

Intracytoplasmic sperm injection for male infertility and consequences for offspring

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Abstract | Intracytoplasmic sperm injection (ICSI) has become the most commonly used method of fertilization in assisted reproductive technology. The primary reasons for its popularity stem from its effectiveness, the standardization of the procedure, which means that it can easily be incorporated into the routine practice of fertility centres worldwide, and the fact that it can be used to treat virtually all forms of infertility. ICSI is the clear method of choice for overcoming untreatable severe male factor infertility, but its (over)use in other male and non-male factor infertility scenarios is not evidence-based. Despite all efforts to increase ICSI efficacy and safety through the application of advanced sperm retrieval and cryopreservation techniques, as well as methods for selecting sperm with better chromatin integrity, the overall pregnancy rates from infertile men remain suboptimal. Treating the underlying male infertility factor before ICSI seems to be a promising way to improve ICSI outcomes, but data remain limited. Information regarding the health of ICSI offspring has accumulated over the past 25 years, and there are reasons for concern as risks of congenital malformations, epigenetic disorders, chromosomal abnormalities, subfertility, cancer, delayed psychological and neurological development, and impaired cardiometabolic profile have been observed to be greater in infants born as a result of ICSI than in naturally conceived children. However, as subfertility probably influences the risk estimates, it remains to be determined to what extent the observed adverse outcomes are related to parental factors or associated with ICSI.

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Intracytoplasmic sperm injection (ICSI) involves the injection of a single spermatozoon into an oocyte cytoplasm using a glass micropipette (FIG. 1a). The method was originally introduced in 1992 as a modification of conventional in vitro fertilization (IVF)¹ (FIG. 1b). The development of ICSI is probably one of the most extraordinary achievements in the field of assisted reproductive technology (ART), apart from IVF itself, enabling men with low sperm quantity and quality, including those with azoospermia, to father a biological child². Currently, ICSI is a well-established laboratory technique used worldwide to treat infertility.

ICSI was originally introduced to overcome the most severe forms of male factor infertility. Since its advent, the use of this method of fertilization has increased steadily, even though the proportion of infertile couples diagnosed with male factor infertility has remained stable³. This observation suggests that ICSI is now widely used, despite the equivocal evidence of its benefit over conventional IVF in couples without male factor infertility^{4,5}. Concerns have also been raised about whether

the indiscriminate use of ICSI leads to adverse health consequences for the resulting offspring⁶.

In this Review, we summarize the current evidence on the use of ICSI, with a primary focus on male factor infertility. We provide a historical overview of ICSI development, consider its indications in both male factor and non-male factor infertility conditions, and describe the technical aspects of ICSI when applied to overcome male factor infertility. We also present and critically discuss the evidence concerning the consequences of ICSI for the health of resulting offspring.

Historical overview of ICSI development

The first sperm injection into the oocyte cytoplasm was done accidentally during a subzonal insemination (SUZI) procedure in 1992 (REF.⁷). At this point, SUZI — a procedure that involves deposition of the spermatozoon into the perivitelline space — was the method of choice for overcoming fertilization problems with standard IVF. As normal fertilization occurred after the unintentional sperm injection into the oolemma, the idea that ICSI

Key points

- Intracytoplasmic sperm injection (ICSI) was introduced to overcome the most severe forms of male factor infertility and has become the most frequently used method of fertilization in assisted reproductive technology (ART).
- Existing evidence does not support ICSI in preference over in vitro fertilization (IVF) in the general non-male factor ART population; however, in couples with unexplained infertility, ICSI is associated with lower fertilization failure rates than IVF.
- Percutaneous and open sperm retrieval methods are highly effective for harvesting sperm from men with obstructive azoospermia; open microsurgical testicular sperm retrieval has been associated with improved sperm retrieval in men with nonobstructive azoospermia.
- Existing evidence indicates that children conceived through ICSI have an increased risk of chromosomal abnormalities, particularly those affecting sex chromosomes, compared with naturally conceived children.
- Whether the risk of cancer is increased among children conceived using ICSI is unclear, but some evidence indicates that certain cancer types are more common in children conceived using ICSI than in naturally conceived children.
- All efforts should be made to evaluate and treat subfertile men, both to improve the safety and efficiency of ICSI and to allow natural conception or the use of less-invasive assisted conception methods when appropriate.

would be a feasible procedure for the treatment of severe male factor infertility emerged².

The first successful series of ICSI was reported by Palermo et al. in 1992 (REF.¹). The authors performed sperm injections in 47 mature oocytes taken from 4 women undergoing IVF. In total, 38 oocytes remained intact after sperm injection, of which 31 were fertilized and resulted in 15 embryos. Four pregnancies were established after embryo transfer to the uterine cavity; two singletons and one twin pregnancy developed until parturition, with the delivery of four healthy babies. All the couples had been referred for ICSI with ejaculated sperm owing to severely impaired spermatozoa characteristics and previous failures of standard IVF and SUZI¹.

In 1994, Tournaye et al. published the first successful series of ICSI using sperm aspirated from the epididymis in 12 patients with obstructive azoospermia due to congenital bilateral absence of the vas deferens (CBAVD)⁸. On the day before oocyte retrieval, the male partners underwent microsurgical epididymal sperm aspiration (MESA) under general anaesthesia. The epididymis was

dissected, the tubules were opened with microscissors, and the epididymal fluid was aspirated. When motile spermatozoa were identified by perioperative microscopic examination, additional fluid containing enough sperm for ICSI was aspirated, and no further tubules were incised. In this series, intracytoplasmic epididymal sperm injection resulted in a fertilization rate of 58%, and out of ten fresh embryo transfers, five pregnancies were achieved. Of these pregnancies, one was a biochemical pregnancy, and two ended in miscarriages, resulting in two ongoing pregnancies, one of which was reported to result in live offspring. Notably, two additional pregnancies were obtained after frozen-thawed embryo transfers. The rate of early pregnancy loss was high (57%), resulting in an ongoing pregnancy rate per sperm aspiration procedure of 21.4%⁸.

Also in 1994, Devroey et al. reported the first study investigating the efficacy of the use of testicular sperm for ICSI⁹. Testicular sperm extraction (TESE) was performed after failed epididymal aspiration in three patients with obstructive azoospermia. Sperm retrievals were carried out on the same day as oocyte retrievals, and free spermatozoa were seen after testicular tissue manipulation. In this series, ICSI with testicular sperm was carried out using 45 mature oocytes, resulting in a fertilization rate of 45.5%. Of the fertilized eggs, 85% achieved the embryo stage and were transferred or cryopreserved. The laboratory procedures were successful, but no pregnancies were obtained⁹. One year later, the first 5 pregnancies after TESE-ICSI were described in a series of 12 patients with CBAVD. These men underwent TESE because retrieving sperm from the epididymis was not possible. In this series, the ongoing implantation and pregnancy rates were 23% and 43%, respectively, but the authors did not report whether these pregnancies resulted in delivery of healthy babies¹⁰.

Later in 1995, Devroey et al.¹¹ reported 15 men with nonobstructive azoospermia treated with TESE-ICSI. Testicular sperm retrieval was carried out using open biopsy on the same day that oocyte retrieval was performed. The extracted specimens were examined, and an additional sample was sent for histological evaluation. In 13 of 15 patients, very small numbers of spermatozoa were found, all of which had severe spermatogenic defects confirmed by histopathology. In this series, testicular sperm injections resulted in a 47.8% fertilization rate. In all, 32 resulting embryos were transferred, and three pregnancies — one set of triplets, one set of twins, and one singleton — were established, with an overall implantation rate of 18.7%¹¹. However, the authors did not report whether the pregnancies resulted in delivery of live infants.

Modern trends: is ICSI overused?

ICSI has become the most common method of fertilization used for ART³. Boulet et al. evaluated data on ART reported to the US National Assisted Reproductive Technology Surveillance System between 1996 and 2012 (REF.⁶). Overall, ICSI was used in 65.1% of all fresh IVF cycles (embryos transferred without being frozen; $n = 1,395,634$) performed. The use of ICSI increased from 36.4% in 1996 to 76.2% in 2012, even though male

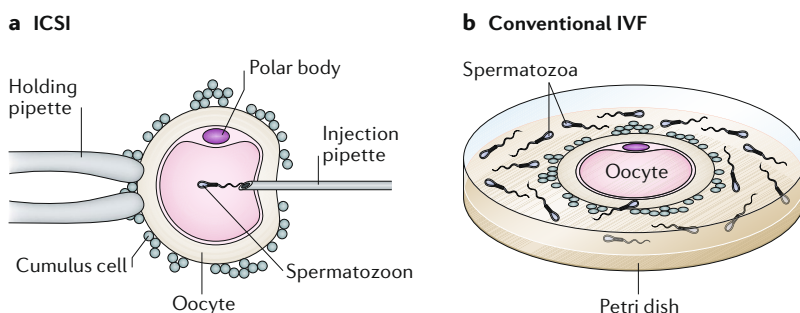


Fig. 1 | Assisted fertilization methods. **a** | Intracytoplasmic sperm injection (ICSI), which involves the injection of a single spermatozoon into an oocyte cytoplasm using a glass micropipette. **b** | Conventional in vitro fertilization (IVF), where oocytes are incubated with sperm in a Petri dish, and the male gamete fertilizes the oocyte naturally.

factor infertility as the stated reason for applying ICSI as a method of fertilization treatment remained essentially unchanged at approximately 36% of cases⁶. Dyer et al. assessed the worldwide ART data generated by the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) between 2008 and 2010. Fertilization was performed using ICSI in approximately 67% of the 4,461,309 ART cycles analysed⁵. Nevertheless, large variation existed between regions, with ICSI being performed in around 55% of ART procedures in Asia, 65% of cases in Europe, 85% of cases in Latin America, and almost 100% of cases in the Middle East⁵.

Possible reasons for the widespread use of ICSI relate to the high level of standardization and validation of the method, which enables rapid incorporation into the routine practice of fertility centres worldwide and the possibility of its use in virtually all causes of infertility². Nonetheless, the global pregnancy rates and delivery rates reported for ICSI remain relatively low, at 28.7% and 18.9%, respectively, for the year 2008; 27.7% and 19.9%, respectively, for 2009; and 26.8% and 20.0%, respectively, for 2010 (REF.⁵). Compared with conventional IVF among couples undergoing ART for non-male factor infertility, ICSI is associated with lower rates of implantation (23.0% versus 25.2%; adjusted relative risk (RR) 0.93; 95% CI 0.91–0.95) and lower rates of live birth (36.5% versus 39.2%; adjusted RR 0.95; 95% CI 0.93–0.97)⁶. In a large retrospective cohort study evaluating 585,065 ART cycles in Australia between 2002 and 2013, Chambers et al. did not find any benefit of ICSI over conventional IVF as a method of fertilization for couples with non-male factor infertility. By contrast, the

adjusted odds of live birth were about 10% lower with ICSI than with IVF¹². As a result, the Practice Committee of the American Society for Reproductive Medicine (ASRM) has advised against the indiscriminate use of ICSI in non-male factor infertility¹³.

Indications for ICSI

Conventional IVF has been successfully used since the birth of Louise Brown in 1978, but its results are sub-optimal when sperm quantity or quality is poor¹. As a single spermatozoon with a functioning centrosome and genome can fertilize an oocyte and generate a healthy embryo, ICSI has become the natural treatment for couples with severe male factor infertility¹⁴ and is also used for a number of non-male factor indications (TABLE 1).

ICSI for male factor infertility

Azoospermia. Azoospermia — a complete absence of spermatozoa in the ejaculate — affects approximately 1% of the male population and 10–15% of men with infertility¹⁵ (BOX 1; FIG. 2). Azoospermia can be classified into two broad categories: obstructive azoospermia and nonobstructive azoospermia. In obstructive azoospermia, complete spermatogenesis is confirmed by histopathological examination, whereas in nonobstructive azoospermia, germ cell aplasia (Sertoli-cell-only (SCO) syndrome), maturation arrest, or hypospermatogenesis is observed. Obstructive azoospermia results from bilateral obstruction of the seminal ducts; as spermatogenesis is normal in men with obstructive azoospermia, sperm can be retrieved from the epididymides or testicles in virtually all cases¹⁶. By contrast, nonobstructive azoospermia is associated with untreatable testicular

Table 1 | Fertilization methods in male factor and non-male factor infertility

Type of infertility	Fertilization method	Refs
Male factor infertility		
Azoospermia	ICSI mandatory	15–20
Severe OAT	ICSI highly recommended	25
Moderate OAT	IVF and ICSI equally effective	26
Isolated teratozoospermia	IVF and ICSI equally effective	32–36
Absolute asthenozoospermia	ICSI mandatory	19,37,38
Globozoospermia	ICSI mandatory	39,40
Antisperm antibodies	IVF and ICSI equally effective	42
Sperm DNA fragmentation	ICSI recommended	49–56,175
Non-male factor infertility		
General non-male factor population	Equally effective, slightly in favour of IVF	4,6,12,57,60
Unexplained infertility	Equally effective, but sibling oocyte studies suggest that ICSI is superior to IVF for fertilization, whereas the reproductive outcome is not significantly different	6,57–60
Poor-quality oocytes and advanced maternal age	Equally effective, slightly in favour of IVF	61,62,65
Poor responders	Equally effective, slightly in favour of IVF	62,65
Preimplantational genetic testing	ICSI highly recommended	13,22,68,69
Tubal ligation	IVF preferable	76
Serodiscordant couples	Equally effective	77,78

ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; OAT, oligoasthenoteratozoospermia.

Box 1 | Terms used to define semen characteristics according to the 2010 WHO manual²³

Azoospermia.

No spermatozoa in the fresh ejaculate even after centrifugation and microscopic examination of the centrifuged pellet.

Oligozoospermia.

Concentration of spermatozoa below the lower reference limit of 15 million per ml.

Asthenozoospermia.

Percentage of progressively motile spermatozoa below the reference limit of 32% (absolute asthenozoospermia refers to the absence of motile spermatozoa in the ejaculate).

Teratozoospermia.

Percentage of morphologically normal spermatozoa below the lower reference limit of 4%.

Oligoasthenozoospermia.

Concentration of spermatozoa and percentage of progressively motile spermatozoa below the reference limits.

Asthenoteratozoospermia.

Percentages of both progressively motile and morphologically normal spermatozoa below the lower reference limits.

Oligoteratozoospermia.

Concentration of spermatozoa and percentage of morphologically normal spermatozoa below the lower reference limits.

Oligoasthenoteratozoospermia.

Concentration of spermatozoa and percentages of progressively motile and morphologically normal spermatozoa below the reference limits.

Necrozoospermia.

Low percentage of live and high percentage of immotile spermatozoa in the ejaculate (complete necrozoospermia refers to the absence of any live spermatozoa in the ejaculate).

Normozoospermia.

Concentration of spermatozoa and percentages of progressively motile and morphologically normal spermatozoa within the reference limits.

Globozoospermia.

Presence of spermatozoa in the ejaculate with small acrosome vesicles or total absence of the acrosomal vesicle.

Leukocytospermia.

Presence of leukocytes in the ejaculate above the threshold value of 1 million per ml.

Cryptozoospermia.

