, J. A., Scott, S. G., Tough, S. C., & Greene, C. A. (2006). A prospective randomized trial of conventional in vitro fertilization versus intracytoplasmic sperm injection in unexplained infertility. *Journal of Assisted Reproduction and Genetics* 23(3), 137–140. <https://doi.org/10.1007/s10815-005-9008-y>
- Gennarelli, G., Carosso, A., Canosa, S., Filippini, C., Cesarano, S., Scarafia, C., Brunod, N., Revelli, A., & Benedetto, C. (2019). ICSI Versus Conventional IVF in Women Aged 40 Years or More and Unexplained Infertility: A Retrospective Evaluation of 685 Cycles with Propensity Score Model. *Journal of Clinical Medicine* 8(10), 1694. <https://doi.org/10.3390/jcm8101694>
- Garrido, N., Gil-Salom, M., Martínez-Jabaloyas, J. M., & Meseguer, M. (2009). First report of the absence of viral load in testicular sperm samples obtained from men with hepatitis C and HIV after washing and their subsequent use. *Fertility and Sterility* 92(3), 1012–1015. <https://doi.org/10.1016/j.fertnstert.2009.01.159>
- Garrido, N., Meseguer, M., Bellver, J., Remohí, J., Simón, C., & Pellicer, A. (2004). Report of the results of a 2 year programme of sperm wash and ICSI treatment for human immunodeficiency virus and hepatitis C virus serodiscordant couples. *Human Reproduction* 19(11), 2581–2586. <https://doi.org/10.1093/humrep/deh460>
- Ghanem, M., Bakr, N. I., Elgayaar, M. A., El Mongy, S., Fathy, H., & Ibrahim, A. H. (2005). Comparison of the outcome of intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia in the first cycle: a report of case series and meta-analysis. *International Journal of Andrology* 28(1), 16–21. <https://doi.org/10.1111/j.1365-2605.2005.00491.x>
- Gkourogianni, A., Kosteria, I., Telonis, A. G., Margeli, A., Mantzou, E., Konsta, M.,

- Loutradis, D., Mastorakos, G., Papassotiriou, I., Klapa, M. I., Kanaka-Gantenbein, C., & Chrousos, G. P. (2014). Plasma metabolomic profiling suggests early indications for predisposition to latent insulin resistance in children conceived by ICSI. *PloS One* 9(4), e94001. <https://doi.org/10.1371/journal.pone.0094001>
- Goldman, M. B., Thornton, K. L., Ryley, D., Alper, M. M., Fung, J. L., Hornstein, M. D., & Reindollar, R. H. (2014). A randomized clinical trial to determine optimal infertility treatment in older couples: the Forty and Over Treatment Trial (FORT-T). *Fertility and Sterility* 101(6), 1572–1574. <https://doi.org/10.1016/j.fertnstert.2014.03.012>
- Gook, D. A., Edgar, D. H. (2007). Human oocyte cryopreservation. *Human Reproduction Update* 13(6), 591-605. <https://doi.org/10.1093/humupd/dmm028>
- Guzick, D. S., Overstreet, J. W., Factor-Litvak, P., Brazil, C.K., Nakajima, S. T., Coutifaris, C., Carson, S. A., Cisneros, P., Steinkampf, M. P., Hill, J. A., Xu, D., & Vogel, D. L. (2001). Sperm morphology, motility, and concentration in fertile and infertile men. *New England Journal of Medicine* 345(19), 1388–1393. <https://doi.org/10.1056/NEJMoa003005>
- Guzick, D.S., Sullivan, M.W., Adamson, G.D., Cedars, M.I., Falk, R.J., Peterson, E.P., Steinkampf, M.P. (1998). Efficacy of treatment for unexplained infertility. *Fertility and Sterility* 70(2), 207–213. [https://doi.org/10.1016/s0015-0282\(98\)00177-0](https://doi.org/10.1016/s0015-0282(98)00177-0)
- Hammit, D. G., Bedia, E., Rogers, P. R., Syrop, C. H., Donovan, J. F., & Williamson, R. A. (1989). Comparison of motility stimulants for cryopreserved human semen. *Fertility and Sterility* 52(3), 495-502. [https://doi.org/10.1016/s0015-0282\(16\)60925-1](https://doi.org/10.1016/s0015-0282(16)60925-1)
- Haas, J., Miller, T. E., Nahum, R., Aizer, A., Kirshenbaum, M., Zilberberg, E., Lebovitz, O., & Orvieto, R. (2021). The role of ICSI vs. conventional IVF for patients with advanced maternal age – a randomised controlled trial. *Journal of Assisted Reproduction and Genetics* 38(1), 95-100. <https://doi.org/10.1007/s10815-020-01990-5>

- Hansen, M., Kurinczuk, J. J., Bower, C., & Webb, S. (2002). The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *New England Journal of Medicine* 346(10), 725-730. <https://doi.org/10.1056/nejmoa010035>
- Hattori, H., Nakajo, Y., Ito, C., Toyama, Y., Toshimori, K., & Kyono, K. (2011). Birth of a healthy infant after intracytoplasmic sperm injection using pentoxifylline-activated sperm from a patient with Kartagener's syndrome. *Fertility and Sterility* 95(7), 2431.e2439–2411. <https://doi.org/10.1016/j.fertnstert.2011.03.074>
- He, F., Wang, M. J., Li, S. L., Zhang, C. Y., & Hu, L. N. (2018). IMSI versus ICSI for male factor infertility: A meta-analysis. *Zhonghua Nan Ke Xue* 24(3), 254-262. (Article in Chinese)
- Hershlag, A., Paine, T., Kvapil, G., Feng, H., & Napolitano, B. (2002). In vitro fertilization intracytoplasmic sperm injection split: an insemination method to prevent fertilization failure. *Fertility and Sterility* 77(2), 229-232. [https://doi.org/10.1016/s0015-0282\(01\)02978-8](https://doi.org/10.1016/s0015-0282(01)02978-8)
- HFEA (2019). Commissioning Guidance for fertility Treatment. <https://www.hfea.gov.uk/media/2920/commissioning-guidance-may-2019-final-version.pdf>
- HFEA (2021). Code of Practice 10th Edition. <https://portal.hfea.gov.uk/media/1527/2019-12-16-code-of-practice-9th-edition-december-2019.pdf>
- Hotaling, J. M., Smith, J. F., Rosen, M., Muller, C. H., & Walsh, T.J. (2011). The relationship between isolated teratozoospermia and clinical pregnancy after in vitro fertilization with or without intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertility and Sterility* 95(3), 1141-1145. <https://doi.org/10.1016/j.fertnstert.2010.09.029>
- Hughes, C. on behalf of the Association of Clinical Embryologists (2012). Association of

