Appendix 7

Online supplementary evidence for section 11 Male infertility

1. Genetic counselling for AZF deletions

After conception, any Y-deletions are transmitted to the male offspring, and genetic counselling is therefore mandatory. In most cases, father and son will have the same microdeletion [1], but occasionally the son may have a more extensive deletion [2]. The extent of spermatogenic failure (still in the range of azoo-/oligo-zoospermia) cannot be predicted entirely in the son, due to the different genetic background and the presence or absence of environmental factors with potential toxicity on reproductive function. A significant proportion of spermatozoa from men with complete AZFc deletion are nullisomic for sex chromosomes [3, 4], indicating a potential risk for any offspring to develop 45,X0 Turner's syndrome and other phenotypic anomalies associated with sex chromosome mosaicism, including ambiguous genitalia [5]. Despite this theoretical risk, babies born from fathers affected by Yq microdeletions are phenotypically normal [1, 6]. This could be due to the reduced implantation rate and a likely higher risk of spontaneous abortion of embryos bearing a 45,X0 karyotype.

Y-chromosome: 'gr/gr' deletion

A new type of Yq deletion, known as the gr/gr deletion, has been described in the AZFc region [7]. This deletion removes half of the gene content of the AZFc region, affecting the dosage of multicopy genes mapping inside this region. This type of deletion confers a 2.5 to 8-fold increased risk for oligozoospermia [1, 8-10]. The frequency of gr/gr deletion in oligozoospermic patients is ~5% [11].

According to four meta-analyses, gr/gr deletion is a significant risk factor for impaired sperm production [9-11]. It is worth noting that both the frequency of gr/gr deletion and its phenotypic expression vary among different ethnic groups, depending on the Y-chromosome background. For example, in some Y haplo-groups, the deletion is fixed and appears to have no negative effect on spermatogenesis. Consequently, the routine screening for gr/gr deletion is still a debated issue, especially in those laboratories serving diverse ethnic and geographic populations. A large multi-centre study has shown that gr/gr deletion is a potential risk factor for testicular germ cell tumours [12]. However, these data need confirmation in an ethnically and geographically matched case-control study setting. For genetic counselling it is worth noting that partial AZFc deletions, gr/gr and b2/b3, may predispose to complete AZFc deletion in the next generation [13].

Autosomal defects with severe phenotypic abnormalities and infertility

Several inherited disorders are associated with severe or considerable generalised abnormalities and infertility (e.g., Prader-Willi syndrome [14], Bardet-Biedl syndrome [15], Noonan's syndrome, Myotonic dystrophy, dominant polycystic kidney disease [16, 17], and 5 α-reductase deficiency [18-21], etc.) Pre-implantation genetic screening may be necessary in order to improve the ART outcomes among men with autosomal chromosomal defects [22, 23].

Sperm chromosomal abnormalities

Sperm can be examined for their chromosomal constitution using FISH both in men with normal karyotype and with anomalies. Aneuploidy in sperm, particularly sex chromosome aneuploidy, is associated with severe damage to spermatogenesis [24-27] and with translocations and may lead to recurrent pregnancy loss (RPL) or recurrent implantation failure [28]. In a large retrospective series, couples with normal sperm FISH had similar outcomes from IVF and ICSI on pre-implantation genetic screening (PGS). However, couples with abnormal FISH had better clinical outcomes after PGS, suggesting a potential contribution of sperm to aneuploidic abnormalities in the embryo [29]. In men with sperm aneuploidy, PGS combined with IVF and ICSI can increase chances of live births [30].

Measurement of Oxidative Stress

Oxidative stress is considered to be central in male infertility by affecting sperm quality, function, as well as the integrity of sperm [31]. Oxidative stress may lead to sperm DNA damage and poorer DNA integrity, which are associated with poor embryo development, miscarriage and infertility [32, 33]. Spermatozoa are vulnerable to oxidative stress and have limited capacity to repair damaged DNA. Oxidative stress is generally associated with poor lifestyle (e.g., smoking) and environmental exposure, and therefore antioxidant regimens and lifestyle interventions may reduce the risk of DNA fragmentation and improve sperm quality [34]. However, these data

have not been supported by RCTs. Furthermore, there are no standardised testing methods for ROS and the duration of antioxidant treatments. Although ROS can be measured by various assays (e.g., chemiluminescence), routine measurement of ROS testing should remain experimental until these tests are validated in RCTs [35].

