Appendix 13
Section 11.7 Assisted Reproductive Technologies

11.7.1 Types of assisted reproductive technology

Assisted reproductive technology consists of procedures that involve the in vitro handling of both human oocytes and sperm, or of embryos, with the objective of establishing pregnancy [1, 2].

Once couples have been prepared for treatment, the following are the steps that make up an ART cycle:

1. Pharmacological stimulation of growth of multiple ovarian follicles, while at the same time other medications is given to suppress the natural menstrual cycle and down-regulate the pituitary gland.
2. Careful monitoring at intervals to assess the growth of the follicles.
3. Ovulation triggering: when the follicles have reached an appropriate size, a drug is administered to bring about final maturation of the eggs.
4. Egg collection (usually with a trans-vaginal US probe to guide the pickup) and, in some cases of male infertility, sperm retrieval.
5. Fertilisation process, which is usually completed by IVF or ICSI.
6. Laboratory procedures follow for embryo culture: culture media, oxygen concentration, co-culture, assisted hatching etc.
7. The embryos are placed into the uterus. Issues of importance here include endometrial preparation, the best timing for embryo transfer, how many embryos to transfer, what type of catheter to use, the use of US guidance, need for bed rest etc.
8. Luteal phase support, for which several hormonal options are available.

Fertility treatments are complex and each cycle consists of several steps. If one of the steps is incorrectly applied, conception may not occur [1].

Several ART techniques are available:

11.7.1.1 Intra-uterine insemination (IUI)

Intra-uterine insemination is an infertility treatment that involves the placement of the prepared sperm into the uterine cavity timed around ovulation. This can be done in combination with ovarian stimulation or in a natural cycle. The aim of the stimulation cycle is to increase the number of follicles available for fertilisation and to enhance the accurate timing of insemination in comparison to the natural cycle IUI [3-5].

Intra-uterine insemination is generally, though not exclusively, used when there is at least one patent fallopian tube with normal sperm parameters and regular ovulatory cycles (unstimulated cycles) and when the female partner is aged < 40 years.

The global pregnancy rate (PR) and delivery rate (DR) for each IUI cycle with the partner’s sperm are 12.0% and 8.0%, respectively. Using donor sperm, the resultant PR and DR per cycle are 17.0% and 12.3%, respectively [6]. The rates of successful treatment cycles for patients decrease with increase in age, and the birth rates across all age groups have remained broadly stable over time. The highest birth rates have been reported in patients younger than 38 years (14% in patients aged < 35 years and 12% in those aged 35-37 years). The rates of successful treatment are low for patients older than 42 years. The multiple pregnancy rate (MPR) for IUI is ~8% [4]. Intra-uterine insemination is not recommended in couples with unexplained infertility, male factor infertility and mild endometriosis, unless the couples have religious, cultural or social objections to proceed with IVF [7].

Intra-uterine insemination with ovarian stimulation is a safer, cheaper, more patient-friendly and non-inferior alternative to IVF in the management of couples with unexplained infertility, male factor infertility and mild endometriosis, unless the couples have religious, cultural or social objections to proceed with IVF [7].

11.7.1.2 In vitro fertilisation (IVF) involves using controlled ovarian hyperstimulation to recruit multiple oocytes during each cycle from the female partner. Follicular development is monitored ultrasonically, and ova are
harvested before ovulation with the use of US-guided needle aspiration. The recovered oocytes are mixed with processed semen to perform IVF. The developing embryos are incubated for 2-3 days in culture and then placed trans-cervically into the uterus.

The rapid refinement of embryo cryopreservation methods has resulted in better perinatal outcomes of frozen-thawed embryo transfer (FET) and makes it a viable alternative to fresh embryo transfer (ET) [10, 11]. Frozen-thawed embryo transfer seems to be associated with lower risk of gestational complications than fresh ET. Individual approaches remain appropriate to balance the options of FET or fresh ET at present [12].

Generally, only 20%-30% of transferred embryos result in clinical pregnancies. The global PR and DR per aspiration for non-donor IVF is 24.0% and 17.6%, respectively [6].

According to the NICE guidelines, IVF treatment is appropriate in cases of unexplained infertility for women who have not conceived after 2 years of regular unprotected sexual intercourse [13].

11.7.1.3 Intracytoplasmic sperm injection

Intracytoplasmic sperm injection is a procedure through which a single sperm is injected directly into an egg using a glass micropipette.

The difference between ICSI and IVF is the method used to achieve fertilisation. In conventional IVF, oocytes are incubated with sperm in a Petri dish, and the male gamete fertilises the oocyte naturally. 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 immobilise 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].