- clinical embryologists - guidelines on good practice in clinical embryology laboratories
2012. *Human Fertility (Camb)* 15(4), 174-189.
<https://doi.org/10.3109/14647273.2012.747891>
- Huszar, G., Ozkavukcu, S., Jakab, A., Celik-Ozenci, C., Sati, G.L., & Cayli, S. (2006).
Hyaluronic acid binding ability of human sperm reflects cellular maturity and fertilizing
potential: selection of sperm for intracytoplasmic sperm injection. *Current Opinions in
Obstetrics and Gynecology* 18(3), 260-267.
<https://doi.org/10.1097/01.gco.0000193018.98061.2f>
- Hwang, J. L., Lin, Y. H., & Tsai, Y. L. (2000). In vitro maturation and fertilization of
immature oocytes: A comparison study of fertilization techniques. *Journal of Assisted
Reproduction and Genetics* 17(1), 39-43. <https://doi.org/10.1023/a:1009450014064>
- Ibrahim, A. A., Awad, H. A., El-Haggag, S., & Mitawi, B. A. (1977). Bilateral testicular
biopsy in men with varicocele. *Fertility and Sterility* 28(6), 663–667.
[https://doi.org/10.1016/s0015-0282\(16\)42620-8](https://doi.org/10.1016/s0015-0282(16)42620-8)
- Inoue, O., Kuji, N., Ito, H., Yamada, M., Hamatani, T., Oyadomari, A., Kato, S., Hanabusa,
H., Isaka, K., & Tanaka, M. (2017). Clinical efficacy of a combination of Percoll
continuous density gradient and swim-up techniques for semen processing in HIV-1
serodiscordant couples. *Asian Journal of Andrology* 19(2), 208–213.
<https://doi.org/10.4103/1008-682X.173442>
- Jakab, A., Sakkas, D., Delpiano, E., Cayli, S., Kovanci, E., Ward, D., Revelli, A., & Huszar,
G. (2005). Intracytoplasmic sperm injection: a novel selection method for sperm with
normal frequency of chromosomal aneuploidies. *Fertility and Sterility* 84(6), 1665-1673.
<https://doi.org/10.1016/j.fertnstert.2005.05.068>
- Jeyendran, R. S., Van der Ven, H. H., Perez-Pelaez, M., Crabo, B. G., & Zaneveld, L. J.
(1984). Development of an assay to assess the functional integrity of the human sperm

- membrane and its relationship to other semen characteristics. *Journal of Reproduction and Fertility* 70(1), 219–228. <https://doi.org/10.1530/jrf.0.0700219>
- Joergensen, M. W., Labouriau, R., Hindkjaer, J., Stougaard, M., Kolevraa, S., Bolund, L., Agerholm, I. E., & Sunde, L. (2015). The parental origin correlates with the karyotype of human embryos developing from tripronuclear zygotes. *Clinical and Experimental Reproductive Medicine* 42(1), 14-21. <https://doi.org/10.5653/cerm.2015.42.1.14>
- Johnson, L. N. C., Sasson, I. E., Sammel, M. D., & Dokras, A. (2013). 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. *Fertility and Sterility* 100(3), 704–711. <https://doi.org/10.1016/j.fertnstert.2013.04.038>
- Jun, S. H., O'Leary, T., Jackson, K. V., & Racowsky, C. (2006). Benefit of intracytoplasmic sperm injection in patients with a high incidence of triploidy in a prior in vitro fertilization cycle. *Fertility and Sterility* 86(4), 825-829. <https://doi.org/10.1016/j.fertnstert.2006.03.043>
- Kamali-Dolat Abadi, M., Tavalae, M., Shahverdi, A., & Nasr-Esfahani, M. H. (2016). Evaluation of PLC ζ and PAWP expression in globozoospermic individuals. *Cell Journal* 18(3), 438-445. <https://doi.org/10.22074/cellj.2016.4572>
- Kanto, S., Sugawara, J., Masuda, H., Sasano, H., Arai, Y., & Kyono, K. (2008). Fresh motile testicular sperm retrieved from nonobstructive azoospermic patients has the same potential to achieve fertilization and pregnancy via ICSI as sperm retrieved from obstructive azoospermic patients. *Fertility and Sterility* 90(5), 2010.e5-2010.e7. <https://doi.org/10.1016/j.fertnstert.2008.01.049>
- Karpman, E., Williams, D. H., & Lipshultz, L. I. (2005). IVF and ICSI in male infertility: update on outcomes, risks, and costs. *The Scientific World Journal* 16(5), 922-932.

<https://doi.org/10.1100/tsw.2005.117>

Kilani, Z. M., Shaban, M. A., Ghunaim, S. D., Keilani, S. S., & Dakkak, A. I (1998). Triplet pregnancy and delivery after intracytoplasmic injection of round-headed spermatozoa.

Human Reproduction 13(8), 2177-2179. <https://doi.org/10.1093/humrep/13.8.2177>

Kilani, Z., Ismail, R., Ghunaim, S., Mohamed, H., Hughes, D., Brewis, I., & Barratt, C.L.

(2004). Evaluation and treatment of familial globozoospermia in five brothers. *Fertility*

and Sterility 82(5), 1436-1439. <https://doi.org/10.1016/j.fertnstert.2004.03.064>

Kim, H. J., Yoon, H. J., Jang, J. M., Lee, W. D., Yoon, S. H., & Lim, J. H. (2017). Evaluation of human embryo development in in vitro fertilization- and intracytoplasmic sperm

injection- fertilized oocytes: A time – lapse study. *Clinical and Experimental*

Reproductive Medicine 44(2), 90-95. <https://doi.org/10.5653/cerm.2017.44.2.90>

Kinzer, D. R., Barrett, C. B., & Powers, R. D. (2008). Prognosis for clinical pregnancy and

delivery after total fertilization failure during conventional in vitro fertilization or

intracytoplasmic sperm injection. *Fertility and Sterility* 90(2), 284-288.

<https://doi.org/10.1016/j.fertnstert.2007.06.025>

Kirby, E. W., Wiener, L. E., Rajanahally, S., Crowell, K., & Coward, R. M. (2016).

Undergoing varicocele repair before assisted reproduction improves pregnancy rate and

live birth rate in azoospermic and oligospermic men with a varicocele: a systematic review

and meta-analysis. *Fertility and Sterility* 106(6), 1338–1343.

<https://doi.org/10.1016/j.fertnstert.2016.07.1093>

Kirkman-Brown, J., Pavitt, S., Khalaf, Y., Lewis, S., Hooper, R., Bhattacharya, S.,

Coomarasamy, A., Sharma, V., Brison, D., Forbes, G., West, R., Pacey, A., Brian, K.,

Cutting, R., Bolton, V., & Miller, D. (2019). Sperm selection for assisted reproduction by

prior hyaluronan binding: the HABSelect RCT. *NIHR Journals Library* Feb 2019.

<https://doi.org/10.3310/eme06010>

- Kissin, D. M., Zhang, Y., Boulet, S. L., Fountain, C., Bearman, P., Schieve, L., Yeargin-Allsopp, M., & Jamieson, D. J. (2015). Association of assisted reproductive technology (ART) treatment and parental infertility diagnosis with autism in ART-conceived children. *Human Reproduction* 30(2), 454-465. <https://doi.org/10.1093/humrep/deu338>
- Knoester, M., Vandenbroucke, J. P., Helmerhorst, F. M., van der Westerlaken, L. A., Walther, F. J., & Veen, S. (2007). Matched follow-up study of 5–8 year old ICSI-singletons: comparison of their neuromotor development to IVF and naturally conceived singletons. *Human Reproduction* 22(6), 1638-1646. <https://doi.org/10.1093/humrep/dem040>
- Kohn, T. P., Kohn, J. R., & Lamb, D. J. (2018). Role of sperm morphology in deciding between various assisted reproduction technologies. *European Urology Focus* 4(3), 311-313. <https://doi.org/10.1016/j.euf.2018.07.029>
- Kovacic, B., Vlaisavljevic, V., & Reljic, M. (2006). Clinical use of pentoxifylline for activation of immotile testicular sperm before ICSI in patients with azoospermia. *Journal of Andrology* 27(1), 45-52. <https://doi.org/10.2164/jandrol.05079>
- Krausz, C., & Riera-Escamilla, A. (2018). Genetics of male infertility. *Nature Reviews Urology* 15(6), 369-384. <https://doi.org/10.1038/s41585-018-0003-3>
- Kruger, T. F., Acosta, A. A., Simmons, K. F., Swanson, R. J., Matta, J. F., & Oehninger, S. (1988). Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertility and Sterility* 49(1), 112-117. [https://doi.org/10.1016/s0015-0282\(16\)59660-5](https://doi.org/10.1016/s0015-0282(16)59660-5)
- Lacamara, C., Ortega, C., Villa, S., Pommer, R., & Schwarze, J. E. (2017). Are children born from singleton pregnancies conceived by ICSI at increased risk for congenital malformations when compared to children conceived naturally? A systematic review and meta-analysis. *JBRA Assisted Reproduction* 21(3), 251-259. <https://doi.org/10.5935/1518-0557.20170047>