No spermatozoa in the fresh ejaculate, but spermatozoa are observed after microscopic examination of centrifuged pellet.

disorders that result in spermatogenic failure. Focal spermatogenesis occurs in 30–60% of men with non-obstructive azoospermia even if they display signs of SCO syndrome or maturation arrest on histopathology; in such cases, sufficient numbers of sperm can be obtained for ICSI¹⁷. The two methods most commonly used to harvest sperm in men with obstructive azoospermia and nonobstructive azoospermia are percutaneous acquisition and open surgery (which can be performed with or without the aid of microsurgery)¹⁸. After retrieval of epididymal or testicular sperm, ICSI is used instead of conventional IVF, as the retrieved gametes are unable to fertilize the oocytes by conventional IVF^{19,20}.

Oligoasthenoteratozoospermia. Despite the overall consensus that ICSI should be used in men with severe male factor infertility, it can be challenging to determine when a male factor becomes a mandatory indication for ICSI. Conventional semen analysis is still widely used for determining the severity of male infertility and recommending the use of ICSI, but it is well known that traditional semen evaluation is limited in that regard, as it does not evaluate the functional aspects of sperm, including genomic integrity²¹. Sperm count, motility, and morphology are the commonly used parameters to assess the need for ICSI in preference to

conventional IVF²² (FIG. 2). However, high-quality data comparing live birth rates from ICSI and IVF in couples with oligoasthenoteratozoospermia²³ (BOX 1) are lacking, as discussed recently by Cissen and co-workers²⁴. Nevertheless, a meta-analysis of 9 randomized controlled trials (RCTs) involving 332 treatment cycles in couples in which the male partner had isolated teratozoospermia (2 trials) or oligoasthenoteratozoospermia (7 trials) showed that the risk ratio for achieving fertilization was 1.9 in favour of ICSI compared with conventional IVF (95% CI 1.4–2.5)²⁵. In this study, the number needed to treat (NNT) by ICSI to avoid one cycle of complete fertilization failure by IVF was 3.1 (95% CI 1.7–12.4)²⁵. However, more recent data have called into question these observations, as no differences in fertilization, implantation, and pregnancy rates were observed between conventional IVF and ICSI groups in men with moderate oligoasthenoteratozoospermia (sperm concentration between 5×10^6 per ml and 15×10^6 per ml and progressive motility $<32\%$)²⁶.

Isolated teratozoospermia. Sperm morphology results have been widely used to select candidates for ICSI. In 1986, Kruger et al. were the first to propose the use of strict criteria to classify sperm abnormalities and to recommend ICSI when the proportion of normal sperm in

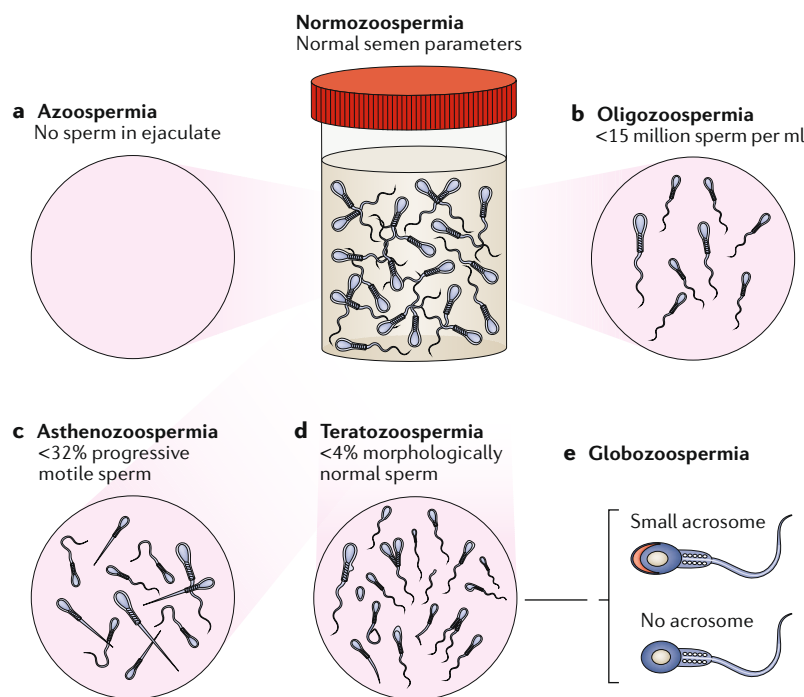


Fig. 2 | **Seminal alterations associated with male infertility.** a | Azoospermia. b | Oligozoospermia. c | Asthenozoospermia. d | Teratozoospermia. e | Globozoospermia.

a given ejaculate was low²⁷. These authors reported that in patients with <4% normal sperm morphology, the fertilization rate after conventional IVF was <8%, and in patients with normal sperm morphology between 4% and 14%, the fertilization rate was >60%²⁸. Subsequently, the data were corroborated by other researchers who found that at least 5% of sperm needed to be morphologically normal in order to achieve an adequate fertilization rate with the use of conventional IVF^{29,30}. Given these observations, it became commonplace to recommend ICSI over conventional IVF in patients with teratozoospermia (defined as <5% morphologically normal sperm)^{25,31} (BOX 1; FIG. 2). However, a recent meta-analysis evaluating the relationship between teratozoospermia and ART outcomes indicated no association between isolated teratozoospermia and the outcomes of IVF or ICSI³². In this meta-analysis, the authors aggregated the data from four studies^{32–36} that included 2,853 IVF or ICSI cycles, of which 673 men had severe teratozoospermia (<5% morphologically normal sperm) and 2,183 did not. The odds of clinical pregnancy in couples in which the male partner had isolated teratozoospermia did not differ regardless of whether conventional IVF (OR 1.04, 95% CI 0.90–1.21) or ICSI (OR 0.95, 95% CI 0.63–1.42) had been carried out³².

Absolute asthenozoospermia. ICSI has been recommended¹⁹ in cases of absolute asthenozoospermia (100% immotile spermatozoa in the ejaculate) and virtual asthenozoospermia (few motile spermatozoa in the ejaculate) (BOX 1; FIG. 2). The former condition is primarily associated with ultrastructural abnormalities of the sperm tail or complete necrozoospermia (no live spermatozoa in the ejaculate), whereas the latter

condition is sometimes seen in freeze–thawed specimens (BOX 1). In absolute asthenozoospermia, evaluating sperm viability is important, as injection of uncharacterized immotile sperm is associated with reduced fertilization and pregnancy rates¹⁹. Several laboratory strategies have been proposed to improve ICSI, including sperm exposure to pentoxifylline or theophylline to improve motility and the hypo-osmotic swelling test and use of the laser to improve selection of viable sperm³⁷. When none of these strategies is effective, sperm can be harvested from the testis, and testicular sperm can be used for injections³⁸.

Globozoospermia. Globozoospermia is characterized by the complete absence of the acrosomal vesicle, an aberrant nuclear membrane, midpiece defects, and a round-headed sperm (FIG. 2). Globozoospermia is a rare genetic condition and is, therefore, untreatable, affecting around 0.1% of infertile men³⁹. Despite having normal sperm count and motility, spermatozoa of men with globozoospermia are unable to fertilize the oocyte naturally. ICSI is the only option in such cases, although fertilization and pregnancy rates are still low owing to the reduced ability of the sperm to activate the oocyte and trigger zygote formation and embryo development⁴⁰. Oocyte activation using mechanical, electrical, and chemical agents has been used to overcome fertilization failure among men with globozoospermia⁴⁰.

Antisperm antibodies. The presence of seminal antisperm antibodies (ASAs) is usually related to a breach of the blood–testis barrier or an obstruction in the male reproductive tract. Elevated levels of ASAs in semen are found in 3–12% of men undergoing infertility evaluation⁴¹. This condition has been associated with testicular torsion, testicular surgery, vasectomy, epididymo-orchitis, testicular carcinoma, cryptorchidism, and infection by HIV⁴². ASAs can potentially impair fertility through effects on sperm motility, sperm capacitation, the acrosome reaction, and sperm–oocyte binding⁴¹. Antibodies directed towards sperm can also act through the release of cytokines that can impair sperm function⁴³. A 2011 meta-analysis that included 16 observational studies, involving 4,209 ART treatments (1,508 IVF and 2,701 ICSI cycles), investigated the association between ASA and pregnancy outcomes in couples who underwent conventional IVF or ICSI. Among men with high levels of ASAs, conventional IVF and ICSI were equally effective in terms of pregnancy rates (odds ratios for failure to achieve a pregnancy were 1.22 (95% CI 0.84–1.77) and 1.00 (95% CI 0.72–1.38), respectively)⁴².

Sperm DNA fragmentation. Given the limitations of conventional semen parameters for making recommendations for ICSI, efforts have been made to identify other useful sperm biomarkers. Sperm DNA integrity has gained interest as it has an essential role in normal embryo development and pregnancy outcome^{20,44}. Sperm DNA fragmentation (SDF) testing has emerged as a complementary diagnostic option to routine semen analysis as it measures the proportion of sperm with damaged chromatin in the neat ejaculate. Using probes

or dyes, sperm DNA breaks can be identified and quantified with the aid of fluorescence microscopy, optical microscopy, or flow cytometry⁴⁵. Notably, SDF is more common in infertile men than in their fertile counterparts^{46,47}. Moreover, despite usually being associated with abnormal semen parameters, an elevated SDF level is not an unusual finding among infertile men with otherwise normal semen parameters⁴⁸.

An increasing body of evidence has indicated that elevated SDF negatively affects the chances of natural and assisted conception⁴⁹. Indeed, SDF testing results might be useful when choosing the method of fertilization, IVF or ICSI⁵⁰. In a 2017 meta-analysis that included 56 studies and 8,068 treatment cycles, Simon et al. showed that clinical pregnancy rates (CPRs) were higher with low (versus high) SDF in couples undergoing IVF (OR 1.65, 95% CI 1.34–2.04; $P < 0.0001$) and ICSI (OR 1.31, 95% CI 1.08–1.59; $P = 0.0068$)⁵¹. By contrast, Osman et al. aggregated the evidence from six studies reporting live birth rate (LBR) and showed that low SDF rates were associated with increased LBRs following IVF (RR 1.27, 95% CI 1.05–1.52; $P = 0.01$) but not ICSI (RR 1.08, 95% CI 0.39–2.96)⁵². As live birth is the primary end point for the couple, these results suggest that in couples with high SDF, ICSI should be recommended in preference over conventional IVF.

Furthermore, the risk of pregnancy loss is increased after both IVF and ICSI among couples whose male partners have high SDF. In a systematic review of 11 studies involving 1,549 IVF and ICSI cycles, the odds of miscarriage increased by 2.48-fold among men with high SDF (95% CI 1.52–4.04; $P < 0.0001$)⁵³. These results have been corroborated by two large studies indicating that SDF was contributory to miscarriage irrespective of the method of fertilization, IVF or ICSI. In one report, Robinson and colleagues evaluated 16 cohort studies comprising 2,969 couples and found that the risk of miscarriage was increased in both IVF and ICSI cycles among women pregnant by male partners with high SDF (RR 2.16, 95% CI 1.54–3.03; $P < 0.00001$)⁵⁴. Likewise, Zhao et al. evaluated 14 publications including 2,756 couples who underwent ART and found an increased risk of miscarriage in patients with high SDF, both overall (OR 2.28, 95% CI 1.55–3.35; $P < 0.0001$) and among those using ICSI (OR 2.68, 95% CI 1.40–5.14; $P = 0.003$)⁵⁵. Collectively, these data suggest that ICSI is superior to IVF as a method of fertilization to overcome SDF-related infertility. However, the risk of miscarriage is still a concern with ICSI using ejaculated sperm⁵⁶.

ICSI for non-male factor infertility

General non-male factor infertility. An early Cochrane review published in 2003 identified a single RCT that compared the use of ICSI with the use of IVF for non-male factor infertility⁴. This study randomly assigned 415 couples to treatment rather than oocytes and was powered to detect a 10% difference in the implantation rate between the two methods of fertilization. The reported result was an implantation rate of 30% for IVF compared with 22% for ICSI ($P = 0.03$)⁵⁷. Although the trial was not powered to investigate reproductive outcomes, no

significant difference was observed regarding the CPR between IVF and ICSI (33% and 26%, respectively). Moreover, IVF was superior to ICSI with regards to fertilization rate (58% versus 47%; $P = 0.0001$), and the overall effect expressed as the NNT was an additional 33 cases of ICSI to overcome a single case of total fertilization failure after IVF. As ICSI involves invasive and time-consuming procedures, this limited positive effect in favour of ICSI regarding total fertilization failure is not enough to recommend ICSI as the first-line treatment for non-male factor infertility. In fact, the most recent published results seem to agree that ICSI leads to a poorer reproductive outcome than does conventional IVF for non-male factor indications, as previously mentioned^{6,12}.

Unexplained infertility. A 2013 systematic review and meta-analysis of 11 RCTs including approximately 12,000 sibling oocytes concluded that ICSI is associated with higher fertilization rates than IVF in couples with unexplained infertility (RR 1.27, 95% CI 1.02–1.58; $P < 0.001$)⁵⁸. Furthermore, a significantly higher total fertilization failure (TFF) rate was observed with IVF than with ICSI (RR 8.22, 95% CI 4.44–15.23; $P < 0.001$). The reported NNT was only five patients with ICSI to avoid one TFF with IVF. The largest study in the meta-analysis investigated the idea of splitting the pool of retrieved oocytes into ICSI and IVF⁵⁹. In this trial, which involved 248 couples with unexplained infertility who had undergone 3 or more unsuccessful intrauterine insemination (IUI) treatments, TFF was reported in 25% of IVF cycles compared with 4% in ICSI cycles. Thus, the authors suggested splitting the oocyte pool between IVF and ICSI for couples with unexplained infertility who had previously undergone unsuccessful IUI treatment⁵⁹. By contrast, a subgroup analysis of 100 couples with unexplained infertility from an RCT that randomly assigned couples and not oocytes reported a higher fertilization rate per oocyte retrieved after IVF (61%) than after ICSI (50%) with no significant difference in the reproductive outcome⁵⁷. Furthermore, in a large retrospective analysis of 112,877 conventional IVF and 205,119 ICSI cycles involving couples with unexplained infertility in the USA between 2008 and 2012, the embryo transfer cancellation rate was not significantly lower with ICSI than with IVF (8.0% and 8.2%, respectively)⁶. Additionally, implantation rate (23% versus 25.2%, $P < 0.001$) and LBR (36.5% versus 39.2%, $P < 0.001$) were lower in the ICSI group than in the IVF group among the cycles that resulted in a fresh embryo transfer⁶. These observations were corroborated by Foong and colleagues, who randomly assigned 60 women with unexplained infertility to conventional IVF or ICSI⁶⁰. In this study, no differences were found between IVF and ICSI in fertilization rate (77.2% versus 82.4%), implantation rate (38.2% versus 44.4%), or LBR (46.7% versus 50%)⁶⁰. Taken together, the evidence from sibling oocyte studies provides a clear indication for ICSI — with or without splitting the oocyte pool — in couples with unexplained infertility undergoing ART, owing to reduced TFF rates and increased fertilization rates. However, a retrospective database study and RCTs

that randomly assigned couples with unexplained infertility to IVF or ICSI concluded that ICSI does not result in an improved reproductive outcome^{6,57,59,60}.

