Intracytoplasmic sperm injection for male infertility and consequences for offspring

Sandro C. Esteves[1,2,3]*, *Matheus Roque*[4], *Giuliano Bedoschi*[5], *Thor Haahr*[3,6] *and Peter Humaidan*[3,6]

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.

[1]*ANDROFERT, Andrology and Human Reproduction Clinic, Campinas, Brazil.*

[2]*Department of Surgery (Division of Urology), Faculty of Medical Sciences, University of Campinas (UNICAMP), Campinas, Brazil.*

[3]*Faculty of Health, Aarhus University, Aarhus, Denmark.*

[4]*ORIGEN, Center for Reproductive Medicine, Rio de Janeiro, Brazil.*

[5]*Division of Reproductive Medicine, Department of Gynecology and Obstetrics, University of São Paulo (USP), São Paulo, Brazil.*

[6]*Fertility Clinic, Skive Regional Hospital, Skive, Denmark.*

**e-mail: s.esteves@androfert.com.br*

https://doi.org/10.1038/s41585-018-0051-8

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 child[2]. 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 stable[3]. 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 offspring[6].

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 emerged[2].

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 SUZI[1].

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 ICSI[9]. 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 obtained[9]. 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 babies[10].

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.

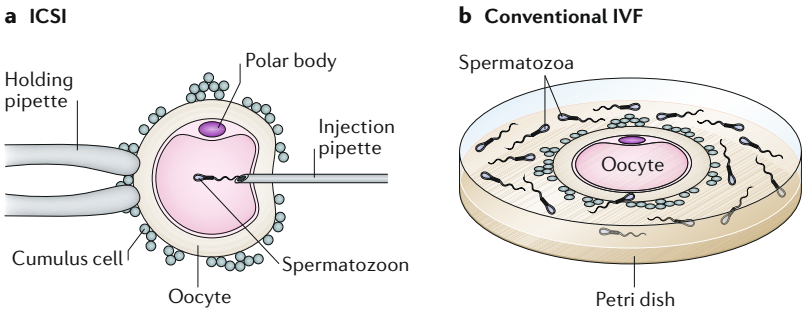


Fig. 1 | **Assisted fertilization methods. a** | Intracytoplasmic sperm injection (ICSI), which involves the injection of a single spermatozoon into an oocyte cytoplasm using a glass micropipette. **b** | Conventional in vitro fertilization (IVF), where oocytes are incubated with sperm in a Petri dish,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

Modern trends: is ICSI overused?

ICSI has become the most common method of fertilization used for ART[3]. 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

factor infertility as the stated reason for applying ICSI as a method of fertilization treatment remained essentially unchanged at approximately 36% of cases[6]. 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 analysed[5]. 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 East[5].

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 infertility[2]. 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 IVF[12]. 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 infertility[13].

Indications for ICSI

Conventional IVF has been successfully used since the birth of Louise Brown in 1978, but its results are suboptimal when sperm quantity or quality is poor[1]. 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 infertility[14] 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 infertility[15] (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 cases[16]. 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 manual**[23]

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 ICSI[17]. 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 integrity[21]. Sperm count, motility, and morphology are the commonly used parameters to assess the need for ICSI in preference to conventional IVF[22] (FIG. 2). However, high-quality data comparing live birth rates from ICSI and IVF in couples with oligoasthenoteratozoospermia[23] (BOX 1) are lacking, as discussed recently by Cissen and co-workers[24]. 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

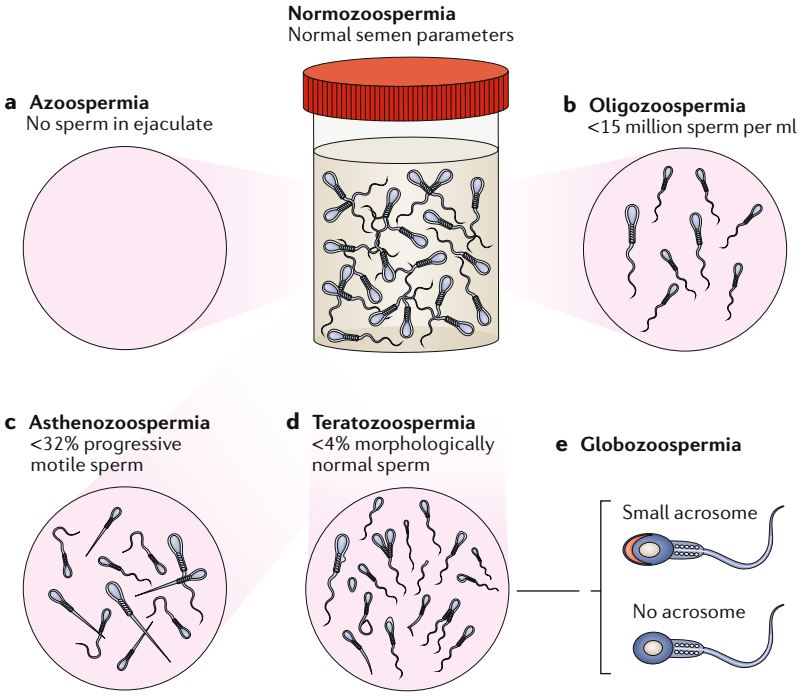


Fig. 2 | **Seminal alterations associated with male infertility. a** | Azoospermia. **b** | Oligozoospermia. **c** | Asthenozoospermia. **d** | Teratozoospermia. **e** | Globozoospermia.

a given ejaculate was low[27]. 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 ICSI[32]. 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 out[32].

Absolute asthenozoospermia. ICSI has been recommended[19] 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 rates[19]. 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 sperm[37]. When none of these strategies is effective, sperm can be harvested from the testis, and testicular sperm can be used for injections[38].

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 men[39]. 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 development[40]. Oocyte activation using mechanical, electrical, and chemical agents has been used to overcome fertilization failure among men with globozoospermia[40].

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 evaluation[41]. This condition has been associated with testicular torsion, testicular surgery, vasectomy, epididymo-orchitis, testicular carcinoma, cryptorchidism, and infection by HIV[42]. ASAs can potentially impair fertility through effects on sperm motility, sperm capacitation, the acrosome reaction, and sperm–oocyte binding[41]. Antibodies directed towards sperm can also act through the release of cytokines that can impair sperm function[43]. 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 cytometry[45]. 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 parameters[48].

An increasing body of evidence has indicated that elevated SDF negatively affects the chances of natural and assisted conception[49]. Indeed, SDF testing results might be useful when choosing the method of fertilization, IVF or ICSI[50]. 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 sperm[56].

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 infertility[4]. 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 IVF[59]. 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 treatment[59]. 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 outcome[57]. 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 transfer[6]. These observations were corroborated by Foong and colleagues, who randomly assigned 60 women with unexplained infertility to conventional IVF or ICSI[60]. 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 transfer[61]. 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 test[65]. 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 abnormalities[67]. 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 functioning[73], neurodevelopmental outcome[74], or cognitive development[75] 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 group[58].

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 spermatozoon[79]. 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 intercourse[77]. 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 different[77]. 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 HCV[78]. 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 embryogenesis[85]. 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 offspring[89]. The notion that impairment of sperm chromatin integrity is associated with potentially correctable conditions, including lifestyle and gonadotoxin exposure, varicocele, and male accessory gland infections[90], 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 damage[93]. 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 controls[90]. In this study, SDF values were higher in men with clinical varicocele (mean 35.7 ± 18.3%) and those with leukocytospermia (mean 41.7 ± 17.6%) than in fertile controls (mean 11.3 ± 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 ICSI[96]. 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 group[97]. 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 repair[98].

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 ± 18.7% versus 50.4 ± 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 rates[103]. These results have been corroborated by a 2015 prospective ICSI study involving men with an increased number of leukocytes in their seminal fluid[104]. 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 capacity[105]. 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 LBRs[106]. 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 quality[107]. 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 ICSI[109]. 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 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 cycles[111]. The authors found no association between male or female BMI and LBR in couples undergoing ICSI[111]. 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 results[113]. 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 patients[114]. 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 outcomes[115]. 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 nonobstructive azoospermia and low levels of total testosterone (<300 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 SRR[119].

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 infertility[122]. 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 ICSI[122]. 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 hormones[123]. 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 attempts[124]. A pregnancy rate of 30.4% was achieved in the treated group, whereas no pregnancies were recorded in the control group, which comprised ten men[124]. 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 weeks[126]. 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*.N680S[126]. 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 dysfunction[127]. 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 cells[130]. 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 vitro[132]. 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 oocytes[14]. 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 micropipette[14] (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 decades[133] (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 antibodies[134]. 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

