EAU Guidelines on
Male Infertility

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European
Association
of Urology

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1.   INTRODUCTION

1.1   Aim
The European Association of Urology (EAU) Guidelines Panel on Male Infertility has prepared these Guidelines to assist urologists and healthcare professionals from related specialties in the treatment of male infertility. Urologists are usually the specialists who are initially responsible for assessing the male when male infertility is suspected. However, infertility can be a multifactorial condition requiring multidisciplinary involvement.

It must be emphasised that clinical guidelines present the best evidence available to the experts. However following guideline recommendations will not necessarily result in the best outcome. Guidelines can never replace clinical expertise when making treatment decisions for individual patients, but rather help to focus decisions - also taking personal values and preferences/individual circumstances of patients into account.

1.2   Publication history
The EAU Male Infertility Guidelines were first published in 2001, followed by full-text updates in 2004, 2007, 2010, 2013, 2014 and 2015.

In 2015 the text was significantly reduced so that only key information was included and re-formatted according to the EAU template for non-oncology Guidelines, so that all Guidelines follow a similar format.

A quick reference document (Pocket Guidelines) is available, both in print and in a number of versions for mobile devices, presenting the main findings of the Male Infertility Guidelines. These are abridged versions which may require consultation together with the full text versions. The Male Infertility Panel published a number of scientific publications in the EAU journal European Urology [1-4]. A separate scientific paper on Vasectomy was published in 2012 [4]. All texts can be viewed and downloaded for personal use at the society website: http://www.uroweb.org/guidelines/online-guidelines/.

1.3   Panel composition
The Male Infertility Guidelines Panel consists of urologists, endocrinologists and gynaecologists with special training in andrology and experience in the diagnosis and treatment of male infertility.

2.   METHODS

References used in this text are graded according to their Level of Evidence (LE) and Guidelines are given a Grade of Recommendation (GR), according to a classification system modified from the Oxford Centre for Evidence-based Medicine Levels of Evidence. Additional methodology information can be found in the general Methodology section of this print, and online at the Eau website: http://www.uroweb.org/guideline/.

A list of Associations endorsing the EAU Guidelines can also be viewed online at the above address. In particular, the Male Infertility Guidelines have been endorsed by the Hellenic Society of Reproductive Medicine.

The recommendations provided in these guidelines are based on a systematic literature search performed by the panel members. The controlled vocabulary of the MeSH database was used alongside a free text protocol, combining "male infertility" with the terms "diagnosis", "epidemiology", "investigations", "treatment", "spermatogenic failure", "genetic abnormalities", "obstruction", "hypogonadism", "varicocele", "cryptorchidism", "testicular cancer", "male accessory gland infection", "idiopathic", "contraception", "ejaculatory dysfunction", and "cryopreservation".

For the 2016 print a scoping search was performed, covering all areas of the guideline, starting from the last cut-off date September 2013, covering 2014 with a cut-off date of January 2015. Embase, Medline and the Cochrane Central Register of Controlled Trials databases were searched, with a limitation to reviews, meta-analysis or meta-analysis of randomized controlled trials.. A total of 609 unique records were identified, retrieved and screened for relevance, of which about 10 publications were selected for inclusion.

2.1   Review
This document was subject to peer review prior to publication in 2015. The decision to re-review is made based on the extent of the revision. A major revision resulting in significant changes to the clinical recommendations presented in the text will warrant re-review.

3. EPIDEMIOLOGY AND AETIOLOGY - GENERAL PRINCIPLES

3.1 Introduction

Definition

"Infertility is the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year", World Health Organization (WHO) [5].

About 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility. One in eight couples encounter problems when attempting to conceive a first child and one in six when attempting to conceive a subsequent child. Three percent of women remain involuntarily childless, while 6% of parous women are not able to have as many children as they would wish [6]. Infertility affects both men and women. In 50% of involuntarily childless couples, a male-infertility-associated factor is found together with abnormal semen parameters. A fertile partner may compensate for the fertility problem of the man and thus infertility usually manifests if both partners have reduced fertility [5]. Male fertility can be reduced as a result of [5]:
- congenital or acquired urogenital abnormalities;
- malignancies;
- urogenital tract infections;
- increased scrotal temperature (e.g. as a consequence of varicocele);
- endocrine disturbances;
- genetic abnormalities;
- immunological factors.