Advanced maternal age, poor-quality oocytes, and poor responders. In a retrospective study of 745 women aged >40 years with non-male factor infertility, Tannus et al. reported that LBRs were similar with the use of ICSI and IVF following fresh embryo transfer⁶¹. However, the cumulative LBR obtained after transferring fresh and frozen-thawed embryos could potentially have favoured IVF, as significantly more cycles with available embryos for cryopreservation were achieved in the IVF group than in the ICSI group (26.4% versus 19.7%, $P=0.04$). These findings seem to be related to an increased rate of mechanical damage to oocytes subjected to sperm injections in this patient population. In fact, a previous retrospective study evaluating 243 patients who were poor responders to ovarian stimulation according to the Bologna criteria reported that the rate of mechanical damage to oocytes was significantly higher when ICSI was applied as the fertilization method than when IVF was used (ICSI degeneration rates were 8% with ICSI for non-male factor infertility and 6.5% for male factor infertility versus an IVF degeneration rate of 0% for non-male factor infertility; $P=0.02$)⁶². Poor ovarian response to ovarian stimulation usually indicates a reduction in follicular response that results in a reduced number of retrieved oocytes^{63,64}. The Bologna criteria define a patient as being a poor ovarian responder when at least two of the following three features are present: advanced maternal age (≥ 40 years) or any other risk factor for poor ovarian response; a previous episode of poor ovarian response (retrieval of <4 oocytes after a conventional ovarian stimulation protocol); and/or an abnormal ovarian reserve test⁶⁵. Furthermore, in a retrospective cohort study involving 425 cycles and 386 poor responders with only one or two oocytes retrieved, Artini et al. observed that CPRs were significantly higher with IVF than with ICSI (32.5% versus 4.8% among women aged <35 years, $P=0.001$; 26.3% versus 7.0% among women aged 35–38 years, $P=0.01$)⁶⁶. By contrast, the conclusion from the retrospective cohort evaluating 243 patients defined as being poor responders according to the Bologna criteria was that IVF and ICSI were equally effective in terms of LBR (5.0% and 4.0% for ICSI in non-male factor and male factor infertility, respectively, versus 3.3% for IVF in non-male factor infertility)⁶².

An important confounder in these retrospective studies is that a potential selection bias cannot be ruled out, as patients were assigned to IVF or ICSI on the basis of fertilization rates and reproductive outcomes in previous cycles, resulting in a poorer a priori prognosis in the ICSI group. Taken together, the existing evidence does not support the use of ICSI for patients with advanced maternal age (≥ 40 years) or poor ovarian response.

Preimplantation genetic testing. Over the past 30 years, preimplantation genetic testing (PGT) has been used in association with ART to analyse the DNA from embryos (cleavage stage or blastocyst) and to determine genetic

abnormalities⁶⁷. ICSI is the preferred method of fertilization over conventional IVF in such cases as it avoids sample contamination from cumulus cells, extraneous sperm attached to the zona pellucida, and non-decondensed sperm within blastomeres that can affect the accuracy of genetic analysis^{13,22,68,69}. The literature remains equivocal about the benefits of performing PGT regarding the health of offspring. Congenital abnormality rates are not significantly different after ICSI–PGT than with IVF or ICSI without PGT or among naturally conceived children^{70–72}. Moreover, no differences have been observed in the psychosocial functioning⁷³, neurodevelopmental outcome⁷⁴, or cognitive development⁷⁵ of children born after ICSI–PGT compared with those born after ICSI alone or those naturally conceived. However, removing cells from an embryo for PGT does not seem to pose an increased risk of health problems in the resulting offspring compared with children born using IVF or ICSI without embryo biopsy and also after natural conception^{70,71}.

Cryopreserved embryos. RCTs comparing the number of cryopreserved embryos created using IVF and ICSI are lacking. However, results from RCTs and retrospective database studies do not suggest that ICSI would result in an increased number of embryos available for cryopreservation^{6,57,60}. Nevertheless, the findings from the sibling oocyte studies showing a lower TFF and a higher fertilization rate with ICSI than with IVF suggest a potentially higher number of cryopreserved embryos in the former group⁵⁸.

Tubal ligation. In a large retrospective study including more than 7,000 cycles, Grimstad et al. compared IVF ($n=3,956$) and ICSI ($n=3,189$) as methods of fertilization in a cohort of women with tubal ligation. For the first autologous fresh cycle, the researchers found that ICSI resulted in a poorer LBR per cycle than IVF (33.0% versus 39.6%, $P<0.0001$)⁷⁶.

Serodiscordant couples. In serodiscordant couples with a seropositive male partner, vertical transmission of HIV or hepatitis C virus (HCV) is extremely rare, if it happens at all, when semen washing is performed before IUI, IVF, or ICSI^{77,78}. Some researchers have argued that ICSI poses less risk of HIV and HCV transmission than IUI and conventional IVF because it uses a single spermatozoon⁷⁹. However, in a 2016 meta-analysis of studies of HIV-discordant couples in which the male partner was infected, which involved 11,585 treatment cycles in 3,994 women, no women or newborns acquired HIV following IUI, IVF, or ICSI with washed semen. In this study, the per cycle HIV transmission (0%) was lower ($P<0.001$) than the historical HIV transmission risk estimate of 0.1% per act of unprotected vaginal intercourse⁷⁷. In addition, the proportions of couples achieving pregnancy by IUI (56.4%, 95% CI 54.2–58.5%) and ICSI (58.1%, 95% CI 55.0–61.1%) were not different⁷⁷. A 2012 study evaluating 85 HCV serodiscordant couples with a seropositive male partner in Italy reported that pregnancy outcomes with IUI and ICSI were similar to those in the Italian ART registry. In this study, IUI

and ICSI were performed with washed sperm, and no mothers or babies born were infected with HCV⁷⁸. These observations indicate that IUI with washed semen offers an effective, safe, and less costly method than ICSI for preventing HIV transmission among HIV-discordant couples wishing to have children.

Managing male factor infertility before ICSI

The striking evolution of ART in the past few decades has clearly influenced urological practice^{18,80}. In the era of ICSI, the work-up of the female partner remains relevant because she is the one who undergoes ovarian stimulation, oocyte collection, and embryo transfer and ultimately carries the embryo and fetus. Remarkable attention is invested in improving embryo quality and pregnancy outcome after ART, but the value of proper male evaluation and treatment is overlooked as ICSI can provide the couple with a baby without the need to explain the nature or cause of underlying male infertility. Despite its obvious success for overcoming all forms of male factor infertility, the LBRs reported for ICSI are only around 30–40%^{6,12}. Furthermore, recent studies suggest that low sperm quality might adversely influence ICSI outcomes^{81–84}. The reasons are not entirely understood, but it has been speculated that the underlying genetic and epigenetic components associated with the impaired sperm characteristics might be the leading cause of poor ICSI outcomes with the use of abnormal sperm. Indeed, it seems that the information concerning the DNA quality is the most important for subsequent syngamy and embryogenesis⁸⁵. Sperm with poor-quality chromatin can hinder fertilization, early embryo development, implantation, and pregnancy through its effects on the integrity of the embryonic genome^{56,86–88}. Moreover, abnormal sperm might pass on *de novo* genetic and epigenetic changes to the conceptus, potentially compromising the health of the offspring⁸⁹. The notion that impairment of sperm chromatin integrity is associated with potentially correctable conditions, including lifestyle and gonadotoxin exposure, varicocele, and male accessory gland infections⁹⁰, has prompted the treatment of these underlying conditions as a means of improving ICSI outcomes.

Varicocelectomy

The negative effect of varicocele on semen characteristics and sperm DNA integrity is well established^{91,92}. The testis can respond to varicocele-associated cell stressors, such as heat stress, ischaemia, or production of vasodilators (for example, nitric oxide), by generating excessive amounts of reactive oxygen species (ROS), which might ultimately lead to sperm chromatin damage⁹³. In a multicentre study involving 593 infertile men attending infertility clinics, sperm chromatin damage rates were higher in all male factor aetiology categories than in fertile controls⁹⁰. In this study, SDF values were higher in men with clinical varicocele (mean $35.7 \pm 18.3\%$) and those with leukocytospermia (mean $41.7 \pm 17.6\%$) than in fertile controls (mean $11.3 \pm 5.5\%$; $P < 0.000$).

A number of studies have shown that varicocelectomy alleviates oxidative stress and enhances sperm

chromatin integrity^{92,94,95}. Varicocele repair has also been suggested to lead to improvements in ART outcomes. In one study, Esteves et al. examined the effect of varicocelectomy in a group of infertile men with clinical varicocele undergoing ICSI⁹⁶. In total, 80 patients underwent subinguinal microsurgical varicocelectomy before ICSI, and 162 had ICSI with untreated varicocele. In this study, the likelihood of achieving clinical pregnancy was significantly increased in the varicocelectomy group (OR 1.82, 95% CI 1.06–3.15; $P = 0.03$), as was the likelihood of a live birth (OR 1.87, 95% CI 1.08–3.25; $P = 0.03$), and the likelihood of miscarriage was decreased (OR 0.433, 95% CI 0.22–0.84; $P = 0.01$). Moreover, a 2016 systematic review and meta-analysis including 4 retrospective studies and 870 cycles comparing ICSI outcomes between treated and untreated men with varicocele showed that LBRs (OR = 2.17, 95% CI 1.55–3.06; $P < 0.00001$) and CPRs (OR = 1.59, 95% CI 1.19–2.12; $P = 0.002$) were increased in the varicocelectomy group⁹⁷. These results have been corroborated by Kirby et al., who aggregated the evidence from 4 studies involving a total of 718 patients with oligozoospermia and clinical varicocele who underwent ICSI. In this study, varicocele repair improved LBR (OR = 1.69, 95% CI 1.02–2.83; $P = 0.042$)⁹⁸. Improvements in sperm function, including sperm chromatin integrity, have been hypothesized to explain the observed beneficial effect of prior varicocelectomy on ICSI outcomes^{97,98}.

Varicocele repair has also been shown to improve sperm retrieval rates (SRRs) in patients with clinical varicocele and nonobstructive azoospermia. A meta-analysis of 3 controlled studies involving 400 patients indicated that the likelihood of harvesting sperm by micro-TESE was increased in men who underwent varicocele repair compared with men with untreated varicocele (OR 2.65, 95% CI 1.69–4.14, $P < 0.001$)⁹⁹. In this study, a tendency towards higher ICSI CPRs and LBRs was observed with the use of testicular sperm retrieved from men with treated varicocele than with the use of sperm from untreated men (clinical pregnancy OR 2.07, 95% CI 0.92–4.65, $P = 0.08$; live birth OR 2.19; 95% CI 0.99–4.83, $P = 0.05$). These results have been corroborated by another meta-analysis involving 202 men with azoospermia and clinical varicocele. In this study, SRRs (OR = 2.509, $P = 0.0001$) and pregnancy rates (OR = 2.33, 95% CI 1.02–5.34; $P = 0.04$) were increased among men who had undergone varicocele repair⁹⁸.

Leukocytospermia

In vitro and in vivo studies confirm that activated seminal leukocytes generate a significant amount of ROS, thereby causing oxidative stress and affecting sperm DNA integrity^{100,101}. Furthermore, SDF rates have been shown to be lower in men with bacteriospermia and leukocytospermia after antibiotic treatment than before antibiotic treatment ($38.6 \pm 18.7\%$ versus $50.4 \pm 19.1\%$; $P < 0.001$)¹⁰².

Nonetheless, the effect of leukocytospermia on ICSI outcomes is poorly studied. In a large retrospective cohort study involving more than 1,900 couples, 3,500 oocyte retrievals, and 802 clinical pregnancies, the authors found no detrimental effect of elevated

seminal leukocyte levels on fertilization, embryo development, or pregnancy rates¹⁰³. These results have been corroborated by a 2015 prospective ICSI study involving men with an increased number of leukocytes in their seminal fluid¹⁰⁴. In both reports, ICSI outcomes were not significantly affected by leukocyte level in the seminal fluid^{103,104}. In the 2015 study, the authors performed multivariate logistic regression analysis and found that the ART outcomes were not significantly different between the leukocytospermia and non-leukocytospermia groups after adjustment for female age, infertility diagnosis, number of previous attempts, treatment protocol, and ART method (IVF or ICSI)¹⁰⁴. Despite these observations, data on ICSI outcomes in treated and untreated men with leukocytospermia are lacking.

Antioxidants

Oral antioxidant therapy, including vitamins E and C, carotenoids, zinc, and selenium, scavenges excess free radicals by enhancing seminal antioxidant capacity¹⁰⁵. Improvements in semen characteristics, oxidative stress markers, SDF, and fertilization rates have been observed with the use of oral antioxidant therapy in men with idiopathic and unexplained infertility or varicocele, although the evidence is not unequivocal^{49,94}. Furthermore, a Cochrane meta-analysis on the use of oral antioxidant therapy in infertile men found that these agents significantly improved pregnancy rates and LBRs¹⁰⁶. This study aggregated the results of 48 RCTs and included a total of 4,179 infertile men, although only 2 trials enrolled men who were undergoing IVF or ICSI. The pooled results from these trials suggested that the use of antioxidants was associated with an increase in LBRs (OR 3.61, 95% CI 1.27–10.29, $P=0.02$; 2 RCTs, 90 patients)¹⁰⁶. Owing to the limited data concerning oral antioxidant therapy in male patients undergoing ART, more research is needed to evaluate not only the clinical utility of oral antioxidant therapy but also the ideal regimen and duration.

Obesity

Obesity has been postulated to be a contributing factor to poor semen quality¹⁰⁷. The mechanisms by which obesity affects male fertility are not entirely understood, but a multifactorial nature is suggested^{107,108}. Increases in peripheral conversion of testosterone to oestrogen due to excess adipose tissue might lead to inhibition of the hypothalamic–pituitary–gonadal axis and result in secondary hypogonadism. Additionally, increased levels of ROS are observed in obese men. Lastly, increased testicular temperatures owing to suprapubic fat might impair spermatogenesis^{107,108}.

In a retrospective cohort study, Bakos et al. evaluated 305 couples undergoing IVF or ICSI cycles and found no association between paternal BMI and fertilization rate, cleavage rate, or the morphological grade of embryos, both overall and after stratifying the method of fertilization, IVF or ICSI¹⁰⁹. However, increased paternal BMI was associated with decreased pregnancy rates ($P<0.01$) and increased miscarriage rates ($P<0.05$), irrespective of whether IVF or ICSI was used. The LBRs per oocyte

retrieval according to paternal BMI were 41.3% for men with normal BMI, 26.4% for overweight men, 22.6% for obese men, and 12.1% for morbidly obese men ($P<0.05$)¹⁰⁹. In another retrospective study evaluating a total of 290 ART cycles, the likelihood of clinical pregnancy after IVF was decreased in couples in which the male partner was overweight (BMI $>25\text{ kg/m}^2$) (OR 0.21, 95% CI 0.07–0.69), but no such association was noted with ICSI (OR 0.75, 95% CI 0.38–1.49)¹¹⁰. The largest cohort study investigating the effects of male and female BMI on the outcomes of IVF and ICSI included 12,061 treatment cycles¹¹¹. The authors found no association between male or female BMI and LBR in couples undergoing ICSI¹¹¹. These results were corroborated by a 2016 meta-analysis that pooled 10 cohort studies involving 5,262 men. In this study, being overweight or obese had no effect on LBR with either IVF (OR 0.91, 95% CI 0.78–1.06) or ICSI (OR 1.00, 95% CI 0.50–1.99)¹¹². At present, the association between BMI and ICSI outcomes remains equivocal, but no study has yet evaluated the impact of paternal weight loss on ICSI outcomes in this population.