- Le, M. T., Nguyen, T. A. T., Nguyen, H. T. T., Nguyen, T. T. T., Nguyen, V. T., Le, D. D., Nguyen, V. Q. H., & Cao, N. T. (2019). Does sperm DNA fragmentation correlate with semen parameters? *Reprod Med Biol* 18(4), 390-396. <https://doi.org/10.1002/rmb2.12297>
- Le Lannou, D., Gastard, E., Guivarch, A., Laurent, M. C., & Poulain, P. (1995). Strategies in frozen donor semen procreation. *Human Reproduction* 10(7), 1765-1774. <https://doi.org/10.1093/oxfordjournals.humrep.a136171>
- Lee, S. H., Lee, J. H., Park, Y.-S., Yang, K., M. & Lim, C. K. (2017). Comparison of clinical outcomes between *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in IVF-ICSI split insemination cycles. *Clinical and Experimental Reproductive Medicine* 44(2), 96-104. <https://doi.org/10.5653/cerm.2017.44.2.96>
- Leslie, G. I., Gibson, F. L., McMahon, C., Cohen, J., Saunders, D. M., & Tennant, C. (2003). Children conceived using ICSI do not have an increased risk of delayed mental development at 5 years of age. *Human Reproduction* 18(10), 2067-2072. <https://doi.org/10.1093/humrep/deg408>
- Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Mindlis, I., Pinotti, R., & Swan, S. H. (2017). Temporal trends in sperm count: A systematic review and meta-regression analysis. *Human Reproduction Update* 23(6), 646–659. <https://doi.org/10.1093/humupd/dmx022>
- Li, Z., Wang, L., Cai, J., & Huang, H. (2006). Correlation of sperm DNA damage with IVF and ICSI outcomes: a systematic review and meta-analysis. *Journal of Assisted Reproduction and Genetics* 23(9-10), 367-376. <https://doi.org/10.1007/s10815-006-9066-9>
- Li, Z., Wang, A. Y., Bowman, M., Hammarberg, K., Farquhar, C., Johnson, L., Safi, N. & Sullivan, E. A. (2018). ICSI does not increase the cumulative live birth rate in non-male factor infertility. *Human Reproduction* 33(7), 1322-1330. <https://doi.org/10.1093/humrep/dey118>

Lipitz, S., Rabinovici, J., Goldenberg, M., Bider, D., Dor, J., & Mashiach, S. (1994).

Complete failure of fertilization in couples with mechanical infertility: implications for subsequent in vitro fertilization cycles. *Fertility and Sterility* 61(5), 863-866.

[https://doi.org/10.1016/s0015-0282\(16\)56697-7](https://doi.org/10.1016/s0015-0282(16)56697-7)

L0ke, Y. J., Galati, J. C., Saffery, R. & Craig J. M. (2015). Association of in vitro fertilization with global and IGF2/H19 methylation variation in newborn twins. *Journal of Developmental Origins of Health and Disease* 6(2), 115-24.

<https://doi.org/10.1017/S2040174415000161>

Loke, Y. J., & Craig, J. M. (2016). Are the effects of IVF on DNA methylation driven by intracytoplasmic sperm injection and male infertility? *Epigenomics* 8(7), 881-884.

<https://doi.org/10.2217/epi-2016-0041>

Ludwig, A. K., Katalinic, A., Thyen, U., Sutcliffe, A. G., Diedrich, K., & Ludwig, M.

(2009b). Physical health at 5.5 years of age of term-born singletons after intracytoplasmic sperm injection: results of a prospective, controlled, single-blinded study. *Fertility and Sterility* 91(1), 115–124. <https://doi.org/10.1016/j.fertnstert.2007.11.037>

Luke, B., Brown, M. B., Wantman, E., Forestieri, N. E., Browne, M. L., Fisher, S. C., Yazdy, M. M., Ethen, M. K., Canfield, M. A., Watkins, S., Nichols, H. B., Farland, L. V., Oehninger, S., Doody, K. J., Eisenberg, M. L., & Baker, V. L. (2021). The risk of birth defects with conception by ART. *Human Reproduction* 36(1), 116-129.

<https://doi.org/10.1093/humrep/deaa272>

Luna, M., Bigelow, C., Duke, M., Ruman, J., Sandler, B., Grunfeld, L., & Copperman, A. B.

(1998). Should ICSI be recommended routinely in patients with four or fewer oocytes retrieved? *Journal of Assisted Reproduction and Genetics* 28(10), 911-915.

<https://doi.org/10.1007/s10815-011-9614-9>

Lundin, K., Söderlund, B., & Hamberger, L. (1997). The relationship between sperm

- morphology and rates of fertilization, pregnancy and spontaneous abortion in an in-vitro fertilization/intracytoplasmic sperm injection programme. *Human Reproduction* 12(12), 2676-2681. <https://doi.org/10.1093/humrep/12.12.2676>
- Macas, E., Imthurn, B., Rosselli, M., & Keller, P. J. (1996). The chromosomal complements of multipronuclear human zygotes resulting from intracytoplasmic sperm injection. *Human Reproduction* 11(11), 2496–2501. <https://doi.org/10.1093/oxfordjournals.humrep.a019147>
- Madaschi, C., de Souza Bonetti, T. C., de Almeida Ferreira Braga, D. P., Pasqualotto, F. F., Iaconelli, A. Jr, & Borges, E. Jr (2008). Spindle imaging: a marker for embryo development and implantation. *Fertility and Sterility* 90(1), 194-198. <https://doi.org/10.1016/j.fertnstert.2007.05.071>
- Madaschi, C., Aoki, T., de Almeida Ferreira Braga, D. P., de Cássia Sávio Figueira, R., Semião Francisco, L., Iaconelli, A. Jr, & Borges, E. Jr. (2009). Zona pellucida birefringence score and meiotic spindle visualization in relation to embryo development and ICSI outcomes. *Reproductive BioMedicine Online* 18(5), 681-686. [https://doi.org/10.1016/s1472-6483\(10\)60014-4](https://doi.org/10.1016/s1472-6483(10)60014-4)
- Maheshwari, A., Muneer, A., Lucky, M., Mathur, R., & McEleny, K. (2020). A review of varicocele treatment and fertility outcomes. *Human Fertility (Camb)* July7, 1–8. <https://doi.org/10.1080/14647273.2020.1785117>
- Malic Voncina, S., Stendqvist, A., Bungum, M., Schyman, T., & Giwercman, A. (2021). Sperm DNA fragmentation index and cumulative live birth rate in a cohort of 2,713 couples undergoing assisted reproduction treatment. *Fertility and Sterility* 16(6), 1483-1490. <https://doi.org/10.1016/j.fertnstert.2021.06.049>
- Mangoli, E., Khalili, M. A., Talebi, A. R., Agha-Rahimi, A., Soleimani, M., Faramarzi, A., & Pouretezari, M. (2019). IMSI procedure improves clinical outcomes and embryo