In 30-40% of cases, no male-infertility-associated factor is found (idiopathic male infertility). These men present with no previous history of diseases affecting fertility and have normal findings on physical examination and endocrine, genetic and biochemical laboratory testing. However, semen analysis might reveal pathological findings in the spermiogram (see 4.2.1). Table 1 summarises the main male-infertility-associated factors. Idiopathic male infertility is assumed to be caused by several factors, including endocrine disruption as a result of environmental pollution, reactive oxygen species, or genetic and epigenetic abnormalities.

Table 1: Male infertility causes and associated factors and percentage of distribution in 10,469 patients [7]

Diagnosis	Unselected patients (n = 12,945)	Azoospermic patients (n = 1,446)
All	100%	11.2%
Infertility of known (possible) cause	42.6%	42.6%
Maldescended testes	8.4	17.2
Varicocele	14.8	10.9
Sperm autoantibodies	3.9	-
Testicular tumour	1.2	2.8
Others	5.0	1.2
Idiopathic infertility	30.0	13.3
Hypogonadism	10.1	16.4
Klinefelter's syndrome (47, XXY)	2.6	13.7
XX male	0.1	0.6
Primary hypogonadism of unknown cause	2.3	0.8
Secondary (hypogonadotropic) hypogonadism	1.6	1.9
Kallmann syndrome	0.3	0.5
Idiopathic hypogonadotropic hypogonadism	0.4	0.4
Residual after pituitary surgery	< 0.1	0.3
Others	0.8	0.8
Late-onset hypogonadism	2.2	-
Constitutional delay of puberty	1.4	-
General/systemic disease	2.2	0.5
Cryopreservation due to malignant disease	7.8	12.5
Testicular tumour	5.0	4.3

Lymphoma	1.5	4.6
Leukaemia	0.7	2.2
Sarcoma	0.6	0.9
Disturbance of erection/ejaculation	2.4	-
Obstruction	2.2	10.3
Vasectomy	0.9	5.3
Cystic fibrosis (CBAVD)	0.5	3.1
Others	0.8	1.9

CBAVD = Congenital Bilateral Absence of the Vas Deferens

3.2 Recommendations on epidemiology and aetiology

Recommendations	GR
To categorise infertility, investigate both partners simultaneously.	C
In the diagnosis and management of male subfertility, include the fertility status of the female partner because this might determine the final outcome.	B
Examine all men diagnosed with fertility problems, including men with abnormal semen parameters for urogenital abnormalities.	C

4. PROGNOSTIC FACTORS AND DIAGNOSTIC EVALUATION - GENERAL PRINCIPLES

4.1 Prognostic factors
Prognostic factors for male infertility are:
- duration of infertility
- primary or secondary infertility
- results of semen analysis and
- age and fertility status of female partner.

The cumulative pregnancy rate is 27% in infertile couples with 2 years of follow-up and oligozoospermia as the primary cause of infertility [8]. Female age is the most important single variable influencing outcome in assisted reproduction [9]. Compared to a woman aged 25 years, the fertility potential of a woman aged 35 years is reduced to 50%, to 25% at 38 years, and less than 5% at over 40 years. In many Western countries, women postpone their first pregnancy until after their education and starting a career.

4.2 Diagnostic evaluation
4.2.1 Semen analysis
A medical history and physical examination are standard assessments in all men, including scrotal ultrasound (US) [10] and semen analysis. A comprehensive andrological examination is indicated if semen analysis shows abnormalities compared with reference values (Table 2). Important treatment decisions are based on the results of semen analysis, therefore, it is essential that the complete laboratory work-up is standardised. Ejaculate analysis has been standardised by the WHO and disseminated by publication of the WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edn.) [11]. It is the consensus that modern spermatology must follow these guidelines.