Empirical medical therapy

Several hormonal and nonhormonal agents have been used empirically to treat male infertility. Gonadotropins, aromatase inhibitors, and selective oestrogen receptor modulators (SERMs) have all been attempted with inconsistent results¹¹³. Gonadotropin therapy is the gold standard treatment for infertile men with hypogonadotropic hypogonadism; a positive pregnancy outcome, either natural or assisted, can often be achieved in this group of patients¹¹⁴. In men with oligozoospermia and excessive aromatase activity, aromatase inhibitors and antioestrogens have been shown to increase sperm counts, but no data are available concerning ICSI outcomes¹¹⁵. Hormone therapy to boost intratesticular testosterone production before sperm retrieval has also been attempted in men with nonobstructive azoospermia with mixed results^{17,116–118}. However, a subset of men with non-obstructive azoospermia and low levels of total testosterone ($<300\text{ ng/dl}$) or a testosterone:oestradiol ratio <10 and, in particular, men with Klinefelter syndrome might benefit from human chorionic gonadotropin-based therapy and SERM, alone or in combination with aromatase inhibitors, to optimize SRR¹¹⁹.

Nevertheless, studies evaluating the role of empirical medical therapy in men with idiopathic infertility as a means of improving ICSI outcomes are scarce^{120,121}. In a 2013 meta-analysis, Attia et al. evaluated 6 RCTs comparing hormone treatment with placebo in a total of 456 men with idiopathic infertility¹²². Increased natural pregnancy rates were noted in the treatment group (OR 4.94, 95% CI 2.13–11.44), but no significant differences were observed in pregnancy rates with hormone treatment among couples undergoing IUI or ICSI¹²². However, the meta-analysis included a single RCT involving only 44 patients evaluating ICSI outcomes in patients who either received or did not receive pretreatment with hormones¹²³. In another small prospective observational study, Caroppo et al.

evaluated the effects of a 3-month course of recombinant human follicle-stimulating hormone (rec-hFSH) in 23 men with idiopathic oligoasthenoteratozoospermia who failed to impregnate their partners after previous ICSI attempts¹²⁴. A pregnancy rate of 30.4% was achieved in the treated group, whereas no pregnancies were recorded in the control group, which comprised ten men¹²⁴. A 2012 RCT evaluated the effects of rec-hFSH treatment (150 international units (IU) on alternate days for 90 days) versus non-antioxidant vitamin supplements on SDF rates in 65 men with idiopathic oligoasthenoteratozoospermia. The SDF rates were significantly lower in the treatment group than in the control group (12.6% versus 23.9%, respectively; $P < 0.05$)¹²⁵. These findings were corroborated by Simoni et al., who conducted a prospective study involving 89 men with idiopathic infertility and elevated SDF rates treated with 150 IU of rec-hFSH every other day for 12 weeks¹²⁶. The authors found a significant decrease ($P = 0.02$) in SDF rates in treated patients who were carriers of the homozygous N polymorphism of *FSHR* p.N680S¹²⁶. ICSI outcomes were not assessed, however, in either study of rec-hFSH.

Collectively, limited data are available concerning the clinical utility of surgical and medical interventions before ICSI. The best available evidence concerns clinical varicocele, in which surgical repair has been shown to improve ICSI pregnancy rates and SRR in selected men. Some evidence suggests that medical therapy used to boost testosterone production might increase the chances of harvesting sperm in nonobstructive azoospermia hypogonadal men, particularly those diagnosed with Klinefelter syndrome. Likewise, treatment of subfertile men with oral antioxidant therapy is suggested to enhance the odds of pregnancy in the overall ICSI population. As for empirical medical therapy using gonadotropins, aromatase inhibitors, or SERM before ICSI, the existing evidence indicates that if any benefit exists, it is limited to men with idiopathic infertility. However, the conclusions above are overwhelmingly based on findings from cohort studies. Given the apparent association between underlying male infertility conditions and impaired ICSI results, a comprehensive urological evaluation remains important to identify the causes of infertility and enable treatment to potentially improve ICSI outcomes. Nonetheless, further research is needed to determine the exact role of medical and surgical interventions for subfertile men undergoing ICSI.

Endocrine disorders

Thyroid dysfunction is associated with altered spermatogenesis, poorer semen quality, and sexual dysfunction¹²⁷. The underlying mechanisms are not entirely understood but seem to involve decreased sex hormone binding globulin (SHBG) levels and secondary hypogonadism^{128,129}. Hyperprolactinaemia might also lead to secondary hypogonadism and infertility. High prolactin levels impair both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) production by inhibiting the pulsatile secretion of gonadotropin-releasing hormone. Furthermore, hyperprolactinaemia has a direct inhibitory effect on spermatogenesis and steroidogenesis

through its actions on prolactin membrane-bound receptors present on Sertoli and Leydig cells¹³⁰. Lastly, diabetes and insulin resistance have been associated with an increased risk of male infertility, particularly when associated with obesity and metabolic syndrome, owing to effects on spermatogenesis and sperm DNA integrity^{108,131}. Notwithstanding the association between endocrine disorders and male infertility, no studies have examined the consequences of such conditions on ICSI outcomes nor the possible benefit of their treatment before ICSI.

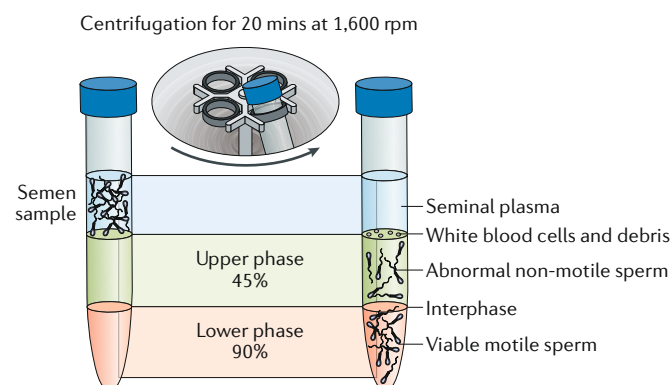
Methods of ICSI

ICSI with ejaculated sperm

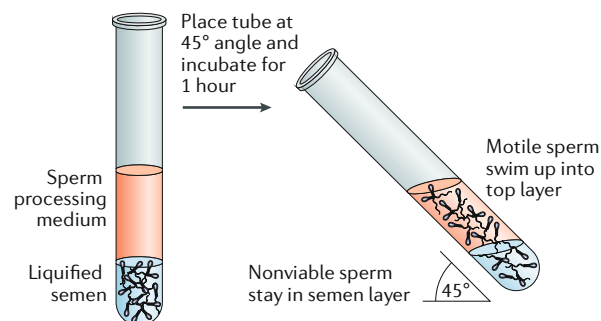
ICSI is similar to conventional IVF in that oocytes and spermatozoa are collected from the partners. A period of ovarian stimulation usually using gonadotropins leads to multifollicular development in the ovary, which is followed by oocyte retrieval, fertilization, and embryo development in vitro¹³². One or more embryos either at the cleavage or blastocyst stage are then transferred to the uterine cavity or cryopreserved. The difference between ICSI and IVF is the method used to achieve fertilization. In conventional IVF, oocytes are incubated with sperm in a Petri dish, and the male gamete fertilizes the oocyte naturally (FIG. 1b). In ICSI, the cumulus–oocyte complexes go through a denudation process in which the cumulus oophorus and corona radiata cells are removed mechanically or by an enzymatic process. This step is essential to enable microscopic evaluation of the oocyte regarding its maturity stage, as ICSI is performed only in metaphase II oocytes¹⁴. A thin and delicate glass micropipette (injection needle) is used to immobilize and pick up morphologically normal sperm selected for injection. A single spermatozoon is aspirated by its tail into the injection needle, which is inserted through the zona pellucida into the oocyte cytoplasm. The spermatozoon is released at a cytoplasmic site sufficiently distant from the first polar body. During this process, the oocyte is held still by a glass micropipette¹⁴ (FIG. 1a).

ICSI is carried out using viable sperm populations. A number of semen processing techniques have been developed to select the optimal sperm fraction for ICSI (FIG. 3). Density gradient centrifugation (DGC) and swim-up procedures have been used as standard semen preparation techniques for ICSI for more than two decades¹³³ (FIG. 3a,b). In general, the methods are based on the principle of removing contaminants (cellular debris, microorganisms, red blood cells, and exfoliating cells). DGC is performed using continuous or discontinuous gradients. In brief, the liquefied ejaculate is usually placed on top of a series of two or three colloidal gradients with different densities (for example, 40% and 80%) and subjected to centrifugation. The highly active spermatozoa move towards the sedimentation gradient and are enriched in the soft pellet at the bottom. The potential advantages of DGC over swim-up procedures include the elimination of leukocytes, debris, and antisperm antibodies¹³⁴. Moreover, a clean fraction of highly motile spermatozoa is obtained, and the method can be applied to low-quality ejaculates. Swim-up procedures are the standard technique for semen preparation in patients

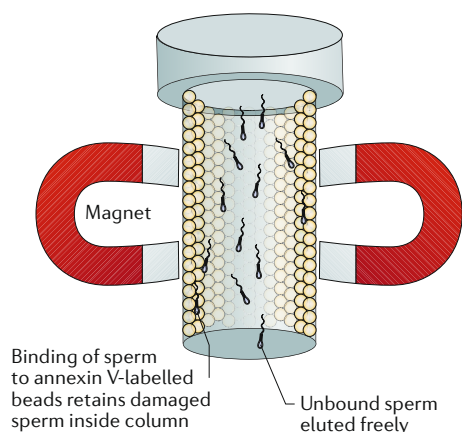
a Density gradient centrifugation



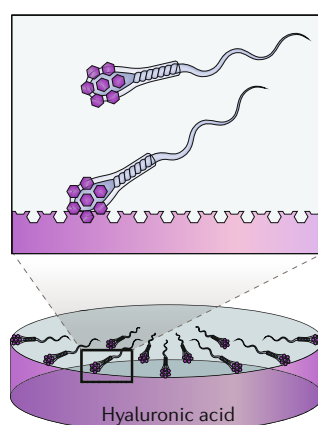
b Swim-up



c Magnetic-activated sperm sorting



d Hyaluronic acid binding



e Motile sperm organelle morphology examination

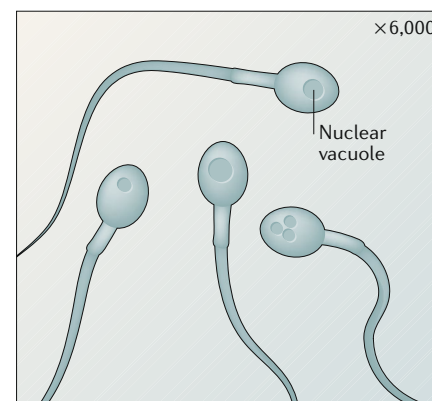


Fig. 3 | Sperm selection methods. **a** | Density gradient centrifugation. Liquefied semen is placed on top of a series of two colloidal gradients with 45% and 90% densities and subjected to centrifugation. The highly active spermatozoa move towards the sedimentation gradient and are enriched in the soft pellet at the bottom. **b** | Swim-up. Liquefied semen is placed beneath an overlay of sperm medium. During the incubation period, motile sperm migrate from the underlayer sperm suspension to the upper layer. The supernatant contains actively motile spermatozoa. **c** | Magnetic-activated sperm cell sorting. The activated magnetic field retains the apoptotic sperm bound to micromagnetic beads coated with annexin V in the column and allows the nonapoptotic healthy sperm cells to flow through the selection column. **d** | Hyaluronic acid binding. Mature sperm bind to hyaluronic acid — a main component of the cumulus cells — whereas immature sperm do not. **e** | Motile sperm organelle morphology examination. Images of the spermatozoa are captured by digitally enhanced light microscope using Nomarski optics that magnify by at least $\times 6,000$. Morphological evaluation is then carried out, and spermatozoa are graded and selected based on the shape and size of nuclear vacuoles. Part **a** adapted from REF.²⁷³, Macmillan Publishers Limited, CC BY 4.0. Part **d** adapted from REF.²⁷⁴ with permission from Ass. Prof. Igor Crha, CS., Faculty of Medicine, Masaryk University, created in collaboration with Service Center for E-Learning, Faculty of Informatics, Masaryk University, Czech Republic.

with normozoospermia. Swim-up involves placing liquefied semen or centrifuged sperm pellets beneath an overlay of sperm medium. During the incubation period, motile sperm migrate from the underlayer sperm suspension to the upper layer. The supernatant contains actively motile spermatozoa¹³⁵. Despite being easy to perform, swim-up might not be suitable for recovering sperm for ICSI in low-quality ejaculates¹³².

Overall, the traditional sperm selection techniques described above are unable to select sperm fractions with optimal DNA integrity and functional characteristics. Advanced sperm selection techniques have now been introduced to optimize the selection of high-quality sperm for ICSI¹³⁶. These selection methods are based on sperm surface charge (electrophoresis and

Zeta potential), apoptosis (magnetic-activated sperm cell sorting (MACS) and glass wool), membrane maturity (hyaluronic acid binding), or ultramorphological sperm assessment¹³⁷ (FIG. 3).

Electrostatic charge-based sperm separation uses an electric field to isolate sperm with different levels of electrostatic potential, which are proportional to the levels of sialic acid residue acquired on the cell surface during maturation¹³⁸. As the membrane glycocalyx of normal mature sperm is rich in sialic acid and the high levels of sialic acid residues on the sperm membrane increase its net negative charge, mature sperm can be selected based on their negative Zeta electrokinetic potential¹³⁹. The method known as Zeta potential is simple and based on the adherence of sperm to surfaces (such as the plastic

tube wall, ICSI needle, and Petri dishes) in a protein-free medium. The Zeta potential was shown to be higher in mature sperm that have low levels of histone retention¹⁴⁰. In an RCT involving a total of 203 ICSI cycles, the Zeta method was compared with DGC. The authors found no difference in fertilization rates ($77.9 \pm 1.9\%$ versus $76.9 \pm 2.1\%$) between groups, but the Zeta group showed a significant increase in the proportion of top quality embryos ($45.8 \pm 3.1\%$ versus $35.4 \pm 4.6\%$; $P = 0.04$) and a higher CPR (39.2% versus 21.8% ; $P = 0.009$)¹⁴¹. However, the implantation rate was not significantly different between the Zeta and DGC groups (21.0% versus 12.7% , respectively)¹⁴¹. Despite preliminary favourable results, the Zeta method is associated with low sperm recovery in patients with oligozoospermia. Furthermore, the method might not be suitable for selecting testicular or caput epididymal sperm because sperm from these sources lack sufficient membrane net electrical charge^{140,142}.