With this technique the oocyte can be fertilised independently of the morphology and/or motility of the spermatozoon injected.

Intracytoplasmic sperm injection is currently the most commonly used ART, accounting for 70–80% of the cycles performed [15].

The procedure was first used in cases of fertilisation failure after standard IVF or when an inadequate number of sperm cells was available. The consistency of fertilisation independent of the functional quality of the spermatozoa has extended the application of ICSI to immature spermatozoa retrieved surgically from the epididymis and testis [16]. Intracytoplasmic sperm injection is the natural treatment for couples with severe male factor infertility and is also used for a number of non-male factor indications (Table 11.1) [17].

The need to denude the oocyte allows assessment of the nuclear maturity of the oocyte. Intracytoplasmic sperm injection is also preferred in conjunction with pre-implantation genetic diagnosis and has recently been used to treat HIV discordant couples, in whom there is a pressing need to minimise exposure of the oocyte to a large number of spermatozoa [16].

The global PR and DR per aspiration for ICSI is 26.2% and 19.0%, respectively [6]. For all ages and with all the different sperm types used, fertilisation after ICSI is at approximately 70%-80% and it ensures a clinical pregnancy rate of up to 45% [15, 16].

Existing evidence does not support ICSI in preference over IVF in the general non-male factor ART population; however, in couples with unexplained infertility, ICSI is associated with lower fertilisation failure rates than IVF [17].
Overall, pregnancy outcomes from ICSI are comparable between epididymal and testicular sperm and also between fresh and frozen–thawed epididymal sperm in men with OA [18]. However, these results are from studies of low evidence [17].

Sperm injection outcomes with fresh or frozen–thawed testicular sperm have been compared in men with NOA. In a meta-analysis of eleven studies and 574 ICSI cycles, no significant difference was observed between fresh and frozen–thawed testicular sperm with regards to fertilisation rate (RR: 0.97, 95% CI: 0.92–1.02) and clinical pregnancy rates (RR: 1.00, 95% CI: 0.75–1.33) [19]. However, no meta-analysis was performed on data regarding implantation, miscarriage, and low-birth rates.

11.7.1.4 Testicular sperm in men with raised DNA fragmentation in ejaculated sperm

The use of testicular sperm for ICSI is associated with possibly improved outcomes compared with ejaculated sperm in men with high sperm DNA fragmentation [17, 20]. Men with unexplained infertility with raised DNA fragmentation may be considered for TESE after failure of ART, although they should be counselled that live-birth rates are under reported in the literature and patients must weigh up the risks of performing an invasive procedure in a potentially normozoospermic or unexplained condition. The advantages of the use of testicular sperm in men with cryptozoospermia have not yet been confirmed in large scale randomised studies [21].

In terms of a practical approach, urologists may offer the use of testicular sperm in patients with high DNA fragmentation. However, patients should be counselled regarding the low levels of evidence for this (i.e., non-randomised studies). Furthermore, testicular sperm should only be used in this setting once the common causes of oxidative stress have been excluded including varicoceles, modifications of dietary/lifestyle factors and treatment of accessory gland infections.

Table 11.1: Fertilisation methods for male factor and non-male factor infertility (adapted from [17])

<table>
<thead>
<tr>
<th>Male Factor Infertility</th>
<th>Fertilisation method</th>
</tr>
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<tbody>
<tr>
<td>Sperm derived from men with azoospermia</td>
<td>ICSI mandatory</td>
</tr>
<tr>
<td>Severe OAT</td>
<td>ICSI highly recommended</td>
</tr>
<tr>
<td>Moderate OAT</td>
<td>IVF and ICSI equally effective</td>
</tr>
<tr>
<td>Isolated teratozoospermia</td>
<td>IVF and ICSI equally effective</td>
</tr>
<tr>
<td>Absolute asthenozoospermia</td>
<td>ICSI mandatory</td>
</tr>
<tr>
<td>Globozoospermia</td>
<td>ICSI mandatory</td>
</tr>
<tr>
<td>Anti-sperm antibodies</td>
<td>IVF and ICSI equally effective</td>
</tr>
<tr>
<td>Sperm DNA fragmentation</td>
<td>ICSI recommended</td>
</tr>
<tr>
<td>Non-male factor infertility</td>
<td></td>
</tr>
<tr>
<td>Unexplained infertility</td>
<td>Equally effective.</td>
</tr>
<tr>
<td>Couples should be informed that ICSI improves fertilisation rates compared to IVF alone, but once fertilisation is achieved the pregnancy rate is no better than with IVF.</td>
<td></td>
</tr>
</tbody>
</table>
It should be noted for clarification that in the absence of male factors, ICSI should not be offered in the first treatment cycle [22].