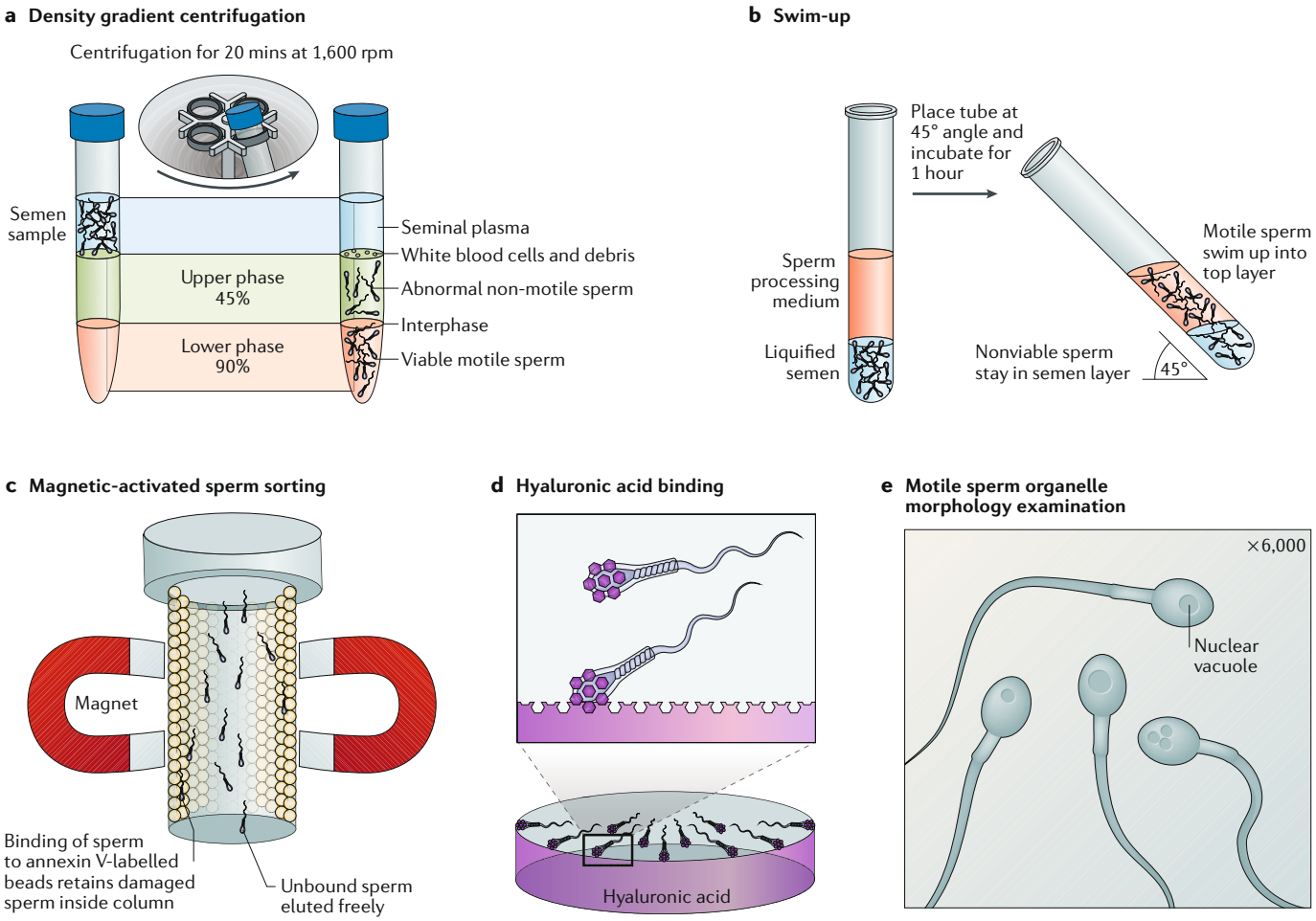


Fig. 3 | **Sperm selection methods. a** | Density gradient centrifugation. Liquefied semen is placed on top of a series of two colloidal gradients with 45% and 90% densities and subjected to centrifugation. The highly active spermatozoa move towards the sedimentation gradient and are enriched in the 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 ×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 spermatozoa[135]. Despite being easy to perform, swim-up might not be suitable for recovering sperm for ICSI in low-quality ejaculates[132].

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 ICSI[136]. 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 assessment[137] (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 maturation[138]. 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 potential[139]. 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 retention[140]. 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 ± 1.9% versus 76.9 ± 2.1%) between groups, but the Zeta group showed a significant increase in the proportion of top quality embryos (45.8 ± 3.1% versus 35.4 ± 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 rates[145]. 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 rate[146].

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 aneuploidies[147] (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 ICSI[148].

Lastly, ultramorphological sperm assessment using motile sperm organelle morphology examination applies high magnification (at least × 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 fertilization[144]. 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 practice[154]. 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 ICSI[155].

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

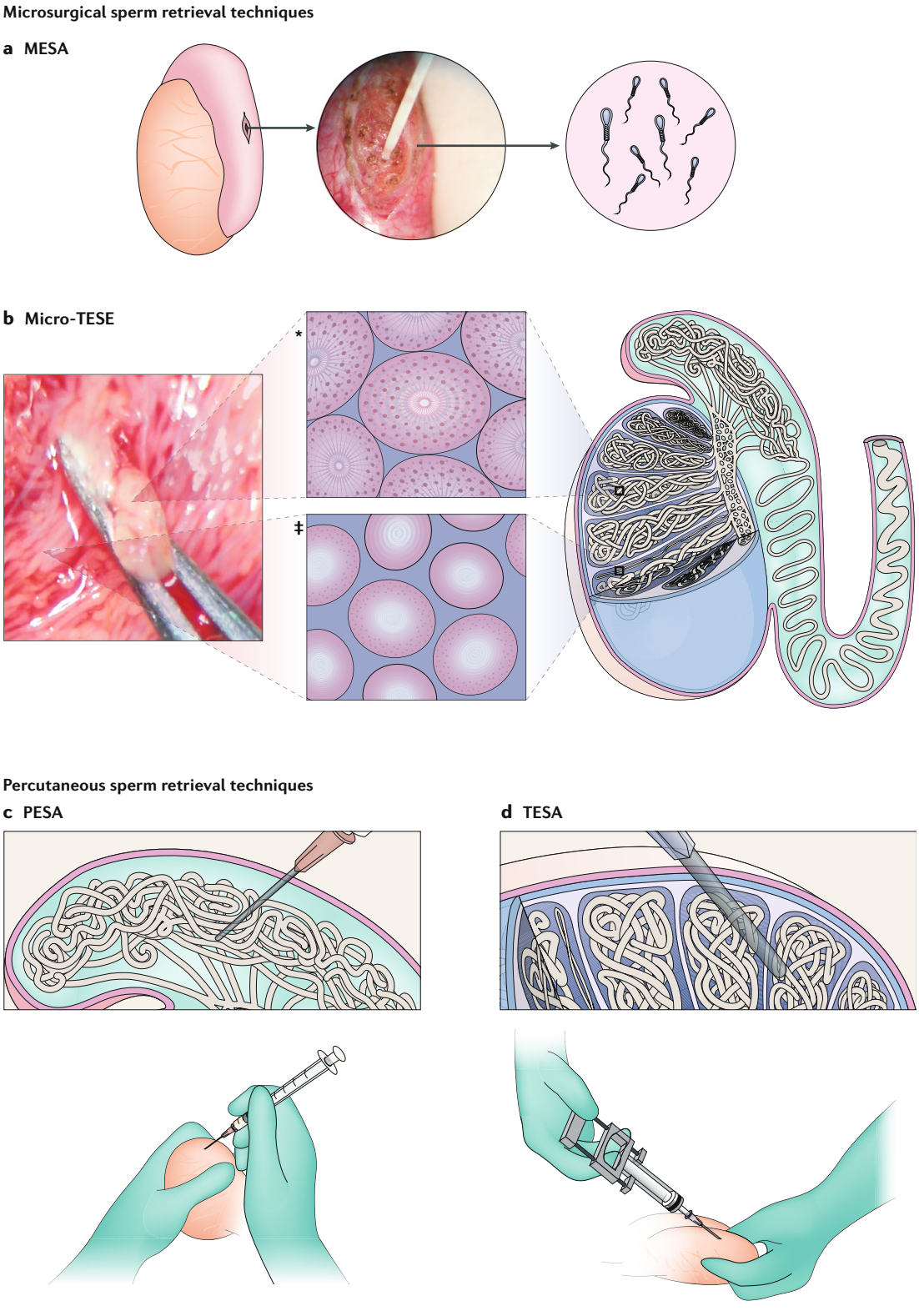
Reconstruction of the seminal tract is a cost-effective treatment in men with obstructive azoospermia, but this approach might not be feasible or desired by the couple[16]. In such cases, sperm retrieval and ICSI with epididymal or testicular sperm offers the possibility of parenthood. Unlike obstructive 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 sperm[18] (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 sperm[156] (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 injection[18].

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].



In men with nonobstructive azoospermia, SRRs are higher and complication rates are lower with micro-TESE than with conventional TESE[158]. 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 nonobstructive 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 sperm[37]. 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 counterparts[17]. 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 sperm[160]. If blood contamination is excessive, the specimen is incubated with erythrocyte-lysing solution to eliminate the contaminating red blood cells[161]. 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 use[37]. 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 azoospermia[164]. 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 sperm[165]. 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 groups[83]. 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 azoospermia[167]. 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 embryo[168].

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 azoospermia[16].

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 ($5–15 \times 10^6$ 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 ± 9.9%) than in the testis (8.3 ± 5.3%; $P < 0.001$); in this group, all sperm injections were performed with testicular sperm. By contrast, SDF rates were 40.9 ± 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; $P = 0.008$), respectively[170]. 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 semen[155]. 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 birth[174]. 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 outcomes[175].

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 cryptozoospermia[176]. 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 available[179]. 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-workers[184]. 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 anecdotal[179]. 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 procedure[185]. 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 option[186].

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 emerged[187].

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 offspring[188]. The sperm nuclear genome includes a central compact toroid comprising protamine-bound DNA that is both transcriptionally and translationally inert[189]. The peripheral compartment, composed of histone-bound DNA, retains the nucleosomal structure and contains promoters for developmentally crucial genes, microRNAs, and signalling factors[190]. 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 aneuploidy[192]. 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 patterns[193]. 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 aneuploidy[194].

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 offspring[200]. 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 naturally[201].

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 pregnancy[202]. 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 IVF[203]. 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 IVF[6]. 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 birthweight[6]; 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.