Outcomes from assisted reproductive technology and long-term health implications to the male and offspring
It is estimated that > 4 million babies have been born with ART since the first baby was conceived by IVF in 1978 [36]. As the number of couples undergoing ART has increased [37, 38], safety concerns related to ART have been raised. Assisted reproductive technology-conceived offspring have poorer prenatal outcomes, such as lower birth weight, lower gestational age, premature delivery, and higher hospital admissions compared with naturally conceived offspring [39, 40]. However, the exact mechanisms resulting in these complications remain obscure. Birth defects have also been associated with children conceived via ART [41-43]. Meta-analyses have shown a 30-40% increase in major malformations linked with ART [44-46]. However, debate continues as to whether the increased risk of birth defects are related to parental age, ART or the intrinsic defects in spermatogenesis in infertile men [47-52].

As for the long-term outcomes, post-natal growth patterns are mostly not associated with ART [41, 53, 54]. However, a number of studies have shown that ART children are taller [55, 56]. This may be important as there is evidence showing that rapid weight gain during early childhood is linked with higher blood pressure levels in children conceived via ART [57]. It is also suggested that ART-conceived children have similar childhood illnesses and hospital services rates as compared with naturally conceived children [58-60]. Some studies have shown an increased risk of retinoblastoma [61] and hepatoblastoma in children after ART. However, these studies have been challenged with other studies that have not supported these findings [62]. The current evidence for cancer risk in children conceived with ART is inadequate and further studies are warranted [63, 64]. Finally, several epigenetic alterations seem to be caused by ART, which might be the molecular basis to some complex traits and diseases [65].

2. Hormonal therapy

Secondary hypogonadism

- Pre-Pubertal-Onset: these conditions require combination therapy with both subcutaneous hCG and FSH or GnRH by pulsed delivery using a subcutaneous pump [66]. GnRH treatment requires a pulsatile secretion using specific devices which may limit patient compliance. Moreover, GnRH therapy should be limited to subjects with a residual pituitary gonadotropic activity [67].
- As for the type of gonadotropin treatment, it is usual to commence hCG first and titrate the dose to achieve testosterone levels within the normal physiological range. However, FSH can be given first or in combination with hCG [68]. Human Chorionic Gonadotrophin is given twice weekly and in patients with congenital secondary hypogonadism in high dose, commencing at 1,000 IU twice weekly. Testosterone levels can be assayed every two weeks with dose increases until ideally mid-range testosterone is achieved. Dose increases can be to 2,000, 3,000, 4,000 and 5,000 IU two or three times a week, until normal testosterone levels are achieved [69-72]. The trophic response of the testes to FSH is variable and it may range from no effect to achieving testicular sizes of 12-15 mL [73]. A trophic response is usually an indication of an increase in spermatogenesis. The production of new spermatogenesis may be evident after 3 months of FSH therapy but could occur even after 18 months of treatment [71-73]. A low baseline sperm concentration does not indicate a poor response to gonadotropin therapy [74]. Semen analysis can be assessed at 3-monthly intervals. Follicle-stimulating hormone therapy prior to GnRH is also effective in stimulating testicular growth and fertility in men with congenital hypogonadotropic hypogonadism (HH) [75]. A larger initial testicular volume is the best prognostic factor for induction of successful spermatogenesis [76].

3. Assisted Reproductive Technologies

3.7.1 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 [77, 78].

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 [77].

Several ART techniques are available:

3.7.1.1 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 [79-81].

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 [82]. 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% [80]. 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 [83].

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 and mild male factor infertility [79, 80]. An RCT showed lower multiple pregnancy rates and comparable live-birth rates in patients treated with IUI with hormonal stimulation when compared to women undergoing IVF with single embryo transfer [84]. Additionally, IUI is a more cost-effective treatment than IVF for couples with unexplained or mild male subfertility [85].