- morphokinetics in patients with different aetiologies of male infertility. *Andrologia* 51(8), e13340. <https://doi.org/10.1111/and.13340>. Epub 2019 Jun 13
- McDowell, S., Kroon, B., Ford, E., Hook, Y., Glujovsky, D., & Yazdani, A. (2014). Advanced sperm selection techniques for assisted reproduction. *Cochrane Database of Systematic Reviews* Oct 28(10):CD010461. <https://doi.org/10.1002/14651858.CD010461.pub2>
- McSwiggin, H. M., & O'Doherty, A. M. (2018). Epigenetic reprogramming during spermatogenesis and male factor infertility. *Reproduction* 156(2), R9–R21. <https://doi.org/10.1530/REP-18-0009>
- Miller, D., Pavitt, S., Sharma, V., Forbes, G., Hooper, R., Bhattacharya, S., Kirkman-Brown, J., Coomarasamy, A., Lewis, S., Cutting, R., Brison, D., Pacey, A., West, R., Brian, K., Griffin, D., & Khalaf, Y. (2019). Physiological, hyaluronan-selected intracytoplasmic sperm injection for infertility treatment (HABSelect): a parallel, two-group, randomised trial. *Lancet* 393(10170), 416-422. [https://doi.org/10.1016/S0140-6736\(18\)32989-1](https://doi.org/10.1016/S0140-6736(18)32989-1)
- Moreno, C., Ruiz, A., Simón, C., Pellicer, A., & Remohí, J. (1998). Intracytoplasmic sperm injection as a routine indication in low responder patients. *Human Reproduction* 13(8), 2126-2129. <https://doi.org/10.1093/humrep/13.8.2126>
- Moskovtsev, S. I., Jarvi, K., Mullen, J. B., Cadesky, K. I., Hannam, T., & Lo, K. C. (2010). Testicular spermatozoa have statistically significantly lower DNA damage compared with ejaculated spermatozoa in patients with unsuccessful oral antioxidant treatment. *Fertility and Sterility* 93(4), 1142-1146. <https://doi.org/10.1016/j.fertnstert.2008.11.005>
- Murugesu, S., Saso, S., Jones, B. P., Bracewell-Milnes, T., Athanasiou, T., Mania, A., Serhal, P., & Ben-Nagi, J. (2017). Does the use of calcium ionophore during artificial oocyte activation demonstrate an effect on pregnancy rate? A meta-analysis. *Fertility and Sterility* 108(3), 468-482. <https://doi.org/10.1016/j.fertnstert.2017.06.029>

National Institute for Health and Care Excellence. (2004). Fertility problems: assessment and treatment (NICE guideline [CG 156; para 1.13]).

<https://www.nice.org.uk/guidance/cg156/chapter/Recommendations#intracytoplasmic-sperm-injection>

Navas, P., Paffoni, A., Intra, G., González-Utor, A., Clavero, A., Gonzalvo, M. C., Díaz, R., Peña, R., Restelli, L., Somigliana, E., Papaleo, E., Castilla, J. A., & Viganò, P. (2017). Obstetric and neo-natal outcomes of ICSI cycles using pentoxifylline to identify viable spermatozoa in patients with immotile spermatozoa. *Reproductive BioMedicine Online* 34(4), 414-421. <https://doi.org/10.1016/j.rbmo.2017.01.009>

Nieschlag, E., Behre, H. M., & Nieschlag, S. (Eds.). (2010). *Andrology: Male Reproductive Health and Dysfunction* (3rd ed.). Springer-Verlag Berlin Heidelberg.

<https://doi.org/10.1007/978-3-540-78355-8>

Niu, X., Ruan, Q., Witz, C. A., & Wang, W. (2020). Comparison of Human Oocyte Activation Between Round-Headed Sperm Injection Followed by Calcium Ionophore Treatment and Normal Sperm Injection in a Patient With Globozoospermia. *Frontiers in Endocrinology (Lausanne)* 11, 183. <https://doi.org/10.3389/fendo.2020.00183>

Nordhoff, V. (2015). How to select immotile but viable spermatozoa on the day of intracytoplasmic sperm injection? An embryologist's view. *Andrology* 3(2), 156–162.

<https://doi.org/10.1111/andr.286>

O'Flynn, N. (2014). Assessment and treatment for people with fertility problems: NICE guideline. *British Journal of General Practice* 64(618), 50–51.

<https://doi.org/10.3399/bjgp14X676609>

Ohlander, S., Hotaling, J., Kirshenbaum, E., Niederberger, C., & Eisenberg, M. L. (2014). Impact of fresh versus cryopreserved testicular sperm upon intracytoplasmic sperm injection pregnancy outcomes in men with azoospermia due to spermatogenic dysfunction:

a meta-analysis. *Fertility and Sterility* 101(2), 344–349.

<https://doi.org/10.1016/j.fertnstert.2013.10.012>

Ombelet, W., Menkveld, R., Kruger, T. F., & Steeno, O. (1995). Sperm morphology assessment: historical review in relation to fertility. *Human Reproduction Update* 1(6), 543-557. <https://doi.org/10.1093/humupd/1.6.543>

Oren-Benaroya, R., Orvieto, R., Gakamsky, A., Pinchasov, M., & Eisenbach, M. (2008). The sperm chemoattractant secreted from human cumulus cells is progesterone. *Human Reproduction* 23(10), 2339-2345. <https://doi.org/10.1093/humrep/den265>

Osman, A., Alsomait, H., Seshadri, S., El-Toukhy, T., & Khalaf, Y. (2015). The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. *Reproductive BioMedicine Online* 30(2), 120-127.

<https://doi.org/10.1016/j.rbmo.2014.10.018>

Pabuccu EG, Caglar GS, Tangal S, Haliloglu AH, Pabuccu R. (2017). Testicular versus ejaculated spermatozoa in ICSI cycles of normozoospermic men with high sperm DNA fragmentation and previous ART failures. *Andrologia* 49(2).

<https://doi.org/10.1111/and.12609>

Paffoni, A., Reschini, M., Pisaturo, V., Guarneri, C., Palini, S., & Viganò, P. (2021). Should rescue ICSI be re-evaluated considering the deferred transfer of cryopreserved embryos in in-vitro fertilization cycles? A systematic review and meta-analysis. *Reproductive Biology and Endocrinology* 19(1), 121. <https://doi.org/10.1186/s12958-021-00784-3>

Palermo, G., Joris, H., Devroey, P., & Van Steirteghem, A. P. (1992). Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 340(8810), 17-18. [https://doi.org/10.1016/0140-6736\(92\)92425-f](https://doi.org/10.1016/0140-6736(92)92425-f)

Palermo, G. D., Cohen, J., & Rozenwaks, Z. (1996). Intracytoplasmic sperm injection: a powerful tool to overcome fertilization failure. *Fertility and Sterility* 65(5), 899-908.