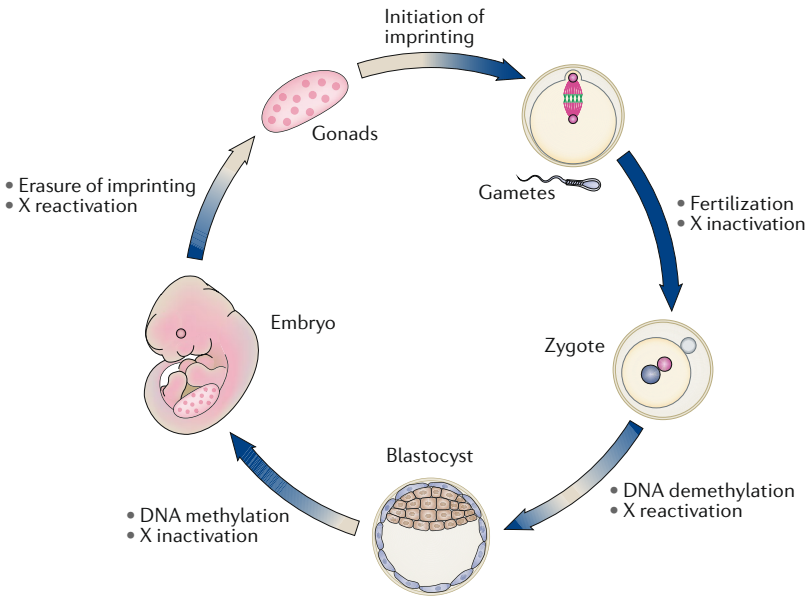


Fig. 5 | **The epigenetic reprogramming cycle.** The two major waves of epigenetic reprogramming occur during gametogenesis and after fertilization. During gametogenesis, the majority of parental epigenetic marks are erased and re-established at the time of oogenesis and spermatogenesis. A second epigenetic 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.

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 transcribed[204]. 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 silencing[205]. Epigenetic activity is well known to be associated with crucial stages of gametogenesis and early embryonic development[206]. 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 allele[207]. 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 population[208], epigenetic diseases, including Prader–Willi syndrome (PWS) and Angelman syndrome (AS), are more common in subfertile populations[209].

Studies have reported DNA methylation defects in embryos originating from ART[210]. 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 ART[211]. Moreover, sperm manipulation and embryo culture conditions might alter the methylation processes, thus increasing the risk of imprinting disorders[190]. 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 men[233]. Chromosomal segregation errors contribute to numerical chromosomal abnormalities in mature germ cells that can be transmitted to the embryo[234]. 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 dysfunctions[235]. 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 sperm[236].

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 procedure[194].

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 infants[242]. Because men with male factor infertility might have impaired Leydig cell function and reduced levels of serum testosterone[247], 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 population[242]. 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 treatment[243]. 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 controls[244]. 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 dysfunction[245].

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 groups[246].

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.

development[248]. 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 risk[249]. 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 rhabdomyosarcoma[251], nervous system tumours and malignant epithelial neoplasms[252], 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 conception[255]. 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 deficits[255]. 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 deficits[256]. 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 outcomes[257].

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 IVF[260]. 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 IVF[260]. 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 adjusted[261]. The mental health of the teenagers, including their behaviour, relationships with colleagues, and social and emotional functioning, was also reassuring[262]. 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 significance[262]. 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 applied[263].

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 mIUI/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 adulthood[264]. Embryos generated from women who are obese or overweight express a compromised developmental and metabolic profile[265]. Oocytes of obese or overweight women tend to be smaller than those of women with normal BMI[265]. 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 trophectoderm, which thereby negatively affects the likelihood of implantation and live birth[266]. 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 offspring[265].

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 naturally[271]. 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 women[272]. These factors have been associated with an impaired cardiometabolic profile in offspring irrespective of whether or not ART had been used[26]. 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.

Published online 2 July 2018

1. Palermo, G., Joris, H., Devroey, P. & Van Steirteghem, A. C. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* **340**, 17–18 (1992).
2. Palermo, G. D., Neri, Q. V. & Rosenwaks, Z. To ICSI or not to ICSI. *Semin. Reprod. Med.* **33**, 92–102 (2015).
3. Jain, T. & Gupta, R. Trends in the use of intracytoplasmic sperm injection in the United States. *N. Engl. J. Med.* **357**, 251–257 (2007).
4. van Rumste, M. M., Evers, J. L. & Farquhar, C. M. ICSI versus conventional techniques for oocyte insemination during IVF in patients with non-male factor subfertility: a Cochrane review. *Hum. Reprod.* **19**, 223–227 (2004).
5. Dyer, S. et al. International Committee for Monitoring Assisted Reproductive Technologies world report: assisted reproductive technology 2008, 2009 and 2010. *Hum. Reprod.* **31**, 1588–1609 (2016).
6. Boulet, S. L. et al. Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. *JAMA* **313**, 255–263 (2015).
7. Pereira, N. Lessons learned in andrology: from intracytoplasmic sperm injection and beyond. *Andrology* **4**, 757–760 (2016).
8. Tournaye, H. et al. Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. *Fertil. Steril.* **61**, 1045–1051 (1994).
9. Devroey, P. et al. Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection. *Fertil. Steril.* **62**, 639–641 (1994).
10. Silber, S. J. et al. High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. *Hum. Reprod.* **10**, 148–152 (1995).
11. Devroey, P. et al. Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum. Reprod.* **10**, 1457–1460 (1995).
12. Chambers, G. M. et al. Population trends and live birth rates associated with common ART treatment strategies. *Hum. Reprod.* **31**, 2632–2641 (2016).
13. Practice Committees of American Society for Reproductive Medicine and Society for Assisted Reproduction Technology. Intracytoplasmic sperm injection (ICSI) for non-male factor infertility: a committee opinion. *Fertil. Steril.* **98**, 1395–1399 (2012).
14. Devroey, P. & Van Steirteghem, A. A review of ten years experience of ICSI. *Hum. Reprod. Update* **10**, 19–28 (2004).
15. Esteves, S. C., Miyaoka, R. & Agarwal, A. An update on the clinical assessment of the infertile male. [corrected]. *Clinics* **66**, 691–700 (2011) erratum 67, 203 (2012).
16. Miyaoka, R. & Esteves, S. C. Predictive factors for sperm retrieval and sperm injection outcomes in obstructive azoospermia: do etiology, retrieval techniques and gamete source play a role? *Clinics* **68**, 111–119 (2013).
17. Esteves, S. C. Clinical management of infertile men with nonobstructive azoospermia. *Asian J. Androl.* **17**, 459–470 (2015).
18. Esteves, S. C., Miyaoka, R., Orosz, J. E. & Agarwal, A. An update on sperm retrieval techniques for azoospermic males. *Clinics* **68**, (Suppl.1) 99–110 (2013).
19. Tournaye, H. Male factor infertility and ART. *Asian J. Androl* **14**, 103–108 (2012).
20. Esteves, S. C. Novel concepts in male factor infertility: clinical and laboratory perspectives. *J. Assist. Reprod. Genet.* **33**, 1319–1335 (2016).
21. Esteves, S. C. et al. Critical appraisal of World Health Organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men. *Urology* **79**, 16–22 (2012).
22. Babayev, S. N., Park, C. W. & Bukulmez, O. Intracytoplasmic sperm injection indications: how rigorous? *Semin. Reprod. Med.* **32**, 283–290 (2014).
23. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th edn 226 (World Health Organization, 2010).
24. Cissen, M. et al. Assisted reproductive technologies for male subfertility. *Cochrane Database Syst. Rev.* **2**, CD000360 (2016).
25. Tournaye, H. et al. Intracytoplasmic sperm injection versus in vitro fertilization: a randomized controlled trial and a meta-analysis of the literature. *Fertil. Steril* **78**, 1030–1037 (2002).
26. Shuai, H. L., Ye, Q., Huang, Y. H. & Xie, B. G. Comparison of conventional in vitro fertilisation and intracytoplasmic sperm injection outcomes in patients with moderate oligoasthenozoospermia. *Andrologia* **47**, 499–504 (2015).
27. Kruger, T. F. et al. Sperm morphologic features as a prognostic factor in in vitro fertilization. *Fertil. Steril* **46**, 1118–1123 (1986).
28. Kruger, T. F. et al. Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertil. Steril* **49**, 112–117 (1998).
29. Gunalp, S., Onculoglu, C., Gurgan, T., Kruger, T. F. & Lombard, C. J. A study of semen parameters with emphasis on sperm morphology in a fertile population: an attempt to develop clinical thresholds. *Hum. Reprod.* **16**, 110–114 (2001).
30. Menkveld, R. et al. Semen parameters, including WHO and strict criteria morphology, in a fertile and subfertile population: an attempt to develop clinical thresholds. *Hum. Reprod.* **16**, 1165–1171 (2001).
31. Plachot, M. et al. Outcome of conventional IVF and ICSI on sibling oocytes in mild male fator infertility. *Hum. Reprod.* **17**, 362–369 (2002).
32. Hotaling, J. M., Smith, J. F., Rosen, M., Muller, C. H. & Walsh, T. J. The relationship between isolated teratozoospermia and clinical pregnancy after in vitro fertilization with or without intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertil. Steril.* **95**, 1141–1145 (2011).
33. Lundin, K., Soderlund, B. & Hamberger, L. The elationship between sperm morphology and rates of fertilization, pregnancy and spontaneous abortion in an in-vitro fertilization/intracytoplasmic sperm injection programme. *Hum. Reprod.* **12**, 2676–2681 (1997).
34. Osawa, Y. et al. Assessment of the dominant abnormal form is useful for predicting the outcome of intracytoplasmic sperm injection in the case of severe teratozoospermia. *J. Assist. Reprod. Genet.* **16**, 436–442 (1999).
35. Dubey, A. et al. The influence of sperm morphology on preimplantation genetic diagnosis cycles outcome. *Fertil. Steril.* **89**, 1665–1669 (2008).
36. Keegan, B. R. et al. Isolated teratozoospermia does not affect in vitro fertilization outcome and is not an indication for intracytoplasmic sperm injection. *Fertil. Steril.* **88**, 1583–1588 (2007).
37. Esteves, S. C. & Varghese, A. C. Laboratory handling of epididymal and testicular spermatozoa: what can be done to improve sperm injection outcome. *J. Hum. Reprod. Sci.* **5**, 233–243 (2012).
38. Povlsen, B. B., Aw, L. D., Laursen, R. J., Esteves, S. C. & Humaidan, P. Pregnancy and birth after intracytoplasmic sperm injection with normal testicular spermatozoa in a patient with azoospermia and tail stump epididymal sperm. *Int. Braz. J. Urol.* **41**, 1220–12255 (2015).
39. Dam, A. H. et al. Globozoospermia revisited. *Hum. Reprod. Update.* **13**, 63–75 (2007).
40. Rubino, P., Viganò, P., Luddi, A. & Piomboni, P. The ICSI procedure from past to future: a systematic review of the more controversial aspects. *Hum. Reprod. Update* **22**, 194–227 (2016).
41. Esteves, S. C., Schneider, D. T. & Verza, S. Jr. Influence of antisperm antibodies in the semen on intracytoplasmic sperm injection outcome. *Int. Braz. J. Urol.* **33**, 795–802 (2007).
42. Zini, A. et al. Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis. *Hum. Reprod.* **26**, 1288–1295 (2011).
43. Chamley, L. W. & Clarke, G. N. Antisperm antibodies and conception. *Semin. Immunopathol.* **29**, 169–184 (2007).
44. Cissen, M. Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: a systematic review and meta-analysis. *PLOS One* **11**, e0165125 (2016).
45. Esteves, S. C., Sharma, R. K., Gosálvez, J. & Agarwal, A. A translational medicine appraisal of specialized andrology testing in unexplained male infertility. *Int. Urol. Nephrol.* **46**, 1037–1052 (2014).