3.7.1.2 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) [86, 87]. 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 [88].

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 [82].

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 [89].

3.7.1.3 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 [90]. 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 [90].

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 [91].

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 [92]. 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) [93].

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 [92].

The global PR and DR per aspiration for ICSI is 26.2% and 19.0%, respectively [82]. 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% [91, 92].

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 [93].

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 [94]. However, these results are from studies of low evidence [93].

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) [95]. However, no meta-analysis was performed on data regarding implantation, miscarriage, and low-birth rates.

3.7.1.4 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 [93, 96]. 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 [97].

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 [93])

	Fertilisation method
Male Factor Infertility	
Sperm derived from men with azoospermia	ICSI mandatory
Severe OAT	ICSI highly recommended
Moderate OAT	IVF and ICSI equally effective
Isolated teratozoospermia	IVF and ICSI equally effective
Absolute asthenozoospermia	ICSI mandatory
Globozoospermia	ICSI mandatory
Anti-sperm antibodies	IVF and ICSI equally effective
Sperm DNA fragmentation	ICSI recommended
Non-male factor infertility	
Unexplained infertility	Equally effective. 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. It should be noted for clarification that in the absence of male factors, ICSI should not be offered in the first treatment cycle [98].
General non-male factor population	Equally effective, slightly in favour of IVF

Poor quality oocytes and advanced maternal age	Equally effective, slightly in favour of IVF
Pre-implantation genetic testing	ICSI highly recommended
Poor responders	Equally effective, slightly in favour of IVF
Tubal ligation	IVF preferable
Sero-discordant couples	Equally effective

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

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 [99]. 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 [100]. 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 [101].

3.7.1.5 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 [102]. 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 [103]. 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) [104]. However, the most recently updated Cochrane review neither supported nor refuted the clinical use of IMSI [105].

3.7.1.5 Physiological ICSI (PICSI) 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 [106]. 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 PICSI 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 PICSI compared with conventional ICSI [106]. A large-sample multicentre randomised trial provided conclusive evidence against the use of PICSI in ART (PICSI live-birth rate vs. ICSI: OR: 1.12, 95% CI: 0.95–1.34) [107]. A time-lapse study found no difference in embryo development dynamics in oocytes fertilised via HA-ICSI vs. conventional ICSI [108].

3.7.1.6 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 [100]. 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 [100, 101]. 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 [109]. 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 [110].

3.7.2 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 [111].

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 [112-114]. 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 [115].

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 [116].

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% [82].

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 [82]. 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 [82].

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 [113, 117, 118]. Additionally, epigenetic disorders and impaired neurodevelopment have been observed in infants born using ICSI compared with naturally conceived children [93]. 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%) [40]. 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 [119-121]. No difference in risk of cancer was found between ART-conceived children and those spontaneously conceived [122].

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 [43], while two meta-analyses demonstrated no difference in risk of congenital malformations between IVF and ICSI conception [46, 123]. 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 [124].