[https://doi.org/10.1016/s0015-0282\(16\)58257-0](https://doi.org/10.1016/s0015-0282(16)58257-0)

Palermo, G. D., Neri, Q. V., & Rosenwaks, Z. (2015). To ICSI or Not to ICSI. *Seminars in Reproductive Medicine* 33(2), 92-102. <https://doi.org/10.1055/s-0035-1546825>

Palmerola, K.L., Vitez, S. F., Amrane, S., Fischer, C. P., & Forman, E. J. (2019). Minimizing mosaicism: assessing the impact of fertilization method on rate of mosaicism after next-generation sequencing (NGS) preimplantation genetic testing for aneuploidy (PGT-A). *Journal of Assisted Reproduction and Genetics* 36(1), 153-157.

<https://doi.org/10.1007/s10815-018-1347-6>

Plachot, M. (2000). Fertilization. *Human Reproduction* 15 Suppl 4: 19-30.

https://doi.org/10.1093/humrep/15.suppl_4.19

Plachot, M., & Crozet, N. (1992). Fertilization abnormalities in human in-vitro fertilization. *Human Reproduction* 7(Suppl 1): 89–94. https://doi.org/10.1093/humrep/7.suppl_1.89

Ponjaert-Kristoffersen, I., Tjus, T., Nekkebroeck, J., Squires, J., Verté, D., Heimann, M., Bonduelle, M., Palermo, G., & Wennerholm, U. B. Collaborative study of Brussels, Göteborg and New York. (2004). Psychological follow-up study of 5-year-old ICSI children. *Human Reproduction* 19(12), 2791-2797.

<https://doi.org/10.1093/humrep/deh511>

Porter, R., Han, T., Tucker, M. J., Graham, J., Liebermann, J., & Scott Sills, E. (2003). Estimation of second polar body retention rate after conventional insemination and intracytoplasmic sperm injection: in vitro observations from more than 5000 human oocytes. *Journal of Assisted Reproduction and Genetics* 20(9), 371-376.

<https://doi.org/10.1023/a:1025481011680>

Povey, A. C., & Stocks, S. J. (2010) Epidemiology and trends in male subfertility. *Human Fertility (Camb)* 13(4), 182-188. <https://doi.org/10.3109/14647273.2010.515700>

Petersen, C. G., Oliveira, J. B., Mauri, A. L., Massaro, F. C., Baruffi, R. L., Pontes, A., &

- Franco, J.G. Jr. (2009). Relationship between visualization of meiotic spindle in human oocytes and ICSI outcomes: a meta-analysis. *Reproductive BioMedicine Online* 18(2), 235-243. [https://doi.org/10.1016/s1472-6483\(10\)60261-1](https://doi.org/10.1016/s1472-6483(10)60261-1)
- Pregl Breznik, B., Kovacic, B., & Vlaisavljevic, V. (2013). Are sperm DNA fragmentation, hyperactivation, and hyaluronan-binding ability predictive for fertilization and embryo development in in vitro fertilization and intracytoplasmic sperm injection? *Fertility and Sterility* 99(5), 1233-1241 <https://doi.org/10.1016/j.fertnstert.2012.11.048>
- Proctor, M., Johnson, N., Van Peperstraten, A., & Philipson, G. (2008). Techniques for surgical retrieval of sperm prior to intracytoplasmic sperm injection (ICSI) for azoospermia. *Cochrane Database of Systematic Reviews* 2008(2), CD002807. <https://doi.org/10.1002/14651858.CD002807.pub3>
- Repping, S., van Weert, J. M., Mol, B. W., de Vries, J. W., & van der Veen, F. (2002). Use of the total motile sperm count to predict total fertilization failure in in vitro fertilization. *Fertility and Sterility* 78(1), 22-28. [https://doi.org/10.1016/s0015-0282\(02\)03178-3](https://doi.org/10.1016/s0015-0282(02)03178-3)
- Retzliff, M. G., & Hornstein, M. D. (2003). Is intracytoplasmic sperm injection safe? *Fertility and Sterility* 80(4), 851-859. [https://doi.org/10.1016/s0015-0282\(03\)01014-8](https://doi.org/10.1016/s0015-0282(03)01014-8)
- Rhemrev, J. P., Lens, J. W., McDonnell, J., Schoemaker, J., & Vermeiden, J. P. (2001). The postwash total progressively motile sperm cell count is a reliable predictor of total fertilization failure during in vitro fertilization treatment. *Fertility and Sterility* 76(5), 884-891. [https://doi.org/10.1016/s0015-0282\(01\)02826-6](https://doi.org/10.1016/s0015-0282(01)02826-6)
- Ribas-Maynou, J., Yeste, M., Becerra-Tomás, N., Aston, K. I., James, E. R., & Salas-Huetos, A. (2021). Clinical implications of sperm DNA damage in IVF and ICSI: updated systematic review and meta-analysis. *Biological Reviews Cambridge Philosophical Society* 96(4), 1284-1300. <https://doi.org/10.1111/brv.12700>
- Rienzi, L., Ubaldi, F., Iacobelli, M., Minasi, M. G., Romano, S., & Greco, E. (2005). Meiotic

- spindle visualization in living human oocytes. *Reproductive BioMedicine Online* 10(2), 192-198. [https://doi.org/10.1016/s1472-6483\(10\)60940-6](https://doi.org/10.1016/s1472-6483(10)60940-6)
- Robinson, J. N., Lockwood, G. M., Dokras, A., Egan, D. M., Ross, C., & Barlow, D. H. (1993). A controlled study to assess the use of in vitro fertilization with donor semen after failed therapeutic donor insemination. *Fertility and Sterility* 59(2), 353-358. [https://doi.org/10.1016/s0015-0282\(16\)55673-8](https://doi.org/10.1016/s0015-0282(16)55673-8)
- Robinson, J. N., Lockwood, G. M., Dokras, A., Egan, D. M., Nicholson, S. C., Ross, C., & Barlow, D. H. (1992). Does isolated teratozoospermia affect performance in in-vitro fertilization and embryo transfer? *Human Reproduction* 9(5), 870-874. <https://doi.org/10.1093/oxfordjournals.humrep.a138608>
- Robinson, L., Gallos, I. D., Conner, S. J., Rajkhowa, M., Miller, D., Lewis, S., Kirkman-Brown, J., & Coomarasamy, A. (2012). The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. *Human Reproduction* 27(10), 2908-17. <https://doi.org/10.1093/humrep/des261>
- Roest, J., Van Heusden, A. M., Zeilmaker, G. H., & Verhoeff, A. (1998). Treatment policy after poor fertilization in the first IVF cycle. *Journal of Assisted Reproduction and Genetics* 15(1), 18-21. <https://doi.org/10.1023/a:1022570120207>
- Rosen, M. P., Shen, S., Dobson, A. T., Fujimoto, V. Y., McCulloch, C. E., & Cedars, M. I. (2006). Oocyte degeneration after intracytoplasmic sperm injection: a multivariate analysis to assess its importance as a laboratory or clinical marker. *Fertility and Sterility* 85(6), 1736-1743. <https://doi.org/10.1016/j.fertnstert.2005.12.017>
- Rubino, P., Vigano, P., Luddi, A., & Piomboni, P. (2016). The ICSI procedure from past to future: a systematic review of the more controversial aspects. *Human Reproduction Update* 22(2), 194-227. <https://doi.org/10.1093/humupd/dmv050>
- Rubio, I., Kuhlmann, R., Agerholm, I. Kirk J., Herrero, J., Escribá, M.J., Bellver, J., &