<table>
<thead>
<tr>
<th>General non-male factor population</th>
<th>Equally effective, slightly in favour of IVF</th>
</tr>
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<tbody>
<tr>
<td>Poor quality oocytes and advanced maternal age</td>
<td>Equally effective, slightly in favour of IVF</td>
</tr>
<tr>
<td>Pre-implantation genetic testing</td>
<td>ICSI highly recommended</td>
</tr>
<tr>
<td>Poor responders</td>
<td>Equally effective, slightly in favour of IVF</td>
</tr>
<tr>
<td>Tubal ligation</td>
<td>IVF preferable</td>
</tr>
<tr>
<td>Sero-discordant couples</td>
<td>Equally effective</td>
</tr>
</tbody>
</table>

ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilisation; OAT = oligo-asthenoteratozoospermia

Intracytoplasmic sperm injection is carried out using viable sperm populations. Several semen processing techniques have been developed to select the optimal sperm fraction for ICSI. Density gradient centrifugation (DGC) and the swim-up procedures have been used as standards for semen preparation for ICSI for more than two decades [23]. However, these traditional sperm selection techniques are unable to select sperm fractions with optimal DNA integrity and functional characteristics. Advanced sperm selection techniques have been introduced to optimise the selection of high-quality sperm for ICSI [24]. 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 ultra-morphological sperm assessment [25].

11.7.1.5 Intra-cytoplasmic morphologically selected sperm injection

Intra-cytoplasmic morphologically selected sperm injection (IMSI) was first introduced in 2002 as a modification of the ICSI technique [26]. This technique increases the magnification of sperm to > 6,000 times; the purpose of which is to perform the motile sperm organelle morphology examination (MSOME), a method used to select spermatozoa that have the choicest morphology in couples with the most severe male factor. Bartoov et al. showed that, for patients with a history of ICSI failure, addition of IMSI resulted in a 60% pregnancy rate, compared with a 30% rate for patients not using IMSI [27]. The pregnancy rate following IVF-IMSI was significantly higher and the miscarriage rate significantly lower, than for the routine IVF-ICSI procedure (60.0% vs. 25.0%, and 14% vs. 40%, respectively) [28]. However, the most recently updated Cochrane review neither supported nor refuted the clinical use of IMSI [29].

11.7.1.6 Physiological ICSI (PICS) technique: a selection based on membrane maturity of sperm

The human oocytes are surrounded by hyaluronic acid (HA), which acts as a natural selector. Only mature sperm that express receptors specific to HA can reach the oocytes and fertilise them. Those sperm have normal shapes, low DNA fragmentation rates, and low frequency of chromosomal aneuploidy [30]. Several studies have attempted to verify whether sperm selection based on HA binding affects IVF outcomes. A meta-analysis included six prospective randomised studies and one retrospective study, all of which used a PICS sperm-selection dish (a plastic culture dish with microdots of HA hydro gel on its inner surface) or the Sperm Slow method (a viscous medium containing HA). No improvements in fertilisation and pregnancy rates were recorded, although embryo quality was superior in PICS compared with conventional ICSI [30]. A large-sample multicentre randomised trial provided conclusive evidence against the use of PICS in ART (PICS live-birth rate vs. ICSI: OR: 1.12, 95% CI: 0.95–1.34) [31]. A time-lapse study found no difference in embryo development dynamics in oocytes fertilised via HA-ICSI vs. conventional ICSI [32].

11.7.1.6 Magnetic-activated cell sorting

Magnetic-activated cell sorting (MACS) is an advanced sperm-selection technique used to isolate sperm that do not show signs of apoptosis and, therefore, are presumed to have a lower rate of DNA damage [24]. Use of
MACS after density gradient centrifugation (DGC) has been found to improve sperm morphology and decrease DNA fragmentation and apoptotic markers, but it reduces motility of the selected sperm [24, 25]. Magnetic-activated cell sorting failed to improve ICSI outcomes compared with DGC or swim-up, although a slightly higher pregnancy rate (RR: 1.5, 95% CI: 1.14–1.98) was observed in MACS patients relative to the control group [33]. No difference in implantation or miscarriage rate was noted (RR: 1.03, 95% CI: 0.8–1.31 and RR: 2, 95% CI: 0.19–20.9, respectively).