46. Majzoub, A., Esteves, S. C., Gosálvez, J. & Agarwal, A. Specialized sperm function tests in varicocele and the future of andrology laboratory. *Asian J. Androl.* **18**, 205–212 (2016).
47. Spano, M. et al. Sperm chromatin damage impairs human fertility. The Danish first pregnancy planner study team. *Fertil. Steril.* **73**, 43–50 (2000).
48. Giwercman, A. et al. Sperm chromatin structure assay as an independent predictor of fertility in vivo: a case-control study. *Int. J. Androl.* **33**, e221–227 (2010).
49. Agarwal, A., Cho, C. L. & Esteves, S. C. Should we evaluate and treat sperm DNA fragmentation? *Curr. Opin. Obstet. Gynecol.* **28**, 164–171 (2016).
50. Practice Committees of American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing: a guideline. *Fertil. Steril.* **99**, 673–677 (2013).
51. Simon, L., Zini, A., Dyachenko, A., Ciampi, A. & Carrell, D. T. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian J. Androl.* **19**, 80–90 (2017).
52. Osman, A., Alsomait, H., Seshadri, S., El-Toukhy, T. & Khalaf, Y. The effect of sperm DNA fragmentation on live birth rate after IVFI or ICSI: a systematic review and meta-analysis. *Reprod. Biomed. Online* **30**, 120–127 (2015).
53. Zini, A., Boman, J. M., Belzile, E. & Ciampi, A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. *Hum. Reprod.* **23**, 2663–2668 (2008).
54. Robinson, L. et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. *Hum. Reprod.* **27**, 2908–2917 (2012).
55. Zhao, J., Zhang, Q., Wang, Y. & Li, Y. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/ intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertil. Steril.* **102**, 998–1005 (2014).
56. Agarwal, A. et al. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. *Transl Androl. Urol.* **5**, 935–950 (2016).
57. Bhattacharya, S. et al. Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. *Lancet* **357**, 2075–2079 (2001).
58. Johnson, L. N. C., Sasson, I. E., Sammel, M. D. & Dokras, A. Does intracytoplasmic sperm injection improve the fertilization rate and decrease the total fertilization failure rate in couples with well-defined unexplained infertility? A systematic review and meta-analysis. *Fertil. Steril.* **100**, 704–711 (2013).
59. Bungum, L., Bungum, M., Humaidan, P. & Andersen, C. Y. A strategy for treatment of couples with unexplained infertility who failed to conceive after intrauterine insemination. *Reprod. Biomed. Online* **8**, 584–589 (2004).
60. Foong, S. C. et al. A prospective randomized trial of conventional in vitro fertilization versus intracytoplasmic sperm injection in unexplained infertility. *J. Assist. Reprod. Genet.* **23**, 137–140 (2006).
61. Tannus, S. et al. The role of intracytoplasmic sperm injection in non-male factor infertility in advanced maternal age. *Hum. Reprod.* **32**, 119–124 (2017).
62. Sfontouris, I. A. et al. Live birth rates using conventional in vitro fertilization compared to intracytoplasmic sperm injection in Bologna poor responders with a single oocyte retrieved. *J. Assist. Reprod. Genet.* **32**, 691–697 (2015).
63. Poseidon Group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. *Fertil. Steril.* **105**, 1452–1453 (2016).
64. Humaidan, P. et al. The novel POSEIDON stratification of 'low prognosis patients in assisted reproductive technology' and its proposed marker of successful outcome. *F1000Res* **5**, 2911 (2016).
65. Ferraretti, A. P. et al. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum. Reprod.* **26**, 1616–1624 (2011).
66. Artini, P. G. et al. Conventional IVF as a laboratory strategy to rescue fertility potential in severe poor responder patients: the impact of reproductive aging. *Gynecol. Endocrinol.* **29**, 997–1001 (2013).
67. Zegers-Hochschild, F. et al. The international glossary on infertility and fertility care, 2017. *Fertil. Steril.* **108**, 393–406 (2017).
68. Thornhill, A. R. et al. ESHRE PGD Consortium 'best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'. *Hum. Reprod.* **20**, 35–48 (2005).
69. Harton, G. L. et al. ESHRE PGD Consortium/ Embryology Special Interest Group—best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/ PGS). *Hum. Reprod.* **26**, 41–46 (2011).
70. Beukers, F. et al. Morphologic abnormalities in 2-year-old children born after in vitro fertilization/ intracytoplasmic sperm injection with preimplantation genetic screening: follow-up of a randomized controlled trial. *Fertil. Steril.* **99**, 408–413 (2013).
71. Desmyttere, S. et al. Growth and health outcome of 102 2-year-old children conceived after pre-implantation genetic diagnosis or screening. *Early Hum. Dev.* **85**, 755–759.
72. Liebaers, I. et al. Report on a consecutive series of 581 children born after blastomere biopsy for preimplantation genetic diagnosis. *Hum. Reprod.* **25**, 275–282 (2010).
73. Winter, C., Van Acker, F., Bonduelle, M. & Desmyttere, S. Psychosocial development of full term singletons, born after preimplantation genetic diagnosis (PGD) at preschool age and family functioning: a prospective case-controlled study and multi-informant approach. *Hum. Reprod.* **30**, 1122–1136 (2015).
74. Middelburg, K. J. et al. Neurological condition of infants born after in vitro fertilization with preimplantation genetic screening. *Pediatr. Res.* **67**, 430–434 (2010).
75. Winter, C. et al. Cognitive and psychomotor development of 5- to 6-year-old singletons born after PGD: a prospective case-controlled matched study. *Hum. Reprod.* **29**, 1968–1977 (2014).
76. Grimstad, F. W., Nangia, A. K., Luke, B., Stern, J. E. & Mak, W. Use of ICSI in IVF cycles in women with tubal ligation does not improve pregnancy or live birth rates. *Hum. Reprod.* **31**, 2750–2755 (2016).
77. Zafer, M. et al. Effectiveness of semen washing to prevent human immunodeficiency virus (HIV) transmission and assist pregnancy in HIV-discordant couples: a systematic review and meta-analysis. *Fertil. Steril.* **105**, 645–655 (2016).
78. Savasi, V., Oneta, M., Parrilla, B. & Cetin, I. Should HCV discordant couples with a seropositive male partner be treated with assisted reproduction techniques (ART)? *Eur. J. Obstet. Gynecol. Reprod. Biol.* **167**, 181–184 (2013).
79. Wu, M. Y. & Ho, H. N. Cost and safety of assisted reproductive technologies for human immunodeficiency virus-1 discordant couples. *World J. Virol.* **4**, 142–146 (2015).
80. Esteves, S. C., Miyaoka, R. & Agarwal, A. Surgical treatment of male infertility in the era of intracytoplasmic sperm injection - new insights. *Clinics* **66**, 1463–1478 (2011).
81. Strassburger, D. et al. Very low sperm count affects the result of intracytoplasmic sperm injection. *J. Assist Reprod. Genet.* **17**, 431–436 (2000).
82. Mitchell, V. et al. Outcome of ICSI with ejaculated spermatozoa in a series of men with distinct ultrastructural flagellar abnormalities. *Hum. Reprod.* **21**, 2065–2074 (2006).
83. Esteves, S. C. & Agarwal, A. Reproductive outcomes, including neonatal data, following sperm injection in men with obstructive and nonobstructive azoospermia: case series and systematic review. *Clinics* **68**, 141–150 (2013).
84. Esteves, S. C. et al. Comparison of sperm retrieval and reproductive outcome in azoospermic men with testicular failure and obstructive azoospermia treated for infertility. *Asian J. Androl.* **16**, 602–606 (2014).
85. Gosálvez, J., Lopez-Fernandez, C., Fernandez, J. L., Esteves, S. C. & Johnston, S. D. Unpacking the mysteries of sperm DNA fragmentation: ten frequently asked questions. *J. Reprod. Biotechnol. Fertil.* **4**, 1–16 (2015).
86. Hamada, A., Esteves, S. C., Nizza, M. & Agarwal, A. Unexplained male infertility: diagnosis and management. *Int. Braz. J. Urol.* **38**, 576–594 (2012).
87. Sakkas, D. & Alvarez, J. G. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil. Steril.* **93**, 1027–1036 (2010).
88. Avendaño, C., Franchi, A., Duran, H. & Oehninger, S. DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. *Fertil. Steril.* **94**, 549–557 (2010).
89. Aitken, R. J., Bronson, R., Smith, T. B. & De Iullis, G. N. The source and significance of DNA damage in human spermatozoa: a commentary on diagnostic strategies and straw man fallacies. *Mol. Hum. Reprod.* **19**, 474–485 (2013).
90. Esteves, S. C. et al. Diagnostic accuracy of sperm DNA degradation index (DDSi) as a potential noninvasive biomarker to identify men with varicocele-associated infertility. *Int. Urol. Nephrol.* **47**, 1471–1477 (2015).
91. Agarwal, A., Sharma, R., Harlev, A. & Esteves, S. C. Effect of varicocele on semen characteristics according to the new 2010 World Health Organization criteria: a systematic review and meta-analysis. *Asian J. Androl.* **18**, 163–170 (2016).
92. Wang, Y. J., Zhang, R. Q., Lin, Y. J., Zhang, R. G. & Zhang, W. L. Relationship between varicocele and sperm DNA damage and the effect of varicocele repair: a meta-analysis. *Reprod. Biomed. Online.* **25**, 307–314 (2012).
93. Agarwal, A., Hamada, A. & Esteves, S. C. Insight into oxidative stress in varicocele-associated male infertility: part 1. *Nat. Rev. Urol.* **9**, 678–690 (2012).
94. Hamada, A., Esteves, S. C. & Agarwal, A. Insight into oxidative stress in varicocele-associated male infertility: part 2. *Nat. Rev. Urol.* **10**, 26–37 (2013).
95. Zini, A. & Dohle, G. Are varicoceles associated with increased deoxyribonucleic acid fragmentation? *Fertil. Steril.* **96**, 1283–1287 (2011).
96. Esteves, S. C., Oliveira, F. V. & Bertolla, R. P. Clinical outcome of intracytoplasmic sperm injection in infertile men with treated and untreated clinical varicocele. *J. Urol.* **184**, 1442–1446 (2010).
97. Esteves, S. C., Roque, M. & Agarwal, A. Outcome of assisted reproductive technology in men with treated and untreated varicocele: systematic review and meta-analysis. *Asian J. Androl.* **18**, 254–258 (2016).
98. Kirby, E. W., Wiener, L. E., Rajanahally, S., Crowell, K. & Coward, R. M. Undergoing varicocele repair before assisted reproduction improves pregnancy rate and live birth rate in azoospermic and oligospermic men with a varicocele: a systematic review and meta-analysis. *Fertil. Steril.* **106**, 1338–1343 (2016).
99. Esteves, S. C., Miyaoka, R., Roque, M. & Agarwal, A. Outcome if varicocele repair in men with nonobstructive azoospermia: a systematic review and meta-analysis. *Asian J. Androl.* **18**, 246–253 (2016).
100. Kovalski, N. N., de Lamirande, E. & Gagnon, C. Reactive oxygen species generated by human neutrophils inhibit sperm motility: protective effect of seminal plasma and scavengers. *Fertil. Steril.* **58**, 809–816 (1992).
101. Moskovtsev, S. I., Willis, J., White, J. & Mullen, J. B. Leukocytospermia: a relationship to sperm deoxyribonucleic acid integrity in patients evaluated for male factor infertility. *Fertil. Steril.* **88**, 737–740 (2007).
102. Moskovtsev, S. I. et al. Cause specific treatment in patients with high sperm DNA damage resulted in significant DNA improvement. *Syst. Biol. Reprod. Med.* **55**, 109–115 (2009).
103. Barraud-Lange, V. et al. Seminal leukocytes are good samaritans for spermatozoa. *Fertil. Steril.* **96**, 1315–1319 (2011).
104. Ricci, G. et al. Effect of seminal leukocytes on in vitro fertilization and intracytoplasmic sperm injection outcomes. *Fertil. Steril* **104**, 87–93 (2015).
105. Agarwal, A. & Sekhon, L. H. Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: is it justified? *Indian J. Urol.* **27**, 74–85 (2011).
106. Showell, M. G. et al. Antioxidants for male subfertility. *Cochrane Database Syst. Rev.* **12**, CD007411 (2014).
107. Morrison, C. D. & Brannigan, R. E. Metabolic syndrome and infertility in men. *Best Pract. Res. Clin. Obstet. Gynaecol.* **29**, 507–515 (2015).
108. Kasturi, S. S., Tannir, J. & Brannigna, R. E. The metabolic syndrome and male infertility. *J. Androl.* **29**, 251–259 (2008).
109. Bakos, H. W. et al. Paternal body mass index is associated with decreased blastocyst development and reduced live birth rate following assisted reproductive technology. *Fertil. Steril.* **95**, 1700–1704 (2011).
110. Keltz, J. et al. Overweight men: clinical pregnancy after ART is decreased in IVF but not in ICSI cycles. *J. Assist. Reprod. Genet.* **27**, 539–544 (2010).
111. Wang, X. et al. Effects of female and male body mass indices on the treatment outcomes and neonatal birth weights associated with in vitro fertilization/