References

1. Krausz, C*., et al.* EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. Andrology, 2014. 2: 5.
2. Stuppia, L*., et al.* A quarter of men with idiopathic oligo-azoospermia display chromosomal abnormalities and microdeletions of different types in interval 6 of Yq11. Hum Genet, 1998. 102: 566.
3. Le Bourhis, C*., et al.* Y chromosome microdeletions and germinal mosaicism in infertile males. Mol Hum Reprod, 2000. 6: 688.
4. Siffroi, J.P*., et al.* Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. Hum Reprod, 2000. 15: 2559.
5. Patsalis, P.C*., et al.* Effects of transmission of Y chromosome AZFc deletions. Lancet, 2002. 360: 1222.
6. Krausz, C*., et al.* Y chromosome and male infertility: update, 2006. Front Biosci, 2006. 11: 3049.
7. Repping, S*., et al.* Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet, 2003. 35: 247.
8. Giachini, C*., et al.* Partial AZFc deletions and duplications: clinical correlates in the Italian population. Hum Genet, 2008. 124: 399.
9. Navarro-Costa, P*., et al.* The AZFc region of the Y chromosome: at the crossroads between genetic diversity and male infertility. Hum Reprod Update, 2010. 16: 525.
10. Stouffs, K*., et al.* What about gr/gr deletions and male infertility? Systematic review and meta-analysis. Hum Reprod Update, 2011. 17: 197.
11. Bansal, S.K*., et al.* Gr/gr deletions on Y-chromosome correlate with male infertility: an original study, meta-analyses, and trial sequential analyses. Sci Rep, 2016. 6: 19798.
12. Nathanson, K.L*., et al.* The Y deletion gr/gr and susceptibility to testicular germ cell tumor. Am J Hum Genet, 2005. 77: 1034.
13. Zhang, F*., et al.* Partial deletions are associated with an increased risk of complete deletion in AZFc: a new insight into the role of partial AZFc deletions in male infertility. J Med Genet, 2007. 44: 437.
14. Haltrich, I. Chromosomal Aberrations with Endocrine Relevance (Turner Syndrome, Klinefelter Syndrome, Prader-Willi Syndrome). Exp Suppl, 2019. 111: 443.
15. Tsang, S.H*., et al.* Ciliopathy: Bardet-Biedl Syndrome. Adv Exp Med Biol, 2018. 1085: 171.
16. Mieusset, R*., et al.* The spectrum of renal involvement in male patients with infertility related to excretory-system abnormalities: phenotypes, genotypes, and genetic counseling. J Nephrol, 2017. 30: 211.
17. Luciano, R.L*., et al.* Extra-renal manifestations of autosomal dominant polycystic kidney disease (ADPKD): considerations for routine screening and management. Nephrol Dial Transplant, 2014. 29: 247.
18. Van Batavia, J.P*., et al.* Fertility in disorders of sex development: A review. J Pediatr Urol, 2016. 12: 418.
19. Kosti, K*., et al.* Long-term consequences of androgen insensitivity syndrome. Maturitas, 2019. 127: 51.
20. Hsieh, M.H*., et al.* The genetic and phenotypic basis of infertility in men with pediatric urologic disorders. Urology, 2010. 76: 25.
21. Okutman, O*., et al.* Genetic evaluation of patients with non-syndromic male infertility. J Assist Reprod Genet, 2018. 35: 1939.

22. Luo, K.*, et al.* Next-generation sequencing analysis of embryos from mosaic patients undergoing in vitro fertilization and preimplantation genetic testing. Fertility and sterility, 2019. 112: 291.
23. Kohn, T.P.*, et al.* Reproductive outcomes in men with karyotype abnormalities: Case report and review of the literature. Canadian Urological Association journal = Journal de l'Association des urologues du Canada, 2015. 9: E667.
24. Johnson, M.D. Genetic risks of intracytoplasmic sperm injection in the treatment of male infertility: recommendations for genetic counseling and screening. Fertil Steril, 1998. 70: 397.
25. Gianaroli, L.*, et al.* Frequency of aneuploidy in sperm from patients with extremely severe male factor infertility. Hum Reprod, 2005. 20: 2140.
26. Pang, M.G.*, et al.* The high incidence of meiotic errors increases with decreased sperm count in severe male factor infertilities. Hum Reprod, 2005. 20: 1688.
27. Tempest, H.G.*, et al.* Cytogenetic risks in chromosomally normal infertile men. Curr Opin Obstet Gynecol, 2009. 21: 223.
28. Baccetti, B.*, et al.* Ultrastructural studies of spermatozoa from infertile males with Robertsonian translocations and 18, X, Y aneuploidies. Hum Reprod, 2005. 20: 2295.
29. Rodrigo, L.*, et al.* Sperm chromosomal abnormalities and their contribution to human embryo aneuploidy. Biol Reprod, 2019. 101: 1091.
30. Kohn, T.P.*, et al.* Genetic counseling for men with recurrent pregnancy loss or recurrent implantation failure due to abnormal sperm chromosomal aneuploidy. J Assist Reprod Genet, 2016. 33: 571.
31. Agarwal, A.*, et al.* Sperm DNA damage assessment: a test whose time has come. Fertil Steril, 2005. 84: 850.
32. Zini, A.*, et al.* Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril, 2001. 75: 674.
33. Iommiello, V.M.*, et al.* Ejaculate oxidative stress is related with sperm DNA fragmentation and round cells. Int J Endocrinol, 2015. 2015: 321901.
34. Bisht, S.*, et al.* Oxidative stress and male infertility. Nat Rev Urol, 2017. 14: 470.
35. Agarwal, A.*, et al.* Oxidation-reduction potential as a new marker for oxidative stress: Correlation to male infertility. Investig Clin Urol, 2017. 58: 385.
36. Lu, Y.*, et al.* Long-term follow-up of children conceived through assisted reproductive technology*. J Zhejiang Univ Sci B, 2013. 14: 359.
37. Kushnir, V.A.*, et al.* Systematic review of worldwide trends in assisted reproductive technology 2004-2013. Reprod Biol Endocrinol, 2017. 15: 6.
38. Rinaudo, P.*, et al.* Transitioning from Infertility-Based (ART 1.0) to Elective (ART 2.0) Use of Assisted Reproductive Technologies and the DOHaD Hypothesis: Do We Need to Change Consenting? Semin Reprod Med, 2018. 36: 204.
39. Kallen, B.*, et al.* In vitro fertilization in Sweden: child morbidity including cancer risk. Fertil Steril, 2005. 84: 605.
40. Schieve, L.A.*, et al.* Low and very low birth weight in infants conceived with use of assisted reproductive technology. N Engl J Med, 2002. 346: 731.
41. Bonduelle, M.*, et al.* A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. Hum Reprod, 2005. 20: 413.
42. El-Chaar, D.*, et al.* Risk of birth defects increased in pregnancies conceived by assisted human reproduction. Fertil Steril, 2009. 92: 1557.
43. Davies, M.J.*, et al.* Reproductive technologies and the risk of birth defects. N Engl J Med, 2012. 366: 1803.