Sperm selection by MACS is based on the principle that apoptotic sperm exhibit externalization of the phospholipid phosphatidylserine (FIG. 3c). Annexin V, a calcium-dependent phospholipid-binding protein with a high affinity for phosphatidylserine, is applied to separate apoptotic from nonapoptotic sperm^{143,144}. A meta-analysis of five RCTs compared ICSI outcomes between MACS and conventional sperm selection techniques. A higher pregnancy rate was observed with MACS (4 studies; RR 1.50, 95% CI 1.14–1.98), but no differences were noted with regards to implantation and miscarriage rates¹⁴⁵. Furthermore, the clinical utility of MACS for improving ICSI outcomes was questioned in a prospective ICSI study involving 237 couples that found no benefit of MACS sperm selection on LBR or implantation rate¹⁴⁶.

Hyaluronic acid sperm binding relies on the assumption that human sperm that bind to hyaluronic acid exhibit normal shape, minimal DNA fragmentation, and low frequency of chromosomal aneuploidies¹⁴⁷ (FIG. 3d). A Cochrane review evaluated the effect of sperm selection using hyaluronic acid binding on ICSI outcomes. Two RCTs compared sperm selection by hyaluronic acid binding with conventional ICSI. No differences were observed in CPRs (RR 1.01, 95% CI 0.84–1.22, 1 RCT, 482 women), LBRs (RR 1.16, 95% CI 0.65–2.05, 1 RCT, 99 women), and miscarriage rates (RR 0.76, 95% CI 0.24–2.44, 1 RCT, 41 women)¹⁴⁵. These results were corroborated by a larger meta-analysis of 7 studies and 1,437 ICSI cycles. The authors did not find improvements in fertilization rate (RR 1.02, 95% CI 0.99–1.06) or pregnancy rate (RR 1.10, 95% CI 0.93–1.30) with sperm selection using hyaluronic acid compared with conventional sperm selection for ICSI¹⁴⁸.

Lastly, ultramorphological sperm assessment using motile sperm organelle morphology examination applies high magnification (at least $\times 6,000$) to select sperm based on organelle morphology (FIG. 3e). Morphological anomalies in the sperm head–neck attachment have been suggested to be linked to abnormal centriolar function and might be associated with defective fertilization¹⁴⁴. However, the studies comparing intracytoplasmic morphologically selected sperm injection (IMSI) with conventional ICSI have yielded conflicting results. Some

investigators have demonstrated improvements in blastocyst development^{149,150}, implantation rate, and CPR with IMSI in couples with male factor infertility^{151–153}, but a 2013 Cochrane review concluded that the results from the existing RCTs do not support the use of IMSI in routine practice¹⁵⁴. In a 2016 retrospective study investigating the effectiveness of IMSI — among other sperm selection methods — in men with high SDF, no differences were observed with regards to LBR, miscarriage rate, or pregnancy rates between IMSI and conventional ICSI¹⁵⁵.

In summary, existing evidence is insufficient to recommend advanced sperm selection techniques for use in ICSI in clinical practice. Further studies are required to determine whether any of these advanced sperm selection techniques could be recommended in specific subgroups of men with low semen quality undergoing ICSI.

ICSI with extracted sperm in azoospermia

Reconstruction of the seminal tract is a cost-effective treatment in men with obstructive azoospermia, but this approach might not be feasible or desired by the couple¹⁶. In such cases, sperm retrieval and ICSI with epididymal or testicular sperm offers the possibility of parenthood. Unlike obstructive azoospermia, non-obstructive azoospermia is usually an untreatable condition associated with spermatogenic failure. In such cases, testicular sperm retrieval and ICSI might be the only hope for biological fatherhood.

Sperm retrieval. Percutaneous epididymal sperm aspiration (PESA) and MESA are the most commonly used methods to harvest epididymal sperm¹⁸ (FIG. 4). By contrast, testicular sperm aspiration (TESA) and open testicular sperm extraction with or without the aid of microsurgery (micro-TESE and TESE, respectively) are the methods used to retrieve testicular sperm¹⁵⁶ (FIG. 4). These procedures can be performed on an outpatient

Fig. 4 | Sperm retrieval methods. a | Microsurgical epididymal sperm aspiration (MESA). A dilated epididymal tubule is dissected and opened. Fluid is aspirated, diluted with sperm medium, and sent to the laboratory for examination. **b |** Microsurgical testicular sperm extraction (micro-TESE). After the testicle is exteriorized, a single large incision is made in an avascular area of the albuginea to expose the seminiferous tubules. The dilated tubules are identified and removed with microforceps. The illustration shows histopathological cross sections of a dilated seminiferous tubule with active spermatogenesis (*) and a thin tubule with germ cell aplasia (†). **c |** Percutaneous epididymal sperm aspiration (PESA). The epididymis is stabilized between the index finger, thumb, and forefinger. A 23 G needle attached to a tuberculin syringe is inserted into the epididymis through the scrotal skin, and fluid is aspirated. **d |** Testicular sperm aspiration (TESA). A 13 G needle connected to a 20 ml syringe fit to the Cameco holder is percutaneously inserted into the testis. Negative pressure is created, and the tip of the needle is moved within the testis to disrupt the seminiferous tubules and sample different areas. The testicular parenchyma is aspirated. Parts **a**, **c**, and **d** reproduced with permission from REF.¹⁸, Clinics, CC BY-NC 3.0. Part **b** reproduced with permission from REF.⁸⁰, Clinics, CC BY-NC 3.0.

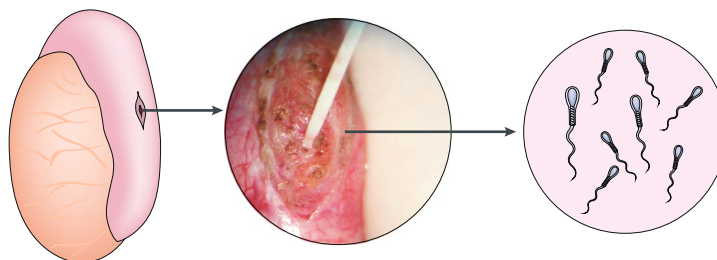
basis with the intention to cryopreserve sperm for future use or in association with oocyte retrieval and immediate sperm injection¹⁸.

In men with obstructive azoospermia, spermatogenesis is normal and sperm can be easily retrieved from the epididymis or testis. The sperm retrieval technique and the cause of obstructive azoospermia seem to have

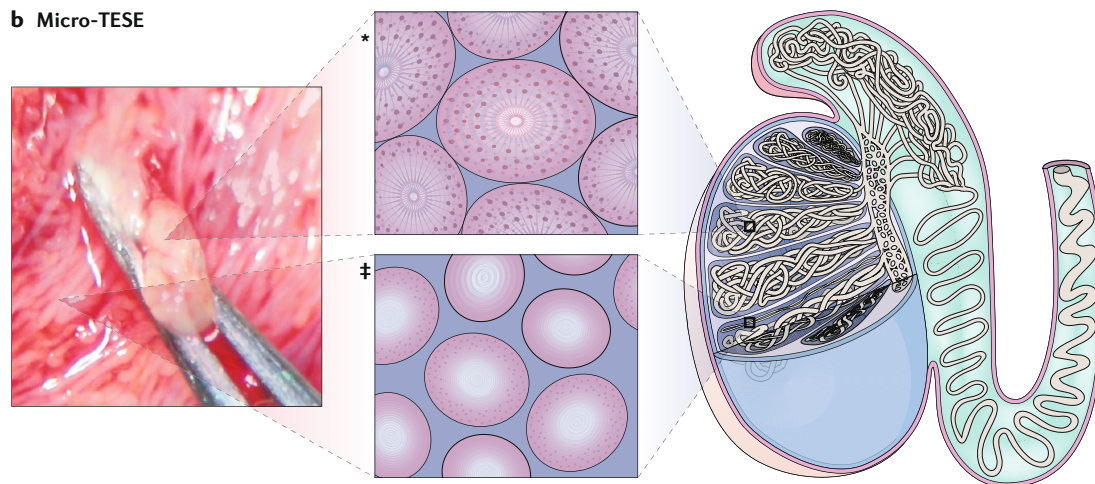
little effect on SRR and ICSI outcomes^{16,157}. However, MESA yields a higher number of motile sperm than does PESA, thereby offering the possibility of cryopreserving larger quantities of sperm that might enable multiple ICSI cycles without the need for repeat sperm retrieval. However, MESA is more technically demanding than percutaneous retrieval methods^{18,156}.

Microsurgical sperm retrieval techniques

a MESA

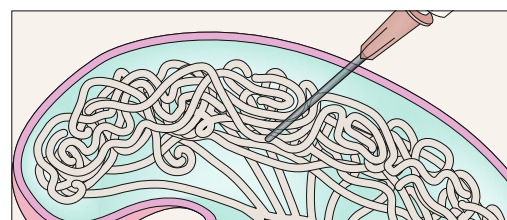


b Micro-TESE

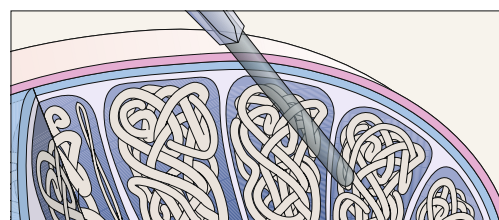


Percutaneous sperm retrieval techniques

c PESA



d TESA



In men with nonobstructive azoospermia, SRRs are higher and complication rates are lower with micro-TESE than with conventional TESE¹⁵⁸. Nonetheless, the likelihood of harvesting sperm with both approaches is related to testicular histology. Men with hypospermatogenesis and maturation arrest have a more favourable outcome than those with SCO syndrome^{17,18,20,84,156,159}. In a study involving a total of 365 men with non-obstructive azoospermia, SRRs were higher in patients with maturation arrest (40.3%) than in those with SCO syndrome (19.5%; $P=0.007$). SRR was lower in both of these categories than in men with hypospermatogenesis (100.0%; $P<0.001$ for both comparisons)¹⁵⁹. In this study, LBRs were lower in men with SCO syndrome (10.0%) than in those with hypospermatogenesis (23.8%) or maturation arrest (22.2%) after adjusting for covariates ($P=0.01$); however, the obstetrical outcomes (gestational age at birth and neonatal birthweight) of resulting offspring were not affected by the testicular histopathology categories.

Laboratory handling of epididymal and testicular sperm. Processing of surgically retrieved spermatozoa differs from the methods for handling ejaculated sperm³⁷. Laboratory technicians should ensure that specimens have minimal or no contaminants, such as red blood cells. Testicular parenchyma or epididymal aspirates should be carefully handled in the IVF laboratory, as these specimens might be more fragile than ejaculated counterparts¹⁷. Laboratory personnel should be well trained to minimize the iatrogenic cellular damage during sperm processing. Testicular tissue processing is carried out by mincing the seminiferous tubules mechanically and/or by incubating the specimens with enzymes (for example, collagenase) to disrupt the tubular structure and release sperm¹⁶⁰. If blood contamination is excessive, the specimen is incubated with erythrocyte-lysing solution to eliminate the contaminating red blood cells¹⁶¹. Lastly, when only immotile spermatozoa are available for ICSI, motility stimulants (for example, pentoxifylline) and other methods of sperm selection (for example, hypo-osmotic swelling test and sperm tail flexibility test) are used to select viable sperm for ICSI. After processing, aliquots of epididymal and testicular suspensions are used fresh for ICSI or are intentionally cryopreserved for future use³⁷. Cryopreservation of surgically retrieved specimens has traditionally been carried out by filling vials with the suspensions followed by vapour liquid nitrogen freezing. A novel method for freezing low sperm quantities has been introduced, in which a few spermatozoa can be frozen in microdroplets using nonbiological closed devices (cell sleepers)^{20,162,163}.

ICSI outcomes. Overall, pregnancy outcomes from ICSI are comparable between epididymal and testicular sperm and also between fresh and frozen-thawed epididymal sperm in men with obstructive azoospermia¹⁶⁴. However, the evidence is not unequivocal as shown by the results of a retrospective cohort study involving 374 men with obstructive azoospermia undergoing ICSI with either epididymal or testicular sperm¹⁶⁵. In this study, LBRs

were higher with epididymal sperm than with testicular sperm (OR 1.82, 95% CI 1.05–3.67)¹⁶⁵.

Sperm injection outcomes with fresh or frozen-thawed testicular sperm have also been compared in men with nonobstructive azoospermia. In a meta-analysis of 11 studies and 574 ICSI cycles, no statistically significant difference was observed between fresh and frozen-thawed testicular sperm with regards to fertilization rate (RR 0.97, 95% CI 0.92–1.02) and CPR (RR 1.00, 95% CI 0.75–1.33)¹⁶⁶. However, no meta-analysis was performed on data regarding implantation rate, miscarriage rate, and LBR.

The classification of azoospermia into obstructive azoospermia and nonobstructive azoospermia has a major influence on ICSI success. In a retrospective cohort study, Esteves and Agarwal compared ICSI outcomes using fresh surgically extracted sperm from men with obstructive azoospermia ($n=182$ cycles) and nonobstructive azoospermia ($n=188$ cycles) and compared the results with those from a general population of infertile men using freshly ejaculated sperm for ICSI ($n=621$ cycles). LBRs were lower in men with nonobstructive azoospermia (21.4%) than in those with obstructive azoospermia (37.5%) and in those from the general male infertility population using ejaculated sperm (32.3%; $P=0.003$). In this study, ICSI outcomes were comparable between obstructive azoospermia and ejaculated sperm groups⁸³. In another cohort study from the same group, ICSI outcomes were compared in men with nonobstructive azoospermia with successful ($n=365$) and failed ($n=40$) sperm retrieval by micro-TESE. In the former group, ICSI was carried out with testicular sperm, whereas in the latter group, donor sperm was used. LBRs in both groups were compared with those from a group of 186 men with obstructive azoospermia in whom epididymal or testicular sperm were used for ICSI. The adjusted odds ratio revealed that the chance of achieving a live birth was lower in men with nonobstructive azoospermia who had successful sperm retrieval than in those with nonobstructive azoospermia in whom donor sperm was used (OR 0.377, 95% CI 0.233–0.609; $P<0.001$) and in men with obstructive azoospermia (OR 0.403, 95% CI 0.241–0.676; $P=0.001$)⁸⁴. According to a large retrospective cohort study involving a total of 1,559 TESE-ICSI cycles, the predictors of a live birth after a successful TESE procedure included lower male LH level, increased male testosterone level, the use of motile spermatozoa for ICSI, and a diagnosis of obstructive azoospermia instead of nonobstructive azoospermia¹⁶⁷. The explanation for the less favourable ICSI outcomes observed with testicular sperm retrieved from men with nonobstructive azoospermia seems to be related to the increased tendency of such cells to carry deficiencies related to the centrioles and genetic material, which ultimately affect their capability to activate the oocytes and trigger the development of a viable embryo¹⁶⁸.