intracytoplasmic sperm injection treatment in China. *Fertil. Steril.* **106**, 460–466 (2016).
112. Le, W., Su, S. H., Shi, L. H., Zhang, J. F. & Wu, D. L. Effect of male body mass index on clinical outcomes following assisted reproductive technology: a meta-analysis. *Andrologia* **48**, 406–424 (2016).
113. Dabaja, E. A. & Schlegel, P. N. Medical treatment of male infertility. *Transl Andol. Urol.* **3**, 9–16 (2014).
114. Fraietta, R., Zylberstejn, D. S. & Esteves, S. C. Hypogonadotropic hypogonadism revisited. *Clinics* **68**, 81–88 (2013).
115. Schlegel, P. N. Aromatase inhibitors for male infertility. *Fertil. Steril.* **98**, 1359–1362 (2012).
116. Hussein, A., Ozgok, Y., Ross, L. & Niederberger, C. Clomiphene administration for cases of nonobstructive azoospermia: a multicenter study. *J. Androl.* **26**, 787–791 (2005).
117. Reifsnyder, J. E., Ramasamy, R., Husseini, J. & Schlegel, P. N. Role of optimizing testosterone before microdissection testicular sperm extraction in men with nonobstructive azoospermia. *J. Urol.* **188**, 535–536 (2012).
118. Shiraishi, K., Ohmi, C., Shimabukuro, T. & Matsuyama, H. Human chorionic gonadotrophin treatment prior to microdissection testicular sperm extraction in non-obstructive azoospermia. *Hum. Reprod.* **27**, 331–339 (2012).
119. Ramasamy, R., Stahl, P. J. & Schlegel, P. N. Medical therapy for spermatogenic failure. *Asian J. Androl.* **14**, 57–60 (2012).
120. Gudeloglu, A., Brahmbhatt, J. V. & Parekattil, S. J. Medical management of male infertility in the absence of a specific etiology. *Semin. Reprod. Med.* **32**, 313–318 (2014).
121. Garg, H. & Kumar, R. Empirical drug therapy for idiopathic male infertility: what is the new evidence? *Urology* **86**, 1065–1075 (2015).
122. Attia, A. M., AbouSetta, A. M. & Al-Inany, H. G. Gonadotrophins for idiopathic male factor subfertility. *Cochrane Database Syst. Rev.* **23**, CD005071 (2013).
123. Baccetti, B. et al. Effect of follicle-stimulating hormone on sperm quality and pregnancy rate. *Asian J. Androl.* **6**, 133–137 (2004).
124. Caroppo, E., Niederberger, C., Vizziello, G. M. & D'Amato, G. Recombinant human follicle stimulating hormone as a pretreatment for idiopathic oligoasthenoteratozoospermic patients undergoing intracytoplasmic sperm injection. *Fertil. Steril.* **80**, 1398–1403 (2003).
125. Colacurci, N. et al. Recombinant human FSH reduces sperm DNA fragmentation in men with idiopathic oligoasthenoteratozoospermia. *J. Androl.* **33**, 588–593 (2012).
126. Simoni, M. et al. Treatment with human, recombinant FSH improves sperm DNA fragmentation in idiopathic infertile men depending on the FSH receptor polymorphism p. N680S: a pharmacogenetic study. *Hum. Reprod.* **31**, 1960–1969 (2016).
127. Patel, N. & Kashanian, J. A. Thyroid dysfunction and male reproductive physiology. *Semin. Reprod. Med.* **34**, 356–360 (2016).
128. Krassas, G. E. et al. A prospective controlled study of the impact of hyperthyroidism on reproductive function in males. *J. Clin. Endocrinol. Metab.* **87**, 3667–3671 (2002).
129. Krajewska-Kulak, E. & Sengupta, P. Thyroid function in male infertility. *Front. Endocrino.* **13**, 174 (2013).
130. Singh, P., Singh, M., Cugati, G. & Singh, A. K. Hyperprolactinemia: an often missed cause of male infertility. *J. Hum. Reprod. Sci.* **4**, 102–103 (2011).
131. Glazer, C. H. et al. Risk of diabetes according to male factor infertility: a register-based cohort study. *Hum. Reprod.* **9**, 1–8 (2017).
132. Esteves, S. C., Schertz, J. C., Verza, Jr, S., Schneider, D. T. & Zabaglia, S. F. A comparison of menotropin, highly-purified menotropin and follitropin alfa in cycles of intracytoplasmic sperm injection. *Reprod. Biol. Endocrinol.* **7**, 111 (2009).
133. Henkel, R. R. & Schill, W. B. Sperm preparation for ART. *Reprod. Biol. Endocrinol.* **1**, 108 (2003).
134. Schneider, D., Feijo, C., Verza, Jr, S. & Esteves, S. Effectiveness of sperm washing by discontinuous density gradient centrifugation to remove antibodies bound to the sperm membrane. *Med. Express.* **1**, 123–126 (2014).
135. Esteves, S. C., Sharma, R. K., Thomas, Jr, A. J. & Agarwal, A. Effect of swim-up sperm washing and subsequent capacitation on acrosome status and functional membrane integrity of normal sperm. *Int. J. Fertil. Womens Med.* **45**, 335–341 (2000).
136. Rappa, K. L. et al. Sperm processing for advanced reproductive technologies: Where are we today? *Biotechnol. Adv.* **34**, 578–587 (2016).
137. Said, T. M. & Land, J. A. Effects of advanced selection methods on sperm quality and ART outcome: a systematic review. *Hum. Reprod. Update* **17**, 719–733 (2011).
138. Simon, L., Ge, S. Q. & Carrell, D. T. Sperm selection based on electrostatic charge. *Methods Mol. Biol.* **927**, 269–278 (2013).
139. Aitken, R. J. et al. Electrophoretic sperm isolation: optimization of electrophoresis conditions and impact on oxidative stress. *Hum. Reprod.* **26**, 1955–1964 (2011).
140. Chan, P. J., Jacobson, J. D., Corselli, J. U. & Patton, W. C. A simple zeta method for sperm selection based on membrane charge. *Fertil. Steril.* **85**, 481–486 (2006).
141. Nasr Esfahani, M. H., Deemeh, M. R., Tavalaee, M., Sekhavati, M. H. & Gourabi, H. Zeta sperm selection improves pregnancy rate and alters sex ratio in male factor infertility patients: a double-blind, randomized clinical trial. *Int. J. Fertil. Steril.* **10**, 253–260 (2016).
142. Stoffel, M. H. et al. Density and distribution of anionic sites on boar ejaculated and epididymal spermatozoa. *Histochem. Cell Biol.* **117**, 441–445 (2002).
143. Gil, M., Sar-Shalom, M., Melendez Sivira, Y., Carreras, R. & Checa, M. A. Sperm selection using magnetic activated cell sorting (MACS) in assisted reproduction: a systematic review and meta-analysis. *J. Assist Reprod. Genet.* **30**, 479–485 (2013).
144. Simopoulou, M. et al. Improving ICSI: a review from the spermatozoon perspective. *Syst. Biol. Reprod. Med.* **62**, 359–371 (2016).
145. McDowell, S. et al. Advanced sperm selection techniques for assisted reproduction. *Cochrane Database Syst. Rev.* **10**, CD010461 (2014).
146. Romany, L. et al. Removal of anexin V – positive sperm cells for intracytoplasmic sperm injection in ovum donation cycles does not improve reproductive outcome: a controlled and randomized trial in unselected males. *Fertil. Steril.* **102**, 1567–1575 (2014).