- Meseguer, M. (2012). Limited implantation success of direct-cleaved human zygotes: a time lapse study. *Fertility and Sterility* 98(6), 1458-1463.
<https://doi.org/10.1016/j.fertnstert.2012.07.1135>
- Ruiz, A., Remohí, J., Minguez, Y., Guanes, P. P., Simón, C., & Pellicer, A. (1997). The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. *Fertility and Sterility* 68(1), 171–173.
[https://doi.org/10.1016/s0015-0282\(97\)81497-5](https://doi.org/10.1016/s0015-0282(97)81497-5)
- Rumbold, A. R., Sevoyan, A., Oswald, T. K., Fernandez, R. C., Davies, M. J., & Moore, V. M. (2019). Impact of male factor infertility on offspring health and development. *Fertility and Sterility* 111(6), 104-1053. <https://doi.org/10.1016/j.fertnstert.2019.05.006>
- Salas-Huetos, A., Bullo, M., & Salas-Salvado, J. (2017). Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies. *Human Reproduction Update* 23(4), 371-389.
<https://doi.org/10.1093/humupd/dmx006>
- Sallam, H. N., Farrag, A., Agameya, A. F., El-Garem, Y., & Ezzeldin, F. (2005). The use of the modified hypo-osmotic swelling test for the selection of immotile testicular spermatozoa in patients treated with ICSI: a randomized controlled study. *Human Reproduction* 20(12), 3435-3440. <https://doi.org/10.1093/humrep/dei249>
- Salman Yazdi, R., Bakhshi, S., Jannat Alipoor, F., Akhoond, M. R., Borhani, S., Farrahi, F., Panah, M. L., & Gilani, M. A. S. (2014). Effect of 830-nm diode laser irradiation on human sperm motility. *Lasers in Medical Science* 29(1), 97–104.
<https://doi.org/10.1007/s10103-013-1276-7>
- Sánchez-Calabuig, M. J., Lopez-Cardona, A. P., Fernández-González, R., Ramos-Ibeas, P., Fonseca Balvís, N., Laguna-Barraza, R., Pericuesta, E., Gutiérrez-Adán, A., & Bermejo-Álvarez, P. (2014). Potential health risks associated to ICSI: insights from animal models

- and strategies for a safe procedure. *Frontiers in Public Health* 2, 241.
<https://doi.org/10.3389/fpubh.2014.00241>
- Sandin, S., Nygren, K. G., Iliadou, A., Hultman, C. M., & Reichenberg, A. (2013). Autism and mental retardation among offspring born after in vitro fertilization. *JAMA* 310(1), 75-84. <https://doi.org/10.1001/jama.2013.7222>
- Savasi, V., Ferrazzi, E., Lanzani, C., Oneta, M., Parrilla, B., & Persico, T. (2007). Safety of sperm washing and ART outcome in 741 HIV-1-serodiscordant couples. *Human Reproduction* 22(3), 772–777. <https://doi.org/10.1093/humrep/del422>
- Schlegel, P. N., Sigman, M., Collura, B., De Jonge, C. J., Eisenberg, M. L., Lamb, D. J., Mulhall, J. P., Niederberger, C., Sandlow, J. I., Sokol, R. Z., Spandorfer, S. D., Tanrikut, C., Treadwell, J. R., Oristaglio, J. T., & Zini, A. (2021). Diagnosis and Treatment of Infertility in Men: AUA/ASRM Guideline PART II. *Journal of Urology* 205(1), 44-51.
<https://doi.org/10.1097/JU.0000000000001520>
- Sfontouris, I. A., Kolibianakis, E. M., Lainas, G. T., Navaratnarajah, R., Tarlatzis, B. C., & Lainas, T.G. (2015). Live birth rates using conventional in vitro fertilization compared to intracytoplasmic sperm injection in Bologna poor responders with a single oocyte retrieved. *Journal of Assisted Reproduction and Genetics* 32(5), 691-697.
<https://doi.org/10.1007/s10815-015-0459-5>
- Shang, Y. L., Zhu, F. X., Yan, J., Chen, L., Tang, W. H., Xiao, S., Mo, W. K., Zhang, Z. G., He, X. J., Qiao, J., Cao, Y. X., & Li, W. (2019). Novel DPY19L2 variants in globozoospermic patients and the overcoming this male infertility. *Asian Journal of Andrology* 21(2), 183-189. https://doi.org/10.4103/aja.aja_79_18
- Shih, K. W., Shen, P. Y., Wu, C. C., & Kang, Y. N. (2019). Testicular versus percutaneous epididymal sperm aspiration for patients with obstructive azoospermia: a systematic review and meta-analysis. *Translational Andrology and Urology* 8(6), 631–640.

<https://doi.org/10.21037/tau.2019.11.20>

Simon, L., Brunborg, G., Stevenson, M., Lutton, D., McManus, J., & Lewis, S. E. (2010).

Clinical significance of sperm DNA damage in assisted reproduction outcome. *Human Reproduction* 25(7), 1594-1608. <https://doi.org/10.1093/humrep/deq103>

Simon, L., Castillo, J., Oliva, R., & Lewis, S. E. (2011). Relationships between human sperm

protamines, DNA damage and assisted reproduction outcomes. *Reproductive BioMedicine Online* 23(6), 724-734. <https://doi.org/10.1016/j.rbmo.2011.08.010>

Simon, L., Zini, A., Dyachenko, A., Ciampi, A., & Carrell, D. T. (2017). A systematic review

and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian Journal of Andrology* 19(1), 80-90.

<https://doi.org/10.4103/1008-682x.182822>

Simopoulou, M., Gkoles, L., Bakas, P., Giannelou, P., Kalampokas, T., Pantos, K., &

Koutsilieris, M. (2016). Improving ICSI: A review from the spermatozoon perspective. *Systems Biology in Reproductive Medicine* 62(6), 359-371.

<https://doi.org/10.1080/19396368.2016.1229365>

Skakkebaek, N. E., Rajpert-De Meyts, E., Buck Louis, G. M., Toppari, J., Andersson, A. M.,

Eisenberg, M. L., Jensen, T. K., Jørgensen, N., Swan, S. H., Sapa, K. J., Ziebe, S.,

Priskorn, L., & Juul, A. (2016). Male reproductive disorders and fertility trends:

Influences of environment and genetic susceptibility. *Physiological Reviews* 96(1), 55–97.

<https://doi.org/10.1152/physrev.00017.2015>

Staessen, C., & Van Steirteghem, A. C. (1997). The chromosomal constitution of embryos

developing from abnormally fertilized oocytes after intracytoplasmic sperm injection and conventional in-vitro fertilization. *Human Reproduction* 12(2), 321–327.

<https://doi.org/10.1093/humrep/12.2.321>

Stone, S., O'Mahony, F., Khalaf, Y., Taylor, A., & Braude, P. (2000). A normal livebirth

after intracytoplasmic sperm injection for globozoospermia without assisted oocyte activation: case report. *Human Reproduction* 15(1), 139-141.

<https://doi.org/10.1093/humrep/15.1.139>

Sullivan, E. A., Zegers-Hochschild, F., Mansour, R., Ishihara, O., de Mouzon, J., Nygren, K. G., & Adamson, G. D. (2013) International committee for monitoring Assisted Reproductive Technologies (ICMART) world report: assisted reproductive technology report 2004. *Human Reproduction* 28(5), 1375-1390.