Finally, another RCT performed on infants conceived via ovum-donation IVF cycles did not report any differences in terms of obstetrical and perinatal outcomes between pregnancies or babies conceived with sperm selected via MACS or swim-up [34].

11.7.2 Safety
The most significant risk of pre-implantation ART treatment is the ovarian hyperstimulation syndrome, a potentially life-threatening condition resulting from excessive ovarian stimulation during ART techniques, ranging from 0.6% to 5% in ART cycles [35].

Other problems include the risk of multiple pregnancies due to the transfer of more than one embryo and the associated risks to mother and baby, including multiple and preterm birth. The most prevalent maternal complications include pre-eclampsia, gestational diabetes, placenta previa, placental abruption, postpartum haemorrhage, and preterm labour and delivery [36-38]. The risks of foetal demise during the third trimester, perinatal mortality, preterm birth, and low birth weight increase with the number of foetuses in the pregnancy. The foetal consequences of preterm birth (cerebral palsy, retinopathy, and broncho-pulmonary dysplasia) and foetal growth restriction (polycythaemia, hypoglycaemia, and necrotising enterocolitis) are significant [39].

The average number of embryos transferred in fresh non-donor IVF and ICSI cycles in 2011 was 1.91, compared with 2.09 in 2008, 2.00 in 2009, and 1.95 in 2010, reflecting a continuing decrease from previous years. The average number of embryos transferred in frozen ET cycles decreased from 1.72 in 2008 to 1.65 in 2009 to 1.60 in 2010 and to 1.59 in 2011 [40].

The global multiple birth rate for fresh cycle transfer has decreased from 21.5% in 2010 to 20.5% in 2011 and for frozen ET cycles from 12.0% to 11.5% [6].

In 2011, the rate of early pregnancy loss was 20.1% after fresh ET, compared with 25.4% after frozen ET. Both rates showed wide regional variation [6]. The multiple birth rates after fresh non-donor ET were 19.6% (twins) and 0.9% (triplets and higher-order births); for frozen ET non-donor cycles, twin and triplet and higher-order birth rates were 11.1% and 0.4%, respectively [6].

Rates of premature delivery and perinatal mortality were lower for frozen ETs than for fresh ETs. The global preterm DR after non-donor fresh ET was 19.1%, and after frozen ET was 13.1%. The perinatal mortality rate per 1,000 births after non-donor fresh ET was 16.3 and after frozen ET was 8.6.

In terms of potential adverse effect, ICSI-conceived offspring has a greater neonatal morbidity, obstetric complications and congenital malformations, compared with spontaneous conception [37, 41, 42]. Additionally, epigenetic disorders and impaired neurodevelopment have been observed in infants born using ICSI compared with naturally conceived children [17]. Among singleton infants born at 37 weeks of gestation or later, those following IVF had a risk of low birth weight that was 2.6 times (95% CI: 2.4–2.7) greater than in the general population (absolute risk of low birth weight with spontaneous vs. resulting from IVF was 2.5% vs. 6.5%) [43]. Singleton infants after IVF were 39% more likely (adjusted RR: 1.39, 95% CI: 1.21–1.59) to have a non-chromosomal birth defect (particularly gastrointestinal and musculoskeletal) compared with all other singleton births. No single ART procedure (e.g., ICSI, fresh, or frozen ETs) was found to substantially increase the risk of birth defects.

Analyses from the Massachusetts Outcome Study of ART reported a 50% increase (adjusted prevalence ratio of 1.5, 95% CI: 1.3–1.6) in birth defects in infants after IVF vs. spontaneous pregnancy, and a 30% increase (adjusted prevalence ratio of 1.3, 95% CI: 1.1–1.5) in birth defects in infants after subfertility vs. spontaneous pregnancy.
No difference in risk of cancer was found between ART-conceived children and those spontaneously conceived [47].

Health differences between ICSI and IVF conceptions have not been comprehensively assessed and results are contradictory. Some authors found a significantly reduced risk of birth defects in IVF compared to ICSI conceived infants [48], while two meta-analyses demonstrated no difference in risk of congenital malformations between IVF and ICSI conception [49, 50]. Data about ICSI- and IVF-conceived adolescents or young adults are scarce but it seems that there is no difference in outcomes between the two techniques. Further research into health outcomes in adolescence and adulthood is required before conclusions can be drawn about the long-term safety of ICSI compared to IVF [51].
References

13. NICE. Fertility Problems: Assessment and Treatment Guidelines.