In summary, percutaneous and open sperm retrieval methods are highly effective for harvesting sperm from men with obstructive azoospermia. Open testicular sperm retrieval, in particular using a microsurgical approach, has been associated with an increased success

of sperm retrieval and a low rate of complications in men with nonobstructive azoospermia. The type of azoospermia, sperm status (fresh or frozen–thawed), and sperm source (epididymal or testicular) might influence ICSI outcomes. The type of azoospermia seems to be a major determinant of ICSI success, as the reproductive outcome is less favourable when ICSI is performed with testicular sperm from men with nonobstructive azoospermia than in those with obstructive azoospermia and in those from the general male infertility population using ejaculated sperm. By contrast, sperm status (fresh versus frozen–thawed) does not seem to have a major effect on ICSI results. The results of ICSI with fresh or frozen–thawed spermatozoa seem to be independent of sperm source (ejaculate, epididymis, or testis) and type of azoospermia (obstructive azoospermia versus nonobstructive azoospermia). Lastly, sperm source (epididymis versus testis) has no apparent effect on ICSI outcomes in men with obstructive azoospermia¹⁶.

Extracted sperm from non-azoospermic men

Evidence indicates that sperm chromatin integrity is higher in testicular sperm than in ejaculated sperm^{87,169,170}. Studies examining paired ejaculated and testicular specimens from the same men showed that SDF is threefold to fivefold lower in the testis than in the ejaculate^{87,170–173}. Furthermore, data indicate that ICSI with testicular sperm (Testi-ICSI) might be advantageous in select infertile men without azoospermia, particularly those with high SDF in semen^{155,169,170,173}. In a prospective comparative study, Esteves et al.¹⁷⁰ assessed ICSI outcomes with the use of ejaculated or testicular sperm in a cohort of 172 infertile men with elevated SDF. The authors enrolled infertile men with mild-to-moderate idiopathic oligozoospermia ($5\text{--}15 \times 10^6$ spermatozoa per ml) presenting with persistent high SDF ($>30\%$) despite the use of oral antioxidant therapy for 3 months. On the day of sperm collection for ICSI, SDF was reassessed using the sperm chromatin dispersion (SCD) test both in ejaculated and testicular specimens. The rates of SDF in the group of men who underwent sperm retrieval were fivefold higher in the semen ($40.7 \pm 9.9\%$) than in the testis ($8.3 \pm 5.3\%$; $P < 0.001$); in this group, all sperm injections were performed with testicular sperm. By contrast, SDF rates were $40.9 \pm 10.2\%$ in the group of patients who underwent ICSI with ejaculated sperm. Miscarriage rates were lower and LBRs were higher in the couples who underwent sperm injections with testicular sperm than in those in whom ICSI was performed with ejaculated sperm. The adjusted RRs for miscarriage and live birth between the testicular sperm group and the ejaculated sperm group were 0.29 (95% CI 0.10–0.82; $P = 0.019$) and 1.76 (95% CI 1.15–2.70; $P = 0.008$), respectively¹⁷⁰. In this study, the NNT with testicular rather than ejaculated sperm to obtain an additional live birth per fresh transfer cycle was 4.9 (95% CI 2.8–16.8). In another study, Bradley et al. retrospectively evaluated ICSI outcomes in a group of infertile men without azoospermia with high levels of SDF in semen¹⁵⁵. Sperm injections were performed with either ejaculated sperm or testicular sperm. In the ejaculated

sperm group, the authors applied interventions to deselect sperm with DNA fragmentation — IMSI and hyaluronic acid sperm selection ICSI (also known as physiological ICSI (PICSI)) — and compared outcomes with those of a control group that had no interventions. They also compared the results of ICSI using ejaculated sperm with intervention ($n = 220$ cycles), ICSI using ejaculated sperm without intervention ($n = 80$ cycles), and ICSI using testicular sperm (Testi-ICSI) ($n = 148$ cycles). Higher LBRs ($P < 0.05$) were obtained with Testi-ICSI (49.8%) than with IMSI (28.7%) or PICSI (38.3%). The lowest LBRs (24.2%) occurred when no intervention was carried out to deselect sperm with DNA fragmentation ($P = 0.020$)¹⁵⁵. Use of Testi-ICSI has been postulated to bypass post-testicular DNA fragmentation caused by oxidative stress during sperm transit through the epididymis. As a consequence, the chances of oocyte fertilization by genomically intact testicular spermatozoa are increased, thus resulting in an increased possibility of formation of a normal embryonic genome and an increased likelihood of achieving a live birth¹⁷⁴. A study published in 2017 aggregated the evidence concerning the use of testicular sperm for ICSI in men with high SDF in semen, and using a meta-analysis, the authors showed that testicular sperm have lower DNA fragmentation than ejaculated sperm and that the use of testicular sperm for ICSI is associated with improved outcomes¹⁷⁵.

However, whether the use of testicular sperm in preference to ejaculated sperm for ICSI is beneficial for men with cryptozoospermia (BOX 1) has not been confirmed. In a meta-analysis including 5 cohort studies with 272 ICSI cycles, no differences were observed in ICSI fertilization rates (RR 0.91, 95% CI 0.78–1.06) or pregnancy rates (RR 0.53; 95% CI 0.19–1.42) when comparing Testi-ICSI with ejaculated ICSI in men with cryptozoospermia¹⁷⁶. Testi-ICSI does seem to be beneficial over ICSI with ejaculated sperm to infertile men with high levels of SDF in the semen, with improvements reported in rates of pregnancy, miscarriage, and live birth^{155,170,177}. Notwithstanding the potential benefit of Testi-ICSI in this patient group, there are risks involved with sperm retrieval. The risks are low ($<5\%$) but include infection, haematoma, and testicular atrophy^{17,18,156,160,164,170,174,175}. Identification of the male factor associated with SDF might facilitate treatment aimed at alleviating SDF, thus potentially enabling natural conception or ICSI with ejaculated sperm^{56,178}.

Intracytoplasmic spermatid injection

Spermatids are the earliest male germ cells with a haploid set of chromosomes. The use of spermatids for ICSI has been attempted when mature sperm are not available¹⁷⁹. In animal models, spermatid injection has resulted in delivery of healthy offspring, but the process is far less efficient and efficacious than ICSI with mature sperm^{180,181}. Vanderzwalmen et al. were the first to report the use of spermatid injection in humans in 1995 (REF.¹⁸²). In the mid-1990s, the first deliveries after spermatid injection were reported by Tesarik et al.¹⁸³ and Fischel and co-workers¹⁸⁴. More than 20 years since these first observations, the clinical efficacy of spermatid injection

in humans has been disappointing overall; in fact, the birth of healthy infants by spermatid injection has been merely anecdotal¹⁷⁹. Several ethical and safety concerns have also been raised related to the injection of immature gametes, including genetic and epigenetic risks, which prompted the UK government to ban spermatid injection. In the USA, the ASRM Practice Committee classified spermatid injection as an experimental procedure¹⁸⁵. In 2015, Japanese scientists reported the birth of 14 babies after refinement of spermatid injection by activation with electric current. The resulting offspring were found to have no notable physical, mental, or epigenetic problems. However, the number of children born to date is too small and the follow-up period too short to consider this approach a safe option¹⁸⁶.

Risks and sequelae to offspring health

The widespread use of ICSI has raised concerns about the health and well-being of resulting offspring owing to its invasive nature that circumvents natural selection mechanisms and because of the related infertility conditions. The primary goal of ICSI is to obtain healthy infants, but concerns regarding a possible increased risk of congenital and urogenital malformations, epigenetic disorders, chromosomal abnormalities, infertility, cancer, delayed psychological and neurological development, and impaired cardiometabolic profile compared with naturally conceived children have emerged¹⁸⁷.

Parental sperm defects rather than the method of ART have been implicated as primary responsible factors for a possible increase in the conditions mentioned above. Indeed, the integrity of the sperm genome and epigenome is essential for the birth of healthy offspring¹⁸⁸. The sperm nuclear genome includes a central compact toroid comprising protamine-bound DNA that is both transcriptionally and translationally inert¹⁸⁹. The peripheral compartment, composed of histone-bound DNA, retains the nucleosomal structure and contains promoters for developmentally crucial genes, microRNAs, and signalling factors¹⁹⁰. The histone-bound DNA is highly susceptible to environmental insults, especially oxidative damage. Also, the sperm epigenome is maintained through the retention of histones, the compaction of considerable portions of the genome by protamines, DNA methylation, and covalent histone modifications. As the male gamete loses most cytosolic antioxidants during spermiogenesis, the cell is highly vulnerable to free radical-induced DNA damage. Low levels of key DNA repair enzymes might explain the persistence of DNA damage in ejaculated sperm from subfertile men exposed to in vitro conditions^{20,93}. The fertilization of oocytes by such sperm through ICSI might result in an increased risk of fertilization failure, embryo arrest, miscarriage, congenital malformations, childhood cancers, and perinatal morbidity^{56,191}.

Additionally, concerns exist about the invasive nature of the ICSI procedure. The sperm injection technique might compromise nuclear decondensation of spermatozoa, possibly leading to embryo aneuploidy¹⁹². Also, the microinjection pipette used to inject the spermatozoon into the oocyte cytoplasm may accidentally disrupt

the oocyte meiotic spindle, possibly leading to abnormal chromosomal segregation patterns¹⁹³. Furthermore, handling oocytes outside the incubator for prolonged periods of time, as occurs in ICSI, can alter, even slightly, the temperature and pH, which might increase the rates of stress-induced aneuploidy¹⁹⁴.

Congenital malformations

The evidence concerning the risk of congenital malformations among children conceived through ART is mixed (TABLE 2). Some evidence indicates that ART offspring are at increased risk compared with those naturally conceived^{195–197}, but other studies failed to show an increased risk of overall congenital malformation^{198,199} or urogenital abnormalities among the resulting offspring²⁰⁰. Along the same lines, a meta-analysis evaluating congenital malformations rates among twin pregnancies found no differences between infants conceived through ART treatments and those conceived naturally²⁰¹.

Nevertheless, the meta-analyses mentioned above^{195–201} included both prospective and retrospective cohort studies, and some patient cohorts were small. Furthermore, the definition of malformation was not uniform among studies, and some studies included control groups comprising fertile populations, which might overestimate the risk of congenital malformations in the comparison group solely because of factors associated with subfertility. Notably, a 2006 Danish national birth cohort study suggested that the increase in congenital malformation rates is related to the diagnosis of infertility or its determinants, such as a longer time to pregnancy²⁰². According to this study, the risk of congenital malformations is increased by 20% in children conceived naturally by couples diagnosed with subfertility compared with children conceived by fertile couples.

Another important question is whether the ICSI procedure, in particular, affects the risk of congenital malformations and long-term health of the offspring when used for non-male indications. The literature is scant regarding this issue, most likely because the use of ICSI for non-male indications has found its way into reproductive medicine only in the past two decades, driven by either concern of fertilization failure during the first IVF attempt or financial considerations. In a retrospective cohort study focusing on autism in live-born infants conceived using ART in California between 1997 and 2006 ($n = 42,383$), the authors reported that ICSI in non-male factor infertility was associated with an increased risk of autism (adjusted HR 1.57; 95% CI 1.18–2.09) when compared with conventional IVF²⁰³. Furthermore, data from the US National ART Surveillance System from 1996 to 2012 reported that ICSI in non-male factor infertility was associated with lower birthweight as well as a lower multiple birth rate than with conventional IVF⁶. In this study, the outcomes of ICSI for male versus non-male infertility indications were not different with regard to implantation rate, pregnancy rate, miscarriage rate, multiple LBR, preterm delivery rate, and low birthweight⁶; however, congenital malformations and other

Table 2 | Studies evaluating congenital malformations in ART and non-ART offspring

Study	Design	Study group (n)	Control group (n)	Outcome measures	Findings	
					ART versus NC infants	ICSI versus IVF infants
Rimm et al. (2011) ¹⁹⁹	Meta-analysis after adjustment	ART infants (NR)	NC infants (NR)	Major congenital malformations after adjusting for the effect of subfertility	No difference in risk of congenital malformations between ART and NC infants after adjustment (RR 1.01; 95% CI 0.82–1.23)	NR
Rossi and D'Addario (2011) ²⁰¹	Systematic review and meta-analysis	ART twins (1,556)	NC twins (2,729)	Congenital malformations in twins	No difference in risk of congenital malformations between ART and NC twins (RR 1.15; 95% CI 0.80–1.63)	NR
Pandey et al. (2012) ¹⁹⁵	Systematic review and meta-analysis	ART infants (4,382)	NC infants (5,324)	Congenital malformations in singletons	Infants conceived by ART had a higher risk of congenital malformations than their NC counterparts (RR 1.67; 95% CI 1.33–2.09)	NR
Wen et al. (2012) ¹⁹⁸	Systematic review and meta-analysis	ART infants (124,468); ICSI infants (27,754)	NC infants (unknown) and IVF infants (46,890)	Congenital malformations	Higher risk of congenital malformations among ART infants than NC infants (RR 1.37; 95% CI 1.26–1.48)	- No difference in risk of congenital malformations between IVF and ICSI (RR 1.05; 95% CI 0.91–1.20) - Increased risk of congenital malformations among IVF infants versus NC infants (RR 1.30; 95% CI 1.17–1.46) and ICSI infants versus NC infants (RR 1.58; 95% CI 1.27–1.95)
Hansen et al. (2013) ¹⁹⁶	Systematic review and meta-analysis	ART infants (92,671)	NC infants (3,870,760)	Congenital malformations and major congenital malformations	- Higher risk of congenital malformations (RR 1.32; 95% CI 1.24–1.42) and major congenital malformations (RR 1.42; 95% CI 1.29–1.56) in ART infants than NC infants - Increased risk of congenital malformations in ART singletons compared with NC singletons (RR 1.36; 95% CI 1.30–1.43) and ART twins compared with NC twins (RR 1.26; 95% CI 0.99–1.60)	NR
Qin et al. (2015) ¹⁹⁷	Systematic review and meta-analysis	ART infants (119,874)	NC infants (1,212,320)	Congenital malformations	- Increased risk of congenital malformations in ART infants versus NC infants (RR 1.33; 95% CI 1.24–1.43) - Increased risk of congenital malformations in ART infants confirmed when data restricted to singletons (RR 1.38; 95% CI 1.30–1.47), major congenital malformations (RR 1.47; 95% CI 1.29–1.68), matched and adjusted studies (RR 1.37; 95% CI 1.27–1.47), and high-quality studies (RR 1.40; 95% CI 1.27–1.55) - Increased risk of congenital malformations in twins (RR 1.18; 95% CI 1.06–1.32) and multiple births in ART infants compared with NC infants (RR 1.16; 95% CI 1.05–1.27)	- Outcomes of IVF versus ICSI infants not reported - Increased risk of congenital malformations in IVF versus NC infants (RR 1.29; 95% CI 1.19–1.41) and ICSI versus NC infants (RR 1.41; 95% CI 1.25–1.60) - Increased risk of congenital malformations in IVF singletons versus NC singletons (RR 1.33; 95% CI 1.22–1.44) and ICSI singletons versus NC singletons (RR 1.42; 95% CI 1.31–1.53) - No difference in risk of congenital malformations between IVF and NC twins (RR 1.14; 95% CI 0.99–1.32) or ICSI and NC twins (RR 1.11; 95% CI 0.92–1.34) - Higher risk of congenital malformations in IVF multiples than NC multiples (RR 1.15; 95% CI 1.01–1.32) but no difference in risk between ICSI and NC multiples (RR 1.13; 95% CI 0.96–1.33)

Table 2 (cont.) | Studies evaluating congenital malformations in ART and non-ART offspring

Study	Design	Study group (n)	Control group (n)	Outcome measures	Findings	
					ART versus NC infants	ICSI versus IVF infants
Massaro et al. (2015) ²⁰⁰	Systematic review and meta-analysis	ICSI infants (12,270)	IVF infants (24,240)	Genitourinary congenital malformations	NR	• Higher risk of congenital malformation involving the genitourinary tract in ICSI infants than IVF infants (RR 1.27; 95% CI 1.02–1.59) • No risk difference when only studies with low risk of bias were included (n=4; RR 1.28; 95% CI 1.00–1.64) • No risk difference when data were restricted to hypospadias (RR 1.21; 95% CI 0.87–1.69) or cryptorchidism (RR 1.39; 95% CI 0.97–2.00)

ART, assisted reproductive technology; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; NC, naturally conceived; NR, not reported; RR, relative risk.

abnormalities among ICSI offspring were not assessed according to whether male infertility was present. Collectively, the association between an increased risk of congenital disabilities among children conceived with medical assistance remains equivocal. The overall quality of meta-analyses evaluating malformation among children conceived using ART is moderate or low mainly owing to heterogeneity, study design, and selection bias. However, the risk of congenital malformations seems to be higher in infants conceived using ART than in naturally conceived infants, particularly

in reports evaluating singletons and in meta-analyses restricted to high-quality studies. A call for continued monitoring is, therefore, justified. ICSI as a method of fertilization does not seem to increase the risk of congenital abnormalities compared with conventional IVF, possibly with the exception of hypospadias, which is linked with paternal subfertility^{196,198}. New studies should take into account the infertility cause and reproductive history to identify if the likely increased risk of congenital malformations is a result of ART technique or the infertility factor. Whether the ICSI procedure per se has an adverse effect on the offspring is still a question open for further research. The widespread use of ICSI for non-male factor indications should enable researchers to report important neonatal and long-term outcomes based on ICSI registers.