<https://doi.org/10.1093/humrep/det036>

Supramaniam, P. R., Granne, I., Ohuma, E. O., Lim, L. N., McVeigh, E., Venkatakrishnan, R., Becker, C. M., & Mittal, M. (2020). ICSI does not improve reproductive outcomes in autologous ovarian response cycles with non-male factor subfertility, *Human Reproduction* 35(3), 583-594 <https://doi.org/10.1093/humrep/dez301>

Swan, S. H., Elkin, E. F., & Fenster, L. (1997). Have sperm densities declined? A reanalysis of global trend data. *Environmental Health Perspectives* 105(11), 1228–1232.

<https://doi.org/10.1289/ehp.971051228>

Swiatecka, J., Bielawski, T., Anchim, T., Leśniewska, M., Milewski, R., & Wołczyński, S. (2014.). Oocyte zona pellucida and meiotic spindle birefringence as a biomarker of pregnancy rate outcome in IVF-ICSI treatment. *Ginekologia Polska* 85(4), 264-271.

<https://doi.org/10.17772/gp/1722>

Szczygiel, M. A., Kusakabe, H., Yanagimachi, R., & Whittingham, D. G. (2002).

Intracytoplasmic sperm injection is more efficient than in vitro fertilization for generating mouse embryos from cryopreserved spermatozoa. *Biology of Reproduction* 67(4), 1278-1284. <https://doi.org/10.1095/biolreprod67.4.1278>

Tanaka, A., Nagayoshi, M., Takemoto, Y., Tanaka, I., Kusunoki, H., Watanabe, S., Kuroda, K., Takeda, S., Ito, M., & Yanagimachi, R. (2015). Fourteen babies born after round

- spermatid injection into human oocytes. *Proceedings of the National Academy of Science USA* 112(47), 14629-14634. <https://doi.org/10.1073/pnas.1517466112>
- Tanaka, A., Suzuki, K., Nagayoshi, M., Tanaka, A., Takemoto, Y., Watanabe, S., Takeda, S., Irahara, M., Kuji, N., Yamagata, Z., & Yanagimachi, R. (2018). Ninety babies born after round spermatid injection into oocytes: survey of their development from fertilization to 2 years of age. *Fertility and Sterility* 110(3), 443-451. <https://doi.org/10.1016/j.fertnstert.2018.04.033>
- Tanii, I., Aradate, T., Matsuda, K., Komiya, A., & Fuse, H. (2011). PACAP-mediated sperm-cumulus cell interaction promotes fertilization. *Reproduction* 141(2), 163-171. <https://doi.org/10.1530/rep-10-0201>
- Tannus, S., Son, W. Y., Gilman, A., Younes, G., Shavit, T., & Dahan, M. H. (2017). The role of Intracytoplasmic sperm injection in non-male factor infertility in advanced maternal age. *Human Reproduction* 32(1), 119-124. <https://doi.org/10.1093/humrep/dew298>
- Taşdemir, I., Taşdemir, M., & Tavukçuoğlu, S. (1998). Effect of pentoxifylline on immotile testicular spermatozoa. *Journal of Assisted Reproduction and Genetics* 15(2), 90-92. <https://doi.org/10.1007/bf02766832>
- Tavalaee, M., & Nasr-Esfahani, M. H. (2016). Expression profile of PLC ζ , PAWP, and TR-KIT in association with fertilization potential, embryo development, and pregnancy outcomes in globozoospermic candidates for intra-cytoplasmic sperm injection and artificial oocyte activation. *Andrology* 4(5), 850-856. <https://doi.org/10.1111/andr.12179>
- Taylor, S. L., Yoon, S. Y., Morshedi, M. S., Lacey, D. R., Jellerette, T., Fissore, R. A., & Oehninger, S. (2010). Complete globozoospermia associated with PLC ζ deficiency treated with calcium ionophore and ICSI results in pregnancy. *Reproductive BioMedicine Online* 20(4), 559-564. <https://doi.org/10.1016/j.rbmo.2009.12.024>
- Teixeira, D. M., Hadyme Miyague, A., Barbosa, M. A., Navarro, P. A., Raine-Fenning, N.,

- Nastri, C. O., & Martins, W. P. (2020). Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction. *Cochrane Database of Systematic Reviews* 2(2), CD010167. <https://doi.org/10.1002/14651858.CD010167.pub3>
- te Velde, E., van Baar, A., & van Kooije, R. (1998). Concerns about assisted reproduction. *Lancet* 351(9115), 1524-1525. [https://doi.org/10.1016/s0140-6736\(98\)22021-6](https://doi.org/10.1016/s0140-6736(98)22021-6)
- Tournaye, H. (2003). ICSI: a technique too far? *International Journal of Andrology* 26(2), 63-69. <https://doi.org/10.1046/j.1365-2605.2003.00380.x>
- Tournaye, H., Verheyen, G., Albano, C., Camus, M., Van Landuyt, L., Devroey, P., & Van Steirteghem, A. (2002.) Intracytoplasmic sperm injection versus in vitro fertilization: a randomized controlled trial and a meta-analysis of the literature. *Fertility and Sterility* 78(5), 1030-1037. [https://doi.org/10.1016/s0015-0282\(02\)03377-0](https://doi.org/10.1016/s0015-0282(02)03377-0)
- Tunç, L., Gürocak, S., Sözen, S., Tan, O., Alkibay, T., & Bozkirli, I. (2005). Is varicocele a prognostic factor for determining sperm retrieval rate before testicular sperm extraction? *Archives of Andrology* 51(2), 159–164. <https://doi.org/10.1080/014850190884318>
- Tüttelmann, F., Ruckert, C., & Röpke, A. (2018). Disorders of spermatogenesis: Perspectives for novel genetic diagnostics after 20 years of unchanged routine. *Medical Genetics* 30(1), 12–20. <https://doi.org/10.1007/s11825-018-0181-7>
- Van der Borght, M., & Wyns, C. (2018). Fertility and infertility: Definition and epidemiology. *Clinical Biochemistry* 62, 2-10. <https://doi.org/10.1016/j.clinbiochem.2018.03.012>
- Van der Westerlaken, L., Helmerhorst, F., Dieben, S., & Naaktgeboren, N. (2005). Intracytoplasmic sperm injection as a treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization. *Fertility and Sterility* 83(3), 612-617. <https://doi.org/10.1016/j.fertnstert.2004.08.029>
- Van Rumste, M. M., Evers, J. L., Farquhar, C. M., & Blake, D. A. (2000). Intra-cytoplasmic