44. Rimm, A.A., *et al.* A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. J Assist Reprod Genet, 2004. 21: 437.
45. Hansen, M., *et al.* Assisted reproductive technologies and the risk of birth defects--a systematic review. Hum Reprod, 2005. 20: 328.
46. Wen, J., *et al.* Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. Fertil Steril, 2012. 97: 1331.
47. Rumbold, A.R., *et al.* Impact of male factor infertility on offspring health and development. Fertil Steril, 2019. 111: 1047.
48. La Rovere, M., *et al.* Epigenetics and Neurological Disorders in ART. Int J Mol Sci, 2019. 20.
49. Bertoncelli Tanaka, M., *et al.* Paternal age and assisted reproductive technology: problem solver or trouble maker? Panminerva Med, 2019. 61: 138.
50. Kissin, D.M., *et al.* Association of assisted reproductive technology (ART) treatment and parental infertility diagnosis with autism in ART-conceived children. Hum Reprod, 2015. 30: 454.
51. Pinborg, A., *et al.* Epigenetics and assisted reproductive technologies. Acta Obstet Gynecol Scand, 2016. 95: 10.
52. Jiang, Z., *et al.* Genetic and epigenetic risks of assisted reproduction. Best Pract Res Clin Obstet Gynaecol, 2017. 44: 90.
53. Sutcliffe, A.G., *et al.* A retrospective case-control study of developmental and other outcomes in a cohort of Australian children conceived by intracytoplasmic sperm injection compared with a similar group in the United Kingdom. Fertil Steril, 2003. 79: 512.
54. Belva, F., *et al.* Medical outcome of 8-year-old singleton ICSI children (born >or=32 weeks' gestation) and a spontaneously conceived comparison group. Hum Reprod, 2007. 22: 506.
55. Makhoul, I.R., *et al.* In vitro fertilisation and use of ovulation enhancers may both influence childhood height in very low birthweight infants. Arch Dis Child Fetal Neonatal Ed, 2009. 94: F355.
56. Miles, H.L., *et al.* In vitro fertilization improves childhood growth and metabolism. J Clin Endocrinol Metab, 2007. 92: 3441.
57. Ceelen, M., *et al.* Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8-18 years of IVF children and spontaneously conceived controls born to subfertile parents. Hum Reprod, 2009. 24: 2788.
58. Knoester, M., *et al.* Perinatal outcome, health, growth, and medical care utilization of 5- to 8-year-old intracytoplasmic sperm injection singletons. Fertil Steril, 2008. 89: 1133.
59. Place, I., *et al.* A prospective longitudinal study of the physical, psychomotor, and intellectual development of singleton children up to 5 years who were conceived by intracytoplasmic sperm injection compared with children conceived spontaneously and by in vitro fertilization. Fertil Steril, 2003. 80: 1388.
60. Belva, F., *et al.* Blood pressure in ICSI-conceived adolescents. Hum Reprod, 2012. 27: 3100.
61. Moll, A.C., *et al.* Incidence of retinoblastoma in children born after in-vitro fertilisation. Lancet, 2003. 361: 309.
62. Marees, T., *et al.* Incidence of retinoblastoma in Dutch children conceived by IVF: an expanded study. Hum Reprod, 2009. 24: 3220.
63. Puumala, S.E., *et al.* Parental infertility, infertility treatment and hepatoblastoma: a report from the Children's Oncology Group. Hum Reprod, 2012. 27: 1649.
64. McLaughlin, C.C., *et al.* Maternal and infant birth characteristics and hepatoblastoma. Am J Epidemiol, 2006. 163: 818.
65. Gomes, M.V., *et al.* Abnormal methylation at the KvDMR1 imprinting control region in clinically normal children conceived by assisted reproductive technologies. Mol Hum Reprod, 2009. 15: 471.