Epigenetic disorders

Epigenetics refers to phenotypic changes caused by mechanisms other than changes in the DNA sequence. Epigenetic changes can activate or inhibit genes, determining which proteins will be transcribed²⁰⁴. Epigenetic silencing, for instance, inhibits genes and contributes to differentiated gene expression. In cells, three systems can interact to silence genes: DNA methylation, histone modification, and RNA silencing²⁰⁵. Epigenetic activity is well known to be associated with crucial stages of gametogenesis and early embryonic development²⁰⁶. A fundamental phenomenon is imprinting (FIG. 5), in which imprinted genes exhibit epigenetic modifications, including DNA methylation. Such genes possess differentially methylated regions that are methylated on either the paternal or maternal allele²⁰⁷. For most autosomal genes, expression occurs in both alleles simultaneously. If the allele inherited by one of the parents undergoes genomic imprinting and is, therefore, silenced, only the allele inherited from the other parent is expressed. By contrast, if the remaining allele has a significant deletion or mutation, the individual might present with an epigenetic disease. Despite being rare in the general population²⁰⁸, epigenetic diseases, including Prader–Willi syndrome (PWS) and Angelman syndrome (AS), are more common in subfertile populations²⁰⁹.

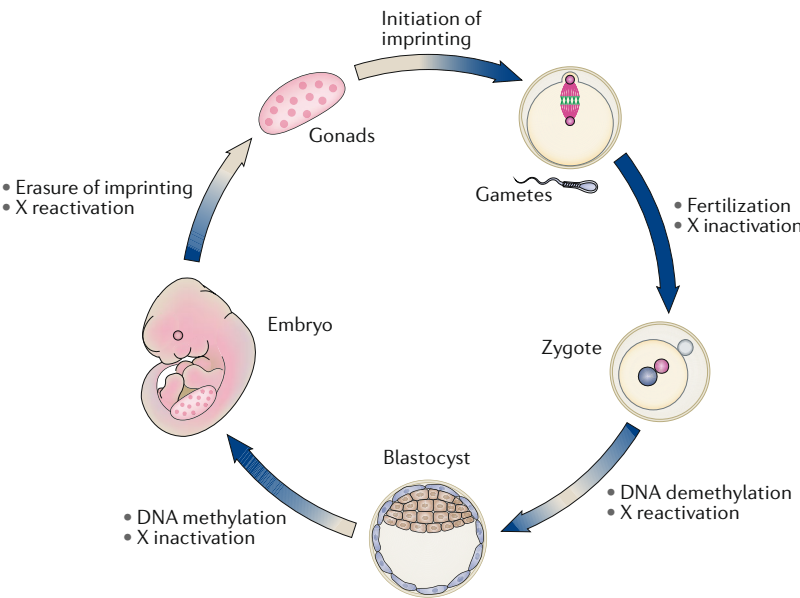


Fig. 5 | The epigenetic reprogramming cycle. The two major waves of epigenetic reprogramming occur during gametogenesis and after fertilization. During gametogenesis, the majority of parental epigenetic marks are erased and re-established at the time of oogenesis and spermatogenesis. A second epigenetic reprogramming wave occurs soon after fertilization with a fast, active paternal demethylation and a slower, passive maternal demethylation. At the blastocyst stage, novel methylation patterns are established in the inner cell mass, while the trophectoderm stays relatively unmethylated. Fluctuation in DNA methylation levels is represented by the differences in the arrows, with the blue colour indicating high methylation levels. Figure reproduced with permission from REF.²⁷⁵, Ann Van Soom.

Studies have reported DNA methylation defects in embryos originating from ART²¹⁰. Such changes have been speculated to result from the ART processes. The altered hormonal milieu associated with ovarian stimulation by exogenous gonadotropins and the retrieval of epigenetically immature oocytes can result in increased epigenetic or imprinting defects in children conceived through ART²¹¹. Moreover, sperm manipulation and embryo culture conditions might alter the methylation processes, thus increasing the risk of imprinting disorders¹⁹⁰. However, embryos with altered methylation patterns might inherit these modifications paternally. Data demonstrate that the aberrant methylation of promoters of specific genes (for example, *DAZL* and *MTHFR*) and general gene classes, such as imprinted loci, is associated with oligozoospermia and azoospermia^{210,212–216} (Supplementary Table 1).

Notwithstanding the observations of differentially methylated patterns between ART and naturally conceived children, the evidence concerning an increased frequency of imprinting and epigenetic diseases among children conceived using ART has been inconclusive^{217–232} (Supplementary Table 2). The heterogeneity in the study populations and tissue samples as well as different laboratory methods might explain the equivocal results. What is apparent, though, is that if differences in imprinting and epigenetic status do occur between naturally and ART-conceived children, the effects are small in magnitude. Detecting these effects, however, will require further controlled studies with standardized methods, in larger and better clinically defined populations. Selecting populations of children from couples with subfertility in both ART and non-ART (exposed and unexposed) is also important in order to explore the influence of fertility problems per se in comparison to the effect of ART with regards to the epigenetic changes in the offspring. The crucial question of whether imprinting and epigenetic diseases are more common after ICSI than conventional IVF remains unresolved.

Chromosomal abnormalities

ICSI has become the method of choice for the treatment of male infertility, but concerns exist that the rates of aneuploidy in the first trimester gestation might be increased owing to the increased frequency of chromosomal abnormalities in spermatozoa of infertile men²³³. Chromosomal segregation errors contribute to numerical chromosomal abnormalities in mature germ cells that can be transmitted to the embryo²³⁴. Indeed, aneuploidies and chromosomal structural aberrations are the main factors involved in fertilization failures, implantation failures, spontaneous abortions, stillbirths, congenital malformations, and mental and behavioural dysfunctions²³⁵. Thus, the risk of chromosomal abnormalities in offspring might increase in infertile couples undergoing ICSI, as this technique can perpetuate aneuploidy through the injection of disomic sperm²³⁶.

In general, the risk of having chromosomal abnormalities, particularly sexual chromosome aneuploidy, is higher in children conceived through ICSI (~1.0%)

than in naturally conceived children (~0.2%) and those conceived with conventional IVF (~0.7%)^{168,194,237–241} (Supplementary Table 3). As these observations occurred predominantly in couples who underwent ART owing to severe male factor infertility, it has been argued that the risk is associated with the low quality of spermatozoa from infertile fathers rather than the ICSI procedure¹⁹⁴.

Infertility

Some studies have evaluated the reproductive potential of children and young adults born through ART, including ICSI^{242–246} (Supplementary Table 4). A longitudinal cohort study assessed serum hormone levels of 3-month-old boys to compare the testicular function of infants born through ART treatments with that of naturally conceived infants²⁴². Because men with male factor infertility might have impaired Leydig cell function and reduced levels of serum testosterone²⁴⁷, these investigators elected to assess the hormone levels of offspring resulting from ICSI at 3 months of age (at which point the pituitary–testicular axis is transiently active) as a predictive marker of adult reproductive function. In their study, no differences were observed in serum reproductive hormone concentrations (FSH, LH, inhibin B, and testosterone) between boys conceived with ICSI and those conceived with IVF in the whole studied population²⁴². However, when considering only boys conceived by ICSI owing to male factor infertility and boys conceived by IVF owing to female factor infertility, boys conceived with ICSI had a significant reduction in serum total and free testosterone levels and an increase in LH:testosterone ratio compared with naturally conceived boys. No differences in serum reproductive hormone concentrations were found between boys conceived with IVF and naturally conceived boys. The authors say that their findings might suggest an impairment in Leydig cell function in boys conceived by ICSI, possibly inherited from their fathers, and raise the need to investigate the implications of these results in adulthood.

A prospective cohort study evaluated semen analysis, hormone serum levels, and physical examination of 18-year-old men who were conceived through an unspecified infertility treatment²⁴³. When compared with the control group of naturally conceived subjects, men born through infertility treatments showed significantly altered semen parameters (reduced sperm concentration, reduced total sperm count, fewer motile sperm, and fewer morphologically normal spermatozoa) and lower testicular volume. Hormone levels (serum testosterone level and free androgen index) were also affected, although the differences were not statistically significant. In another long-term follow-up study of a cohort of men aged 18–22 years conceived through ICSI owing to male infertility, semen analysis revealed lower sperm concentrations and lower sperm motility in young adults born through ART treatments than in naturally conceived controls²⁴⁴. Findings concerning the reproductive endocrine profile of ART offspring are reassuring overall, with mean and median levels of FSH, LH, inhibin B, and testosterone similar to those of the control group. Despite these findings, men conceived through ART treatments were more likely to have inhibin B levels below the 10th percentile and FSH

Table 3 | Risk of cancer in children born after ART treatments

Study	Type	Study group (n)	Control group (n)	Outcome measures	Findings	
					ART versus NC infants	ICSI versus IVF infants
Källén et al. (2010) ²⁵⁴	Retrospective cohort	ART infants (26,692)	NC infants (NR)	Childhood cancer incidence	- Total cancer risk estimate higher for ART-born children than NC children (OR 1.42; 95% CI 1.09–1.87) 53 cases of cancer were reported in children born after ART versus 38 expected cases - Odds ratio for children conceived after ART and who received a cancer diagnosis at <3 years was 1.87 (95% CI 1.27–2.77) and at ≥3 years was 1.32 (95% CI 0.89–1.96)	Among the 53 children who were conceived by ART and developed cancer, 15 were born with the use of ICSI
Hargreave et al. (2013) ²⁵⁰	Systematic review and meta-analysis of cohort and case-control studies	ART infants (649)	NC infants (30,438)	Childhood cancer incidence	- Increased overall risk of cancer among ART children (RR 1.33; 95% CI 1.08–1.63) - Increased risk of leukaemia (RR 1.65; 95% CI 1.35–2.01), neuroblastoma (RR 4.04; 95% CI 1.24–13.18), and retinoblastoma (RR 1.62; 95% CI 1.12–2.35) in ART infants	NR
Williams et al. (2013) ²⁵¹	Retrospective cohort	ART infants (106,013)	Person-years of follow-up (700,705)	Childhood cancer incidence	- No increase in overall risk of cancer among ART infants - Increased risk of hepatoblastoma (SIR 3.64; 95% CI 1.34–7.93, $P=0.02$) and rhabdomyosarcoma (SIR 2.62; 95% CI 1.26–4.82, $P=0.02$) in ART infants, but absolute risks were small (6.21 hepatoblastoma cases per million person-years and 8.82 rhabdomyosarcoma cases per million person-years) - No increased risk of cancer in ART infants from couples who received ART owing to male factor-only (SIR 0.92; 95% CI 0.53–1.49) or female factor-only infertility (SIR 1.04; 95% CI 0.70–1.50)	No increased risk of cancer in IVF (SIR 0.89; 95% CI 0.68–1.14) or ICSI (SIR 1.07; 95% CI 0.70–1.57) infants
Sundh et al. (2014) ²⁵²	Retrospective cohort	ART infants (91,796)	NC infants (358,419)	Childhood cancer incidence	- No increase in overall risk of cancer among ART infants - Increased risk of nervous system tumours (adjusted HR 1.44; 95% CI 1.01–2.05) and malignant epithelial neoplasms (adjusted HR 2.03; 95% CI 1.06–3.89) in ART children, but absolute risk was small (0.46 of 1,000 cases of nervous system tumours and 0.15 of 1,000 cases of malignant epithelial neoplasms) - In ART infants, children with cancer were more often twins ($P=0.046$), had a lower gestational age ($P=0.04$), and had an increased incidence of congenital malformations ($P=0.04$) and chromosomal aberrations ($P<0.0001$) compared with children without cancer	NR
Reigstad et al. (2016) ²⁵³	Retrospective cohort	ART infants (25,782)	NC infants (1,602,876)	Childhood cancer incidence	- No increase in overall risk of cancer among ART infants (HR 1.21; 95% CI 0.90–1.63) - Increased risk of leukaemia (HR 1.67; 95% CI 1.02–2.73) and Hodgkin lymphoma (HR 3.63; 95% CI 1.12–11.72) in ART infants	In analysis stratified by ART method (IVF versus ICSI), no significant differences were detected between groups

ART, assisted reproductive technology; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; NC, naturally conceived; NR, not reported; RR, relative risk; SIR, standardized incidence ratio.

levels above the 90th percentile, which might suggest testicular dysfunction²⁴⁵.

Lastly, data concerning the fertility status of women born through ART treatments are scarce. In one long-term cohort study, women aged 18–22 years who were born through ART had anti-Müllerian hormone and FSH serum levels that were similar to those of a control group consisting of naturally conceived women. Antral follicle count was also similar in the two groups²⁴⁶.

Collectively, these data indicate that male fertility might be impaired in ICSI offspring, most likely owing

to paternal inheritance. As for female fertility, the existing information concerning the reproductive potential of girls born through ICSI in the adult reproductive stage is too scarce to make any assumptions.