- sperm injection versus partial zona dissection, subzonal insemination and conventional techniques for oocyte insemination during in vitro fertilisation. *Cochrane Database of Systematic Reviews* 2000(2): CD001301. <https://doi.org/10.1002/14651858.cd001301>
- Van Rumste, M. M. E., Evers, J. L. H., & Farquhar, C. (2003). Intra-cytoplasmic sperm injection versus conventional techniques for oocyte insemination during in vitro fertilisation in couples with non-male subfertility. *Cochrane Database of Systematic Reviews* 2003(2): CD001301.
- Van Soom, A., Tanghe, S., De Pauw, I., Maes, D., & de Kruif, A. (2002). Function of the cumulus oophorus before and during mammalian fertilization. *Reproduction in Domestic Animals* 37(3), 144-151. <https://doi.org/10.1046/j.1439-0531.2002.00345.x>
- Van Steirteghem, A., Bonduelle, M., Devroey, P., & Liebaers, I. (2002). Follow-up of children born after ICSI. *Human Reproduction Update* 8(2), 111-116. <https://doi.org/10.1002/14651858.CD001301>
- Vaughan, D.A., & Sakkas, D. (2019). Sperm selection methods in the 21st century. *Biology of Reproduction* 101(6), 1076-1082. <https://doi.org/10.1093/biolre/iox032>
- Walls, M., Junk, S., Ryan, J. P., & Hart, R. (2012). IVF versus ICSI for the fertilization of in-vitro matured human oocytes. *Reproductive BioMedicine Online* 25(6), 603-607. <https://doi.org/10.1016/j.rbmo.2012.08.001>
- Wang, J. G., Douglas, N. C., Prosser, R., Kort, D., Choi, J. M., & Sauer, M. V. (2009). Optimization of IVF pregnancy outcomes with donor spermatozoa. *Journal of Assisted Reproduction and Genetics* 26(2-3), 83-91. <https://doi.org/10.1007/s10815-008-9291-5>
- Wang, R., Danhof, N. A., Tjon-Kon-Fat, R. I., Eijkemans, M. J., Bossuyt, P. M., Mochtar, M. H., van der Veen, F., Bhattacharya, S., Mol, B. W. J., & van Wely, M. (2019). Interventions for unexplained infertility: a systematic review and network meta-analysis. *Cochrane Database of Systematic Reviews* 9(9), CD012692.

<https://doi.org/10.1002/14651858.CD012692.pub2>

Wang, W. H., Meng, L., Hackett, R. J., & Keefe, D. L. (2001). Developmental ability of human oocytes with or without birefringent spindles imaged by Polscope before insemination. *Human Reproduction* 16(7), 1464-1468.

<https://doi.org/10.1093/humrep/16.7.1464>

Wennerholm, U. B., Bergh, C., Hamberger, L., Lundin, K., Nilsson, L., Wikland, M., & Kallen, B. (2000). Incidence of congenital malformations in children born after ICSI. *Human Reproduction* 15(4), 944-948. <https://doi.org/10.1093/humrep/15.4.944>

West, R., Coomarasamy, A., Frew, L., Hutton, R., Kirkman-Brown, J., Lawlor, M., Lewis, S., Partanen, R., Payne-Dwyer, A., Roman-Montanana, C., Torabi, F., Tsagdi, S., & Miller, D. (2022). Sperm selection with hyaluronic acid improved live birth outcomes among older couples and was connected to sperm DNA quality, potentially affecting all treatment outcomes. *Human Reproduction* 37(6), 1106-1125.

<https://doi.org/10.1093/humrep/deac058>

Wiser, A., Ghetler, Y., Gonen, O., Piura, E., Berkovits, A., Itskovich, A., Rom, T., & Shulman, A. (2012). Re-evaluation of post-wash sperm is a helpful tool in the decision to perform in vitro fertilisation or intracytoplasmic sperm injection. *Andrologia* 44(2), 73-77.

<https://doi.org/10.1111/j.1439-0272.2010.01107.x>

World Health Organization (2021). WHO laboratory Manual for the Examination and Processing of Human Semen (6th ed). World Health Organization Press, Geneva.

Wu, E.X., Stanar, P., & Ma, S. (2014). X-chromosome inactivation in female newborns conceived by assisted reproductive technologies. *Fertility and Sterility* 101(6), 718-723.

<https://doi.org/10.1016/j.fertnstert.2014.03.010>

Yanagida, K., Fujikura, Y., & Katayose, H. (2008). The present status of artificial oocyte activation in assisted reproductive technology. *Reproductive Medicine and Biology* 7(3),

133-142. <https://doi.org/10.1111/j.1447-0578.2008.00210.x>

Younes, G., Tannus, S., Son, W. Y., & Dahan, M. H. (2019). When to do intracytoplasmic sperm injection: a prospective comparison. *Archives in Gynecology and Obstetrics* 300(5), 1461–1471. <https://doi.org/10.1007/s00404-019-05324-4>

Yovich, J. M., Edirisinghe, W. R., Cummins, J. M., & Yovich, J. L. (1988). Preliminary results using pentoxifylline in a pronuclear stage tubal transfer (PROST) program for severe male factor infertility. *Fertility and Sterility* 50(1), 179–81. [https://doi.org/10.1016/s0015-0282\(16\)60030-4](https://doi.org/10.1016/s0015-0282(16)60030-4)

Yu, X. W., Wei, Z. T., Jiang, Y. T., & Zhang, S. L. (2015). Y chromosome azoospermia factor region microdeletions and transmission characteristics in azoospermic and severe oligozoospermic patients. *International Journal of Clinical and Experimental Medicine* 8(9), 14634–14646. <http://www.ncbi.nlm.nih.gov/pmc/articles/pmc4658835/>

Yu, Z., Wei, Z., Yang, J., Wang, T., Jiang, H., Li, H., Tang, Z., Wang, S., & Liu, J. (2018). Comparison of intracytoplasmic sperm injection outcome with fresh versus frozen-thawed testicular sperm in men with nonobstructive azoospermia: a systematic review and meta-analysis. *Journal of Assisted Reproduction and Genetics* 35(7), 1247–1257. <https://doi.org/10.1007/s10815-018-1206-5>

Zegers-Hochschild, F., Adamson, G. D., Dyer, S., Racowsky, C., de Mouzon, J., Sokol, R., Rienzi, L., Sunde, A., Schmidt, L., Cooke, I. D., Simpson, J. L., & van der Poel, S. (2017). The International Glossary on Infertility and Fertility Care. *Fertility and Sterility* 108(3), 393–406. <https://doi.org/10.1016/j.fertnstert.2017.06.005>

Zhang, Z., Zhu, L., Jiang, H., Chen, H., Chen, Y., & Dai, Y. (2015). Sperm DNA fragmentation index and pregnancy outcome after IVF or ICSI: a meta-analysis. *Journal of Assisted Reproduction and Genetics* 32(1), 17-26. <https://doi.org/10.1007/s10815-014-0374-1>

Zhao, J., Zhang, Q., Wang, Y., & Li, Y. (2014). Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertility and Sterility* 102(4), 998-1005. <https://doi.org/10.1016/j.fertnstert.2014.06.033>

Zhu, J. L., Basso, O., Obel, C., Hvidtjørn, D., & Olsen, J. (2009). Infertility, infertility treatment and psychomotor development: the Danish National Birth Cohort. *Paediatric and Perinatal Epidemiology* 23(2), 98–106. <https://doi.org/10.1111/j.1365-3016.2008.00989.x>

Zollner, U., Schleyer, M., & Steck, T. (1996). Evaluation of a cut-off value for normal sperm morphology using strict criteria to predict fertilization after conventional in-vitro fertilization and embryo transfer in asthenozoospermia. *Human Reproduction* 11(10), 2155-2161. <https://doi.org/10.1093/oxfordjournals.humrep.a019068>

Table 1. Levels of evidence (NICE, 2004)

Grade strength of evidence

A. Requires at least one RCT as part of a body of literature of overall good quality and consistency addressing the specific recommendation. (evidence levels 1a, 1b).

B. Requires the availability of well-controlled clinical studies but has no randomised clinical trials on the topics of recommendations. (evidence levels 2a, 2b, 3).

C. Requires evidence obtained from expert committee reports of opinions and/or clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality. (evidence level 4).

GPP. Good practice point