147. Sakkas, D. Novel technologies for selecting the best sperm for in vitro fertilization and intracytoplasmic sperm injection. *Fertil. Steril.* **99**, 1023–1029 (2013).
148. Bech-Fruchter, R., Shalev, E. & Weiss, A. Clinical benefit using sperm hyaluronic acid binding technique in ICSI cycles: a systematic review and meta-analysis. *Reprod. Biomed. Online.* **32**, 286–298 (2016).
149. Vanderzwalmen, P. et al. Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. *Reprod. Biomed. Online* **17**, 617–627 (2008).
150. Knez, K., Zorn, B., Tomazevic, T., Vrtacnik-Bokal, E. & Virant-Klun, I. The IMSI procedure improves poor embryo development in the same infertile couples with poor semen quality: a comparative prospective randomized study. *Reprod. Biol. Endocrinol.* **9**, 123 (2011).
151. Berkovitz, A. et al. How to improve IVF-ICSI outcome by sperm selection. *Reprod. Biomed. Online* **12**, 634–638 (2006).
152. Setti, A. S. et al. Intracytoplasmic morphologically selected sperm injection is beneficial in cases of advanced maternal age: a prospective randomized study. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **171**, 286–290 (2013).
153. Setti, A. S. et al. Twelve years of MSOME and IMSI: a review. *Reprod. Biomed. Online* **27**, 338–352 (2013).
154. Teixeira, D. M. et al. Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction. *Cochrane Database Syst. Rev.* **7**, CD010167 (2013).
155. Bradley, C. K. et al. Intervention improves assisted conception intracytoplasmic sperm injection outcomes for patients with high levels of sperm DNA fragmentation: a retrospective analysis. *Andrology* **4**, 903–910 (2016).
156. Esteves, S. C., Miyaoka, R. & Agarwal, A. Sperm retrieval techniques for assisted reproduction. *Int. Braz. Urol.* **37**, 570–583 (2011).
157. Esteves, S. C. et al. Reproductive potential of men with obstructive azoospermia undergoing percutaneous sperm retrieval and intracytoplasmic sperm injection according to the cause of obstruction. *J. Urol.* **189**, 232–237 (2013).
158. Deruyver, Y., Vanderschueren, D. & Van der Aa, F. Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: a systematic review. *Andrology* **2**, 20–24 (2014).
159. Esteves, S. C. & Agarwal, A. Re: sperm retrieval rates and intracytoplasmic sperm injection outcomes for men with non-obstructive azoospermia and the health of resulting offspring. *Asian J. Androl.* **16**, 642 (2014).
160. Esteves, S. C. Microdissection testicular sperm extraction (micro-TESE) as a sperm acquisition method for men with nonobstructive azoospermia seeking fertility: operative and laboratory aspects. *Int. Braz. J. Urol.* **39**, 440 (2013).
161. Esteves, S. C., Verza, Jr. S. et al. in *Practical Manual of In Vitro Fertilization* (eds Nagy, Z. P., Varghese, A. C., Agarwal A.) 207–220 (Springer, New York, 2012).
162. Endo, Y. et al. Simple vitrification for small numbers of human spermatozoa. *Reprod. Biomed. Online.* **24**, 301–307 (2012).
163. Coetzee, K., Ozgur, K., Berkkanoglu, M., Bulut, H. & Isikli, A. Reliable single sperm cryopreservation in Cell Sleepers for azoospermia management. *Andrologia* **48**, 203–210 (2016).
164. Van Peperstraten, A., Proctor, M. L., Johnson, N. P. & Philipson, G. Techniques for surgical retrieval of sperm prior to intra-cytoplasmic sperm injection (ICSI) for azoospermia. *Cochrane Database Syst Rev.* **16**, CD002807 (2008).
165. Van Wely, M., Barbey, N., Meissner, A., Repping, S. & Silber, S. J. Live birth rates after MESA or TESE in men with obstructive azoospermia: is there a difference? *Hum. Reprod.* **30**, 761–766 (2015).
166. Ohlander, S., Hotaling, J., Kirshenbaum, E., Niederberger, C. & Eisenberg, M. L. Impact of fresh versus cryopreserved testicular sperm upon intracytoplasmic sperm injection pregnancy outcomes in men with azoospermia due to spermatogenic dysfunction: a meta-analysis. *Fertil. Steril.* **101**, 344–349 (2014).
167. Meijerink, A. M. et al. Prediction model for live birth in ICSI using testicular extracted sperm. *Hum. Reprod.* **31**, 1942–1951 (2016).
168. Vozdova, M., Heracek, J., Sobotka, V. & Rubes, J. Testicular sperm aneuploidy in non-obstructive azoospermic patients. *Hum. Reprod.* **27**, 2233–2239 (2012).
169. Greco, E. et al. Efficient treatment of infertility due to sperm DNA dmage by ICSI with testicular spermatozoa. *Hum. Reprod.* **20**, 226–230 (2005).
170. Esteves, S. C., Sánchez-Martín, F., Sánchez-Martín, P., Schneider, D. T. & Gosálvez, J. Comparison of reproductive outcome in oligozoospermic men with high sperm DNA fragmentation undergoing intracytoplasmic sperm injection with ejaculated and testicular sperm. *Fertil. Steril.* **104**, 1398–1405 (2015).
171. Moskovtsev, S. I. et al. Testicular spermatozoa have statistically significantly lower DNA damage compared with ejaculated spermatozoa in patients with unsuccessful oral antioxidant treatment. *Fertil. Steril.* **93**, 1142–1146 (2010).
172. Moskovtsev, S. I. et al. A comparison of ejaculated and testicular spermatozoa aneuploidy rates in patients with high sperm DNA damage. *Syst. Biol. Reprod. Med.* **58**, 142–148 (2012).
173. Mehta, A., Bolyakov, A., Schlegel, P. N. & Paduch, D. A. Higher pregnancy rates using testicular sperm in men with severe oligospermia. *Fertil. Steril.* **104**, 1382–1387 (2015).
174. Esteves, S. C., Roque, M. & Garrido, N. Use of testicular sperm for intracytoplasmic sperm injection in men with high sperm DNA fragmentation: a SWOT analysis. *Asian J. Androl.* **20**, 1–8 (2018).
175. Esteves, S. C., Roque, M., Bradley, C. K. & Garrido, N. Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis. *Fertil. Steril.* **108**, 456–467 (2017).
176. Abhyankar, N., Kathrins, M. & Niederberger, C. Use of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with cryptozoospermia: a meta-analysis. *Fertil. Steril.* **105**, 1469–1475 (2016).
177. Pabuccu, E. G., Caglar, G. S., Tangal, S., Haliloglu, A. H. & Pabuccu, R. Testicular versus ejaculated spermatozoa in ICSI cycles of normozoospermic men with high sperm DNA fragmentation and previous ART failures. *Andrologia* **49**, https://doi.org/10.1111/and.12609 (2017).
178. Zini, A., Bach, P. V., Al-Malki, A. H. & Schlegel, P. N. Use of testicular sperm for ICSI in oligozoospermic couples: how far should we go? *Hum. Reprod.* **32**, 7–13 (2017).
179. Vloeberghs, V., Verheyen, G. & Tournaye, H. Intracytoplasmic spermatid injection and in vitro maturation: fact or fiction? *Clinics* **68**, 151–156 (2013).