Cancer

Childhood cancer is one of the leading causes of death in children in both developing and developed countries. The aetiology of childhood cancer remains poorly understood, but it has been hypothesized that some malignancies are initiated in the early stages of fetal

Table 4 | Studies evaluating psychological and neurodevelopmental features in ICSI offspring

Study	Design	Study group (n)	Control group (n)	Outcome measures	Findings	
					ART versus NC infants	ICSI versus IVF infants
Tsai et al. (2011) ²⁵⁹	Retrospective cohort	Children born after TESE–ICSI (48)	Children born after ICSI using fresh ejaculated sperm from men with severe OAT (19)	Perinatal outcomes and development of children assessed at age 1–7 years	No evidence of differences in development of children after TESE–ICSI or ICSI using sperm from men with severe OAT	NR
Sandin et al. (2013) ²⁶⁰	Prospective cohort	Children born after ART (30,959)	NC children (2,541,125)	Risk of autistic disorder and mental retardation in offspring	ART was not associated with autistic disorder (adjusted RR 1.14; 95% CI 0.94–1.39) but was associated with a small but statistically significant increased risk of mental retardation (adjusted RR 1.18; 95% CI 1.01–1.36) compared with NC children	• ICSI using ejaculated sperm from men with male factor infertility in fresh embryo transfers was associated with an increased risk of mental retardation (adjusted RR 1.47; 95% CI 1.03–2.09) compared with IVF • Mental retardation was also associated with ICSI with frozen embryos among children born prematurely (multiples or singletons) and with IVF without ICSI with frozen embryos among preterm singleton infants • Autistic disorder (adjusted RR 4.60; 95% CI 2.14–9.88) and mental retardation (adjusted RR 2.35; 95% CI 1.01–5.45) were also associated with ICSI using surgically extracted sperm compared with IVF after a fresh embryo transfer, but the association was not evident among singletons (RR 0.70; 95% CI 0.10–5.16)
Bay et al. (2013) ²⁵⁵	Systematic review	ART infants (NR)	NC infants (NR)	Neurodevelopmental outcomes (cognitive, behavioural, emotional, or psychomotor development) and diagnoses of mental disorders	Neurodevelopmental outcomes of ART infants were overall comparable to those of NC infants	NR
Ilioi et al. (2015) ²⁶¹	Systematic review	Children born after ART that used the parents' own gametes or gamete donation	NC adolescents and standardized normative samples	Adolescent psychological adjustment and parent–adolescent relationship	Children born through ART showed a positive parent–adolescent relationship and were well adjusted	NR
Meijerink et al. (2016) ²⁵⁸	Prospective cohort	Children born after TESE–ICSI (103)	General population	Behavioural, cognitive, and motor performance and physical development assessed at 5 years of age	Behavioural and cognitive performances scored better, but motor performance scored poorer in 5-year-olds born after TESE–ICSI than NC children	NR
Spangmose et al. (2017) ²⁵⁶	National registry-based cohort	ART adolescents (2,836 singletons and 1,930 twins)	NC adolescents (5,660 singletons and 7,064 twins)	Academic performance measured with standardized national test	• The adjusted mean overall test score was significantly lower for ART singletons than NC singletons (–0.15; 95% CI –0.29 to –0.02) • No differences in academic performance were found when ART twins were compared with NC twins nor between ART singletons and ART twins	NR

Table 4 (cont.) | Studies evaluating psychological and neurodevelopmental features in ICSI offspring

Study	Design	Study group (n)	Control group (n)	Outcome measures	Findings	
					ART versus NC infants	ICSI versus IVF infants
Balayla et al. (2017) ²⁶³	Prospective cohort	ART infants (175)	NC infants (1,345)	Cognitive, motor, and language development at 2 years of age	Children born after ART showed no difference in cognitive scores, composite motor scores, or language scores	No difference was observed when IVF and ICSI were compared

ART, assisted reproductive technology; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; NC, naturally conceived; NR, not reported; OAT, oligoasthenoteratozoospermia; RR, relative risk; TESE, testicular sperm extraction.

development²⁴⁸. Consequently, events that precede and occur around the time of conception can have a major role and justify specific assessments.

The first systematic review and meta-analysis that assessed the risk of cancer in children born through ART treatments was published more than 10 years ago and did not find an overall increase in cancer risk²⁴⁹. However, a 2013 systematic review and meta-analysis demonstrated an overall increased risk of cancer in ART offspring compared with naturally conceived children (RR 1.33, 95% CI 1.08–1.63)²⁵⁰. Statistically significant associations were established between fertility treatment and the risks of specific types of cancer, such as leukaemia, neuroblastoma, and retinoblastoma^{250–254} (TABLE 3).

Large longitudinal cohort studies not included in the systematic reviews mentioned above have shown no increase in the overall risk of cancer among children born through ART^{251–253}. In analyses by cancer type, however, an increased risk of specific types of cancer was found, including hepatoblastoma and rhabdomyosarcoma²⁵¹, nervous system tumours and malignant epithelial neoplasms²⁵², and leukaemia and Hodgkin lymphoma^{253,254}, although the absolute risk was small. In general, significant differences in post hoc subanalyses should be interpreted with caution when no significant differences were found in the primary outcome measures. However, different cancers can have different aetiologies, and, therefore, it is conceivable that the risk of cancer for children born after ART treatments might be increased for some specific types of cancer, despite no increase in the overall risk. Whether children born through ART treatments are at an increased risk of developing cancer is still unclear, but limited evidence suggests that certain types of cancer are more common in ART offspring. In analyses stratified by ART method, no significant differences were detected between ICSI and conventional IVF concerning the incidence of childhood cancer^{251,253}.

Psychological and neurological development

The results of large longitudinal cohort studies regarding the association between ART and childhood neurodevelopmental disabilities, including cognitive, motor, and language development, are crucial knowledge for couples that will undergo ART^{249,255–263} (TABLE 4). A 2013 systematic review analysing the long-term neurodevelopment of children conceived through ART treatments indicates that overall neurodevelopment is comparable to that of those born from natural conception²⁵⁵.

However, defining possible neurodevelopmental deficits in such children is complex. First, ART has been associated with a high twin pregnancy rate, which per se represents an increased risk factor for preterm birth, low birthweight, and being small for gestational age. These factors, in turn, are risk factors for neurodevelopmental deficits²⁵⁵. Even when only singletons are evaluated, children conceived through ART have increased rates of preterm birth and fetal growth restriction, thus adding a substantial selection bias that favours the presence of neurodevelopmental deficits²⁵⁶. Another important factor to consider is the effect of subfertility, regardless of ART, on neurodevelopmental differences. Furthermore, the use of different types of controls and analytical approaches for the control of confounding and mediating variables has to be considered by researchers willing to study the effect of infertility and ART on childhood outcomes²⁵⁷.

The long-term effects on neurodevelopment in children born through TESE and ICSI have been poorly studied, but the existing evidence is overall reassuring regarding the development and health of these children^{258,259}. Some evidence does, however, indicate an increased RR of mental retardation among children born after ICSI using ejaculated sperm from fathers with male infertility compared with those born after IVF²⁶⁰. Furthermore, autistic disorder and mental retardation were also associated with the use of ICSI using TESE to overcome azoospermia compared with the use of conventional IVF²⁶⁰. The incidence of these disorders was very small after TESE–ICSI and IVF, but these associations need to be evaluated further.

Data from studies evaluating adolescent psychological adjustment are reassuring overall. Children born through ART treatments have a positive parent–adolescent relationship and are well adjusted²⁶¹. The mental health of the teenagers, including their behaviour, relationships with colleagues, and social and emotional functioning, was also reassuring²⁶². Nevertheless, a study based on national data evaluating academic performance showed that adolescents conceived through ART had a significantly lower mean score than adolescents naturally conceived, but the differences were small and most likely not of clinical significance²⁶². By contrast, a prospective cohort study evaluating cognitive, motor, and language development of ART versus naturally conceived children at 2 years of age showed no difference in cognitive scores, composite motor scores, or language scores, irrespective of whether ICSI or conventional IVF was applied²⁶³.

Table 5 | Studies comparing cardiometabolic profile in ART and naturally conceived infants

Study	Design	Study group (n)	Control group (n)	Outcome measures	Findings of ART versus NC infants
Ceelen et al. (2008) ²⁶⁸	Prospective cohort	ART infants (225)	NC infants (225)	Blood pressure, fasting glucose, and fasting insulin levels	• SBP and DBP levels were higher in ART infants than controls (109 ± 11 versus 105 ± 10 mmHg, $P < 0.001$, and 61 ± 7 versus 59 ± 7 mmHg, $P < 0.001$, respectively) • Higher fasting glucose levels in pubertal ART infants than in controls (5.0 ± 0.4 versus 4.8 ± 0.4 mmol per litre, respectively; $P = 0.005$)
Belva et al. (2012) ²⁷⁰	Cross-sectional study	ICSI infants (217)	NC infants (223)	Resting blood pressure and blood pressure response to a psychological stressor	No statistically significant difference in blood pressure was observed between the groups
Belva et al. (2012) ²⁶⁹	Prospective cohort	ICSI infants (217)	NC infants (223)	Body composition data (anthropometry, skinfold thickness, and circumference)	• Among boys, no statistically significant difference in body composition was observed between the groups • Among girls, ICSI infants had a significantly higher sum of peripheral, central, and total sum of skinfolds and a significantly higher mean mid-upper arm circumference and waist circumference than NC girls (differences between ICSI and NC were 2.8 mm, 3.1 mm, 6.3 mm, 1.5 cm, and 2.1 cm, respectively; all $P < 0.05$)
Gkourogianni et al. (2014) ²⁷¹	Cross-sectional study	ICSI infants (42)	NC infants (42)	Medical history, biochemical levels, and metabolomics analysis	• Lower urea (median 31 (ICSI) versus 34.5 (NC), $P = 0.04$) and lower grade inflammation markers chitinase-3-like protein 1 (CHI3L1; also known as YKL-40) (median 15.1 (ICSI) versus 24.6 (NC), $P = 0.0002$), hsCRP (0.44 ± 0.3 (ICSI) versus 0.78 ± 0.87 (NC), $P = 0.022$) and higher T_3 (median 182.6 ± 25 (ICSI) versus 156.7 ± 30.1 (NC), $P = 0.0001$) in ICSI-conceived children than controls • Plasma metabolomics analysis indicated clear differences between the two groups
Pontesilli et al. (2015) ²⁶⁷	Prospective cohort	Infants conceived from infertile couples through ovulation induction (34), artificial insemination (51), ART (28), or NC after 12 months of unprotected intercourse (220)	NC infants (2,244)	Birthweight, BMI at age 5–6 years, blood pressure, and levels of glucose, cholesterol, triglycerides, HDL, and LDL	• No statistically significant differences in birthweight and BMI at age 5–6 years were observed between the groups • Glucose levels were higher in children conceived by ART than their NC counterparts
Guo et al. (2017) ²⁶⁴	Systematic review and meta-analysis	ART infants (2,112)	NC infants (4,096)	SBP and DBP, cardiovascular function, BMI, and lipid and glucose profiles	• SBP (weighted mean difference 1.88 mmHg; 95% CI 0.27–3.49) and DBP (weighted mean difference 1.51 mmHg; 95% CI 0.33–2.70) were significantly higher in ART infants than in NC infants • Fasting insulin was higher in ART infants than in their NC counterparts (0.38 mIU/L; 95% CI 0.08–0.68) • No statistically significant difference in BMI was found between ART and NC infants

ART, assisted reproductive technology; DBP, diastolic blood pressure; ICSI, intracytoplasmic sperm injection; hsCRP, high-sensitivity C-reactive protein; NC, naturally conceived; NR, not reported; SBP, systolic blood pressure; T_3 , tri-iodothyronine.

Cardiometabolic profile

The potential health risks associated with ICSI are of great importance to public health, as millions of babies worldwide are born through this procedure. All procedures inherent to ART, including ovarian stimulation, gamete manipulation, and embryonic culture, occur in a crucial window for the establishment of genomic methylation patterns. Some researchers have suggested that alterations in DNA methylation affect transcriptional control and potentially increase the risk of cardiometabolic diseases in childhood and adulthood²⁶⁴. Embryos generated from women who are obese or overweight express a compromised developmental and metabolic profile²⁶⁵. Oocytes of obese or overweight women tend to be smaller than those of women with normal BMI²⁶⁵.

These oocytes have a reduced chance of reaching the blastocyst stage, and those that reach this stage have accelerated development, with fewer cells in the trophoderm, which thereby negatively affects the likelihood of implantation and live birth²⁶⁶. These embryos also show notable metabolic changes, with decreased glucose consumption, altered amino acid metabolism profile, and increased triglyceride levels, which could have long-term implications for the offspring²⁶⁵.

Few studies have investigated the cardiometabolic profile of children conceived using ART (TABLE 5). Increased blood pressure and higher fasting glucose levels have been reported in infants conceived through ART compared with naturally conceived counterparts, but the evidence is not unequivocal^{264,267–270}. Furthermore,

metabolomics analysis revealed differences between offspring conceived using ART and those conceived naturally²⁷¹. Nevertheless, an important factor associated with the cardiometabolic profile of children conceived using ART is parental subfertility, which per se might have a major role. Infertile women tend to be older, more obese, and often more nulliparous than fertile women²⁷². These factors have been associated with an impaired cardiometabolic profile in offspring irrespective of whether or not ART had been used²⁶. Lastly, the relationship between the cardiometabolic profile of children conceived using ART and the method of fertilization is yet to be determined.

Conclusions

Twenty-five years since its introduction to overcome severe male factor infertility, ICSI is widely used to treat both male factor and non-male factor infertility. However, superiority of ICSI over conventional IVF in couples without male factor infertility has not been demonstrated. Current efforts are focused on the

development of safer techniques for selecting better quality sperm for injection. Nonetheless, the overall LBRs with ICSI remain in the range of only 30%, and increasing evidence suggests that children born through ICSI have an increased risk of congenital malformations, chromosomal abnormalities, childhood cancer, and disrupted reproductive hormonal profile compared with naturally conceived children. Additionally, epigenetic disorders and impaired neurodevelopment have been observed in infants born using ICSI compared with naturally conceived children. The underlying parental infertility seems to have a major effect on the health of ICSI offspring, but some of these health issues might arise from the ART process as a whole and ICSI in particular. Evaluation and treatment of the underlying male infertility condition are advisable to optimize ICSI outcomes and eventually enable natural conception or the use of less-invasive (and safer) assisted conception methods.

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Author contributions

S.C.E. designed the manuscript, helped in data interpretation and coordination, and drafted the manuscript. M.R., G.B., and T.H. participated in the acquisition of data and drafted the manuscript. P.H. helped in data interpretation and coordination and drafted and revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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Review criteria

An extensive search of studies examining the relationship between intracytoplasmic sperm injection and male infertility was performed using PubMed and MEDLINE. The start and end dates for the search were January 2006 and February 2017, respectively. The overall strategy for study identification and data extraction was based on the following key words: “assisted reproductive technology”, “intracytoplasmic sperm injection”, “male infertility”, “pregnancy outcomes”, and “children”, with the filters “humans” and “English language”. Using the aforementioned criteria, 209 relevant articles were identified. Data that were solely published in conference or meeting proceedings, websites, or books were not included. Citations dated outside the search dates were included only if they provided conceptual content.

Supplementary information

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