66. El Meliegy, A.*, et al.* Systematic review of hormone replacement therapy in the infertile man. Arab Journal of Urology, 2018. 16: 140.
67. Salonia, A.*, et al.* Paediatric and adult-onset male hypogonadism. Nat Rev Dis Primers, 2019. 5: 38.
68. Rastrelli, G.*, et al.* Factors affecting spermatogenesis upon gonadotropin-replacement therapy: a meta-analytic study. Andrology, 2014. 2: 794.
69. Jones, T.H.*, et al.* Diurnal rhythm of testosterone induced by human chorionic gonadotrophin (hCG) therapy in isolated hypogonadotrophic hypogonadism: a comparison between subcutaneous and intramuscular hCG administration. Eur J Endocrinol, 1994. 131: 173.
70. Guo, C.Y.*, et al.* Treatment of isolated hypogonadotropic hypogonadism effect on bone mineral density and bone turnover. J Clin Endocrinol Metab, 1997. 82: 658.
71. Efficacy and safety of highly purified urinary follicle-stimulating hormone with human chorionic gonadotropin for treating men with isolated hypogonadotropic hypogonadism. European Metrodin HP Study Group. Fertil Steril, 1998. 70: 256.
72. Bouloux, P.*, et al.* Efficacy and safety of recombinant human follicle-stimulating hormone in men with isolated hypogonadotropic hypogonadism. Fertil Steril, 2002. 77: 270.
73. Jones, T.H.*, et al.* Self-administered subcutaneous human menopausal gonadotrophin for the stimulation of testicular growth and the initiation of spermatogenesis in hypogonadotrophic hypogonadism. Clin Endocrinol (Oxf), 1993. 38: 203.
74. Burris, A.S.*, et al.* Gonadotropin therapy in men with isolated hypogonadotropic hypogonadism: the response to human chorionic gonadotropin is predicted by initial testicular size. J Clin Endocrinol Metab, 1988. 66: 1144.
75. Dwyer, A.A.*, et al.* Trial of recombinant follicle-stimulating hormone pretreatment for GnRH-induced fertility in patients with congenital hypogonadotropic hypogonadism. J Clin Endocrinol Metab, 2013. 98: E1790.
76. Liu, P.Y.*, et al.* Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. J Clin Endocrinol Metab, 2009. 94: 801.
77. Farquhar, C.*, et al.* Assisted reproductive technology: an overview of Cochrane Reviews. Cochrane Database Syst Rev, 2015: Cd010537.
78. Farquhar, C.*, et al.* Assisted reproductive technology: an overview of Cochrane Reviews. Cochrane Database Syst Rev, 2018. 8: Cd010537.
79. Kandavel, V.*, et al.* Does intra-uterine insemination have a place in modern ART practice? Best Pract Res Clin Obstet Gynaecol, 2018. 53: 3.
80. Veltman-Verhulst, S.M.*, et al.* Intra-uterine insemination for unexplained subfertility. Cochrane Database Syst Rev, 2016. 2: Cd001838.
81. Ombelet, W.*, et al.* Semen quality and prediction of IUI success in male subfertility: a systematic review. Reprod Biomed Online, 2014. 28: 300.
82. Adamson, G.D.*, et al.* International Committee for Monitoring Assisted Reproductive Technology: world report on assisted reproductive technology, 2011. Fertil Steril, 2018. 110: 1067.
83. Wilkes, S. NICE CG156: fertility update. What it means for general practitioners. J Fam Plann Reprod Health Care, 2013. 39: 241.
84. Bensdorp, A.J.*, et al.* Prevention of multiple pregnancies in couples with unexplained or mild male subfertility: randomised controlled trial of in vitro fertilisation with single embryo transfer or in vitro fertilisation in modified natural cycle compared with intrauterine insemination with controlled ovarian hyperstimulation. Bmj, 2015. 350: g7771.
85. Goverde, A.J.*, et al.* Further considerations on natural or mild hyperstimulation cycles for intrauterine insemination treatment: effects on pregnancy and multiple pregnancy rates. Hum Reprod, 2005. 20: 3141.