180. Ogura, A., Yanagimachi, R. & Usui, N. Behaviour of hamster and mouse round spermatid nuclei incorporated into mature oocytes by electrofusion. *Zygote* **1**, 1–8 (1993).
181. Goswami, G., Singh, S. & Devi, M. G. Successful fertilization and embryo development after spermatid injection: a hope for nonobstructive azospermic patients. *J. Hum. Reprod. Sci.* **8**, 175–177 (2015).
182. Vanderzwalmen, P. et al. Fertilization of an oocyte microinseminated with a spermatid in an *in-vitro* fertilization programme. *Hum. Reprod.* **10**, 502–503 (1995).
183. Tesarik, J., Mendoza, C. & Testart, J. Viable embryos from injection of round spermatids into oocyte. *N. Engl. J. Med.* **333**, 525 (1995).
184. Fischel, S., Aslam, I. & Tesarik, J. Spermatid conception: a stage too early, or a time too soon? *Hum. Reprod.* **11**, 1371–1375 (1996).
185. Practice Committees of American Society for Reproductive Medicine and Society for Assisted Reproduction Technology. Round spermatid nucleus injection (ROSNI). *Fertil. Steril.* **90**, S199–201 (2008).
186. Tanaka, A. et al. Fourteen babies born after round spermatid injection into human oocytes. *Proc. Natl Acad. Sci. USA* **112**, 14629–14634 (2015).
187. Aitken, R. J. & De Iuliis, G. N. Origins and consequences of DNA damage in male germ cells. *Reprod. Biomed. Online* **14**, 727–733 (2007).
188. Krawetz, S. A. Paternal contribution: new insights and future challenges. *Nat. Rev. Genet.* **6**, 633–642 (2005).
189. Hamada, A. J., Esteves, S. C. & Agarwal, A. A comprehensive review of genetics and genetic testing in azoospermia. *Clinics* **68** (Suppl. 1), 39–60 (2013).
190. Kumar, M., Kumar, K., Jain, S., Hassan, T. & Dada, R. Novel insights into the genetic and epigenetic paternal contribution to the human embryo. *Clinics* **68** (Suppl. 1), 5–14 (2013).
191. Lewis, S. E. & Aitken, R. J. DNA damage to spermatozoa has impacts on fertilization and pregnancy. *Cell Tissue Res.* **322**, 33–41 (2005).
192. Terada, Y., Luetjens, C. M., Sutovsky, P. & Schatten, G. Atypical decondensation of the sperm nucleus, delayed replication of the male genome, and sex chromosome positioning following intracytoplasmic human sperm injection (ICSI) into golden hamster eggs: does ICSI itself introduce chromosomal anomalies? *Fertil. Steril.* **74**, 454–460 (2000).
193. Van Der Westerlaken, L. A., Helmerhorst, F. M., Hermans, J. & Naaktgeboren, N. Intracytoplasmic sperm injection: position of the polar body affects pregnancy rate. *Hum. Reprod.* **14**, 2565–2569 (1999).
194. Coates, A. et al. Use of suboptimal sperm increases the risk of aneuploidy of the sex chromosomes in preimplantation blastocyst embryos. *Fertil. Steril.* **104**, 866–872 (2015).
195. Pandey, S., Shetty, A., Hamilton, M., Bhattacharya, S. & Maheshwari, A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Hum. Reprod. Update* **18**, 485–503 (2012).
196. Hansen, M., Kurinczuk, J. J., Milne, E., de Klerk, N. & Bower, C. Assisted reproductive technology and birth defects: a systematic review and meta-analysis. *Hum. Reprod. Update* **19**, 330–353 (2013).
197. Qin, J. et al. Assisted reproductive technology and risk of congenital malformations: a meta-analysis based on cohort studies. *Arch. Gynecol. Obstet.* **292**, 777–798 (2015).
198. Wen, J. et al. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. *Fertil. Steril.* **97**, 1331–1337 (2012).
199. Rimm, A. A., Katayama, A. C. & Katayama, K. P. A meta-analysis of the impact of IVF and ICSI on major malformations after adjusting for the effect of subfertility. *J. Assist Reprod. Genet.* **28**, 699–705 (2011).
200. Massaro, P. A., MacLellan, D. L., Anderson, P. A. & Romao, R. L. Does intracytoplasmic sperm injection pose an increased risk of genitourinary congenital malformations in offspring compared to in vitro fertilization? A systematic review and meta-analysis. *J. Urol.* **193**, 1837–1842 (2015).
201. Rossi, A. C. & D'Addario, V. Neonatal outcomes of assisted and naturally conceived twins: systematic review and meta-analysis. *J. Perinat Med.* **39**, 489–493 (2011).
202. Zhu, J. L., Basso, O., Obel, C., Bille, C. & Olsen, J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *BMJ* **333**, 679AE (2006).
203. Kissin, D. M. et al. Association of assisted reproductive technology (ART) treatment and parental infertility diagnosis with autism in ART-conceived children. *Hum. Reprod.* **30**, 454–465 (2015).
204. Tollervey, J. R. & Lunyak, V. V. Epigenetics: judge, jury and executioner of stem cell fate. *Epigenetics* **7**, 823–840 (2012).
205. Geiman, T. M. & Robertson, K. D. Chromatin remodeling, histone modifications, and DNA methylation-how does it all fit together? *J. Cell. Biochem.* **87**, 117–125 (2002).
206. Bunkar, N., Pathak, N., Lohiya, N. K. & Mishra, P. K. Epigenetics: a key paradigm in reproductive health. *Clin. Exp. Reprod. Med.* **43**, 59–81 (2016).
207. Hogg, K. & Western, P. S. Refurbishing the germline epigenome: Out with the old, in with the new. *Semin. Cell Dev. Biol.* **45**, 104–113 (2015).
208. Odom, L. N. & Segars, J. Imprinting disorders and assisted reproductive technology. *Curr. Opin. Endocrinol. Diabetes Obes.* **17**, 517–522 (2010).
209. Gosden, R., Trasler, J., Lucifero, D. & Faddy, M. Rare congenital disorders, imprinted genes, and assisted reproductive technology. *Lancet* **361**, 1975–1977 (2003).
210. Kobayashi, H. et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. *Hum. Mol. Genet.* **16**, 2542–2551 (2007).
211. de Waal, E. et al. Gonadotropin stimulation contributes to an increased incidence of epimutations in ICSI-derived mice. *Hum. Mol. Genet.* **21**, 4460–4472 (2012).
212. Hartmann, S., Bergmann, M., Bohle, R. M., Weidner, W. & Steger, K. Genetic imprinting during impaired spermatogenesis. *Mol. Hum. Reprod.* **12**, 407–411 (2006).
213. Marques, C. et al. Abnormal methylation of imprinted genes in human sperm is associated with oligozoospermia. *Mol. Hum. Reprod.* **14**, 67–74 (2008).
214. Kobayashi, H. et al. DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. *Eur. J. Hum. Genet.* **17**, 1582–1591 (2009).
215. Marques, C. et al. Methylation defects of imprinted genes in human testicular spermatozoa. *Fertil. Steril.* **94**, 585–594 (2010).
216. Boissonnas, C. et al. Specific epigenetic alterations of IGF2-H19 locus in spermatozoa from infertile men. *Eur. J. Hum. Genet.* **18**, 73–80 (2010).
217. Gomes, M. V., Huber, J., Ferriani, R. A., Amaral Neto, A. M. & Ramos, E. S. Abnormal methylation at the KvDMR1 imprinting control region in clinically normal children conceived by assisted reproductive technologies. *Mol. Hum. Reprod.* **15**, 471–477 (2009).
218. Kanber, D., Buiting, K., Zeschnigk, M., Ludwig, M. & Horsthemke, B. Low frequency of imprinting defects in ICSI children born small for gestational age. *Eur. J. Hum. Genet.* **17**, 22–29 (2009).
219. Tierling, S. et al. Assisted reproductive technologies do not enhance the variability of DNA methylation imprints in human. *J. Med. Genet.* **47**, 371–376 (2010).
220. King, J. L. et al. Skewed X inactivation and IVF-conceived infants. *Reprod. Biomed. Online* **20**, 660–663 (2010).
221. Turan, N. et al. Inter- and intra-individual variation in allele-specific DNA methylation and gene expression in children conceived using assisted reproductive technology. *PLOS Genet.* **6**, e1001033 (2010).
222. Wong, E. C., Hatakeyama, C., Robinson, W. P. & Ma, S. DNA methylation at H19/IGF2 ICR1 in the placenta of pregnancies conceived by in vitro fertilization and intracytoplasmic sperm injection. *Fertil. Steril.* **95**, 2524–2526.e3 (2011).
223. Li, L. et al. Evaluation of DNA methylation status at differentially methylated regions in IVF-conceived newborn twins. *Fertil. Steril.* **95**, 1975–1979 (2011).
224. Feng, C. et al. General imprinting status is stable in assisted reproduction-conceived offspring. *Fertil. Steril.* **96**, 1417–1423.e9 (2011).
225. Oliver, V. F. et al. Defects in imprinting and genome-wide DNA methylation are not common in the in vitro fertilization population. *Fertil. Steril.* **97**, 147–153 (2012).
226. Rancourt, R. C., Harris, H. R. & Michels, K. B. Methylation levels at imprinting control regions are not altered with ovulation induction or in vitro fertilization in a birth cohort. *Hum. Reprod.* **27**, 2208–2216 (2012).
227. Puumala, S. E. et al. Similar DNA methylation levels in specific imprinting control regions in children conceived with and without assisted reproductive technology: a cross-sectional study. *BMC Pediatr.* **12**, 33 (2012).
228. Hiura, H. et al. Characterization of DNA methylation errors in patients with imprinting disorders conceived by assisted reproduction technologies. *Hum. Reprod.* **27**, 2541–2548 (2012).
229. Nelissen, E. C. et al. Placentas from pregnancies conceived by IVF/ICSI have a reduced DNA methylation level at the H19 and MEST differentially methylated regions. *Hum. Reprod.* **28**, 1117–1126 (2013).
230. Sakian, S. et al. Altered gene expression of H19 and IGF2 in placentas from ART pregnancies. *Placenta* **36**, 1100–1105 (2015).
231. Melamed, N., Choufani, S., Wilkins-Haug, L. E., Koren, G. & Weksberg, R. Comparison of genome-wide and gene-specific DNA methylation between ART and naturally conceived pregnancies. *Epigenetics* **10**, 474–483 (2015).
232. Vincent, R. N., Gooding, L. D., Louie, K., Chan Wong, E. & Ma, S. Altered DNA methylation and expression of PLAGL1 in cord blood from assisted reproductive technology pregnancies compared with natural conceptions. *Fertil. Steril.* **106**, 739–748.e3 (2016).
233. Mehdi, M. et al. Aneuploidy rate in spermatozoa of selected men with severe teratozoospermia. *Andrologia* **44** (Suppl. 1), 139–143 (2012).
234. Hassold, T. et al. Human aneuploidy: incidence, origin, and etiology. *Environ. Mol. Mutag.* **28**, 167–175 (1996).
235. Morales, C. et al. Cytogenetic study of spontaneous abortions using semi-direct analysis of chorionic villi samples detects the broadest spectrum of chromosome abnormalities. *Am. J. Med. Genet. A* **146A**, 66–70 (2008).
236. Griffin, D. K., Hyland, P., Tempest, H. G. & Homa, S. T. Safety issues in assisted reproduction technology: should men undergoing ICSI be screened for chromosome abnormalities in their sperm? *Hum. Reprod.* **18**, 229–235 (2003).
237. Kushnir, V. A. & Frattarelli, J. L. Aneuploidy in abortuses following IVF and ICSI. *J. Assist Reprod. Genet.* **26**, 93–97 (2009).
238. Magli, M. C. et al. Paternal contribution to aneuploidy in preimplantation embryos. *Reprod. Biomed. Online* **18**, 536–542 (2009).
239. Rodrigo, L. et al. Testicular sperm from patients with obstructive and nonobstructive azoospermia: aneuploidy risk and reproductive prognosis using testicular sperm from fertile donors as control samples. *Fertil. Steril.* **95**, 1005–1012 (2011).
240. Bingol, B., Abike, F., Gedikbasi, A., Tapisiz, O. L. & Gunenc, Z. Comparison of chromosomal abnormality rates in ICSI for non-male factor and spontaneous conception. *J. Assist Reprod. Genet.* **29**, 25–30 (2012).
241. Ghoraeian, P., Mozdarani, H., Aleyasin, A. & Alizadeh-Nili, H. Frequency of sex chromosomal disomy in spermatozoa of normal and oligozoospermic Iranian patients and its effects on fertilisation and implantation rates after ICSI. *Andrologia* **45**, 46–55 (2013).
242. Mau Kai, C. et al. Reduced serum testosterone levels in infant boys conceived by intracytoplasmic sperm injection. *J. Clin. Endocrinol. Metab.* **92**, 2598–2603 (2007).
243. Jensen, T. K. et al. Fertility treatment and reproductive health of male offspring: a study of 1,925 young men from the general population. *Am. J. Epidemiol.* **165**, 583–590 (2007).
244. Belva, F. et al. Semen quality of young adult ICSI offspring: the first results. *Hum. Reprod.* **31**, 2811–2820 (2016).
245. Belva, F. et al. Reproductive hormones of ICSI-conceived young adult men: the first results. *Hum. Reprod.* **32**, 439–446 (2017).
246. Belva, F. et al. Serum reproductive hormone levels and ultrasound findings in female offspring after intracytoplasmic sperm injection: first results. *Fertil. Steril.* **107**, 934–939 (2017).
247. Andersson, A. M., Jorgensen, N., Frydelund-Larsen, L., Rajpert-De Meyts, E. & Skakkebaek, N. E. Impaired Leydig cell function in infertile men: a study of 357 idiopathic infertile men and 318 proven fertile controls. *J. Clin. Endocrinol. Metab.* **89**, 3161–3167 (2004).
248. Lightfoot, T. J. & Roman, E. Causes of childhood leukaemia and lymphoma. *Toxicol. Appl. Pharmacol.* **199**, 104–117 (2004).

249. Raimondi, S., Pedotti, P. & Taioli, E. Meta-analysis of cancer incidence in children born after assisted reproductive technologies. *Br. J. Cancer* **93**, 1053–1056 (2005).
250. Hargreave, M., Jensen, A., Toender, A., Andersen, K. K. & Kjaer, S. K. Fertility treatment and childhood cancer risk: a systematic meta-analysis. *Fertil. Steril.* **100**, 150–161 (2013).
251. Williams, C. L. et al. Cancer risk among children born after assisted conception. *N. Engl. J. Med.* **369**, 1819–1827 (2013).
252. Sundh, K. J. et al. Cancer in children and young adults born after assisted reproductive technology: a Nordic cohort study from the Committee of Nordic ART and Safety (CoNARTaS). *Hum. Reprod.* **29**, 2050–2057 (2014).
253. Reigstad, M. M. et al. Risk of cancer in children conceived by assisted reproductive technology. *Pediatrics* **137**, e20152061 (2016).
254. Källén, B. et al. Cancer risk in children and young adults conceived by in vitro fertilization. *Pediatrics* **126**, 270–276 (2010).
255. Bay, B., Mortensen, E. L. & Kesmodel, U. S. Assisted reproduction and child neurodevelopmental outcomes: a systematic review. *Fertil. Steril.* **100**, 844–853 (2013).
256. Pinborg, A. et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum. Reprod. Update* **19**, 87–104 (2013).
257. Carson, C. et al. Cognitive development following ART: effect of choice of comparison group, confounding and mediating factors. *Hum. Reprod.* **25**, 244–252 (2010).
258. Meijerink, A. M. et al. Behavioral, cognitive, and motor performance and physical development of five-year-old children who were born after intracytoplasmic sperm injection with the use of testicular sperm. *Fertil. Steril.* **106**, 1673–1682.e5 (2016).
259. Tsai, C. C. et al. Clinical outcomes and development of children born after intracytoplasmic sperm injection (ICSI) using extracted testicular sperm or ejaculated extreme severe oligo-astheno-teratozoospermia sperm: a comparative study. *Fertil. Steril.* **96**, 567–571 (2011).
260. Sandin, S., Nygren, K. G., Iliadou, A., Hultman, C. M. & Reichenberg, A. Autism and mental retardation among offspring born after in vitro fertilization. *JAMA* **310**, 75–84 (2013).
261. Ilioi, E. C. & Golombok, S. Psychological adjustment in adolescents conceived by assisted reproduction techniques: a systematic review. *Hum. Reprod. Update* **21**, 84–96 (2015).
262. Spangmose, A. L. et al. Academic performance in adolescents born after ART-a nationwide registry-based cohort study. *Hum. Reprod.* **32**, 447–456 (2017).
263. Balayla, J. et al. Neurodevelopmental outcomes after assisted reproductive technologies. *Obstet. Gynecol.* **129**, 265–272 (2017).
264. Guo, X. Y. et al. Cardiovascular and metabolic profiles of offspring conceived by assisted reproductive technologies: a systematic review and meta-analysis. *Fertil. Steril.* **107**, 622–631 (2017).
265. Leary, C., Leese, H. J. & Sturmey, R. G. Human embryos from overweight and obese women display phenotypic and metabolic abnormalities. *Hum. Reprod.* **30**, 122–132 (2015).
266. Ebner, T. et al. Quantitative and qualitative trophectoderm grading allows for prediction of live birth and gender. *J. Assist Reprod. Genet.* **33**, 49–57 (2016).
267. Pontesilli, M. et al. Subfertility and assisted reproduction techniques are associated with poorer cardiometabolic profiles in childhood. *Reprod. Biomed. Online* **30**, 258–267 (2015).
268. Ceelen, M. et al. Cardiometabolic differences in children born after in vitro fertilization: follow-up study. *J. Clin. Endocrinol. Metab.* **93**, 1682–1688 (2008).
269. Belva, F. et al. Are ICSI adolescents at risk for increased adiposity? *Hum. Reprod.* **27**, 257–264 (2012).
270. Belva, F. et al. Blood pressure in ICSI-conceived adolescents. *Hum. Reprod.* **27**, 3100–3108 (2012).
271. Gkourogianni, A. et al. Plasma metabolomic profiling suggests early indications for predisposition to latent insulin resistance in children conceived by ICSI. *PLOS One* **9**, e94001 (2014).
272. van der Steeg, J. W. et al. Obesity affects spontaneous pregnancy chances in subfertile, ovulatory women. *Hum. Reprod.* **23**, 324–328 (2008).
273. Malvezzi, H. et al. Sperm quality after density gradient centrifugation with three commercially available media: a controlled trial. *Reprod. Biol. Endocrinol.* **12**, 121 (2014).
274. The Center of Assisted Reproduction. Embryological procedures. *Masaryk University* http://www.ivfbrno.cz/en/embryological-procedures/t1046 (2018).
275. Van Soom, A. et al. Epigenetics and the periconception environment in ruminants. *Proc. Belg. R. Acad. Med.* **2**, 1–23 (2013).

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.

Publisher's note

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
Supplementary information is available for this paper at https://doi.org/10.1038/s41585-018-0051-8.