86. Shapiro, B.S.*, et al.* Clinical rationale for cryopreservation of entire embryo cohorts in lieu of fresh transfer. Fertil Steril, 2014. 102: 3.
87. Ozgur, K.*, et al.* Higher clinical pregnancy rates from frozen-thawed blastocyst transfers compared to fresh blastocyst transfers: a retrospective matched-cohort study. J Assist Reprod Genet, 2015. 32: 1483.
88. Sha, T.*, et al.* Pregnancy-related complications and perinatal outcomes resulting from transfer of cryopreserved versus fresh embryos in vitro fertilization: a meta-analysis. Fertil Steril, 2018. 109: 330.
89. NICE. Fertility Problems: Assessment and Treatment Guidelines.
90. Devroey, P.*, et al.* A review of ten years experience of ICSI. Hum Reprod Update, 2004. 10: 19.
91. Rubino, P.*, et al.* The ICSI procedure from past to future: a systematic review of the more controversial aspects. Hum Reprod Update, 2016. 22: 194.
92. Palermo, G.D.*, et al.* Intracytoplasmic sperm injection: state of the art in humans. Reproduction, 2017. 154: F93.
93. Esteves, S.C.*, et al.* Intracytoplasmic sperm injection for male infertility and consequences for offspring. Nat Rev Urol, 2018. 15: 535.
94. Van Peperstraten, A.*, et al.* Techniques for surgical retrieval of sperm prior to intra-cytoplasmic sperm injection (ICSI) for azoospermia. Cochrane Database Syst Rev, 2008: Cd002807.
95. Ohlander, S.*, et al.* 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, 2014. 101: 344.
96. Esteves, S.C.*, et al.* 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, 2017. 108: 456.
97. Abhyankar, N.*, et al.* Use of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with cryptozoospermia: a meta-analysis. Fertil Steril, 2016. 105: 1469.
98. van Rumste, M.M.*, et al.* Intra-cytoplasmic sperm injection versus conventional techniques for oocyte insemination during in vitro fertilisation in patients with non-male subfertility. Cochrane Database Syst Rev, 2003: Cd001301.
99. Henkel, R.R.*, et al.* Sperm preparation for ART. Reprod Biol Endocrinol, 2003. 1: 108.
100. Rappa, K.L.*, et al.* Sperm processing for advanced reproductive technologies: Where are we today? Biotechnol Adv, 2016. 34: 578.
101. Said, T.M.*, et al.* Effects of advanced selection methods on sperm quality and ART outcome: a systematic review. Hum Reprod Update, 2011. 17: 719.
102. Bartoov, B.*, et al.* Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. J Androl, 2002. 23: 1.
103. Bartoov, B.*, et al.* Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. Fertil Steril, 2003. 80: 1413.
104. Berkovitz, A.*, et al.* How to improve IVF-ICSI outcome by sperm selection. Reprod Biomed Online, 2006. 12: 634.
105. Teixeira, D.M.*, et al.* Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction. Cochrane Database Syst Rev, 2020. 2: Cd010167.
106. Beck-Fruchter, R.*, et al.* Clinical benefit using sperm hyaluronic acid binding technique in ICSI cycles: a systematic review and meta-analysis. Reprod Biomed Online, 2016. 32: 286.
107. Miller, D.*, et al.* Physiological, hyaluronan-selected intracytoplasmic sperm injection for infertility treatment (HABSelect): a parallel, two-group, randomised trial. Lancet, 2019. 393: 416.

108. Liu, Y.*, et al.* Intracytoplasmic sperm injection using hyaluronic acid or polyvinylpyrrolidone: a time-lapse sibling oocyte study. Hum Fertil (Camb), 2019. 22: 39.
109. Gil, M.*, et al.* Sperm selection using magnetic activated cell sorting (MACS) in assisted reproduction: a systematic review and meta-analysis. J Assist Reprod Genet, 2013. 30: 479.
110. Romany, L.*, et al.* Obstetric and perinatal outcome of babies born from sperm selected by MACS from a randomized controlled trial. J Assist Reprod Genet, 2017. 34: 201.
111. D'Angelo, A.*, et al.* Coasting (withholding gonadotrophins) for preventing ovarian hyperstimulation syndrome. Cochrane Database Syst Rev, 2017. 5: Cd002811.
112. Guler, I.*, et al.* Impact of testicular histopathology as a predictor of sperm retrieval and pregnancy outcome in patients with nonobstructive azoospermia: correlation with clinical and hormonal factors. Andrologia, 2016. 48: 765.
113. Pandey, S.*, et al.* Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. Hum Reprod Update, 2012. 18: 485.
114. Committee on Practice Bulletins. Practice Bulletin No. 169: Multifetal Gestations: Twin, Triplet, and Higher-Order Multifetal Pregnancies. Obstet Gynecol., 2016. 128: 131.
115. Institute of Medicine Committee on Understanding Premature, B.*, et al.*, The National Academies Collection: Reports funded by National Institutes of Health, in *Preterm Birth: Causes, Consequences, and Prevention*, R.E. Behrman & A.S. Butler, Editors. 2007, National Academies Press (US)

National Academy of Sciences.: Washington (DC).
116. Dyer, S.*, et al.* International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010. Hum Reprod, 2016. 31: 1588.
117. Hansen, M.*, et al.* Assisted reproductive technology and birth defects: a systematic review and meta-analysis. Hum Reprod Update, 2013. 19: 330.
118. Qin, J.*, et al.* Assisted reproductive technology and risk of congenital malformations: a meta-analysis based on cohort studies. Arch Gynecol Obstet, 2015. 292: 777.
119. Jain, T.*, et al.* 30 years of data: impact of the United States in vitro fertilization data registry on advancing fertility care. Fertil Steril, 2019. 111: 477.
120. Boulet, S.L.*, et al.* Assisted Reproductive Technology and Birth Defects Among Liveborn Infants in Florida, Massachusetts, and Michigan, 2000-2010. JAMA Pediatr, 2016. 170: e154934.
121. Liberman, R.F.*, et al.* Assisted Reproductive Technology and Birth Defects: Effects of Subfertility and Multiple Births. Birth Defects Res, 2017. 109: 1144.
122. Spaan, M.*, et al.* Risk of cancer in children and young adults conceived by assisted reproductive technology. Hum Reprod, 2019. 34: 740.
123. Lie, R.T.*, et al.* Birth defects in children conceived by ICSI compared with children conceived by other IVF-methods; a meta-analysis. Int J Epidemiol, 2005. 34: 696.
124. Catford, S.R.*, et al.* Long-term follow-up of intra-cytoplasmic sperm injection-conceived offspring compared with in vitro fertilization-conceived offspring: a systematic review of health outcomes beyond the neonatal period. Andrology, 2017. 5: 610.