Table 2: Lower reference limits (5th centiles and their 95% CIs) for semen characteristics

Parameter	Lower reference limit (range)
Semen volume (mL)	1.5 (1.4-1.7)
Total sperm number (10^6/ejaculate)	39 (33-46)
Sperm concentration (10^6/mL)	15 (12-16)
Total motility (PR + NP)	40 (38-42)
Progressive motility (PR, %)	32 (31-34)
Vitality (live spermatozoa, %)	58 (55-63)
Sperm morphology (normal forms, %)	4 (3.0-4.0)
Other consensus threshold values	
pH	> 7.2
Peroxidase-positive leukocytes (10^6/mL)	< 1.0
Optional investigations	
MAR test (motile spermatozoa with bound particles, %)	*< 50*
Immunobead test (motile spermatozoa with bound beads, %)	*< 50*
Seminal zinc (µmol/ejaculate)	*≥ 2.4*
Seminal fructose (µmol/ejaculate)	*≥ 13*
Seminal neutral glucosidase (mU/ejaculate)	*≤ 20*

CIs = confidence intervals; MAR = mixed antiglobulin reaction NP = non-progressive; PR = progressive.

4.2.1.1   Frequency of semen analysis

If the results of semen analysis are normal according to WHO criteria, one test is sufficient. If the results are abnormal in at least two tests, further andrological investigation is indicated. It is important to differentiate between the following:
- oligozoospermia: < 15 million spermatozoa/mL;
- asthenozoospermia: < 32% progressive motile spermatozoa;
- teratozoospermia: < 4% normal forms.

Often, all three anomalies occur simultaneously, which is defined as oligo-astheno-teratozoospermia (OAT) syndrome. As in azoospermia, in extreme cases of oligozoospermia (spermatozoa < 1 million/mL), there is an increased incidence of obstruction of the male genital tract and genetic abnormalities.

*4.2.2   **Recommendations for the diagnostic evaluation of male infertility***

Recommendations	GR
Perform semen analyses according to the guidelines of the WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edn).	A*
Perform further andrological assessment when semen analysis is abnormal in at least two tests.	A*
Adhere to the 2010 WHO Manual for the standardised investigation, diagnosis and management of the infertile male for diagnosis and evaluation of male subfertility.	C

*Upgraded following panel consensus.

5. CONDITIONS CAUSING MALE INFERTILITY

5.1 Primary Spermatogenic Failure

5.1.1 Aetiology
The causes of testicular deficiency are summarised in Table 3.

Table 3: Causes of testicular deficiency

Factors	Causes
Congenital	Anorchia
	Testicular dysgenesis/cryptorchidism
	Genetic abnormalities (karyotype, Y-chromosome deletions)
Acquired	Trauma
	Testicular torsion
	Post-inflammatory forms, particularly mumps orchitis
	Exogenous factors (medications, cytotoxic or anabolic drugs, irradiation, heat)
	Systemic diseases (liver cirrhosis, renal failure)
	Testicular tumour
	Varicocele
	Surgery that may compromise vascularisation of the testes and lead to testicular atrophy
Idiopathic	Unknown aetiology
	Unknown pathogenesis

5.1.2 Diagnostic evaluation
Routine investigations include semen analysis and hormonal determinations. Other investigations may be required depending on the individual situation.

Typical findings from the history and physical examination of a patient with testicular deficiency are:
- cryptorchidism (uni-or bilateral)
- testicular torsion and trauma
- genitourinary infection
- exposure to environmental toxins
- gonadotoxic medication (anabolic drugs, SSRIs, etc)
- exposure to radiation or cytotoxic agents
- testicular cancer
- absence of testes
- abnormal secondary sexual characteristics
- gynaecomastia
- abnormal testicular volume and/or consistency
- varicocele.

5.1.2.1 Semen analysis
In NOA, semen analysis shows normal ejaculate volume and azoospermia after centrifugation. A recommended method is semen centrifugation at 3000 g for 15 min and a thorough microscopic examination by phase contrast optics at ×200 magnification of the pellet. All samples can be stained and re-examined microscopically [11].

5.1.2.2 Hormonal determinations
In men with testicular deficiency, Hypergonadotropic hypogonadism is usually present, with high levels of follicle-stimulating hormone (FSH) and luteinising hormone (LH), and sometimes low levels of testosterone. Generally, the levels of FSH correlate with the number of spermatogonia: when spermatogonia are absent or markedly diminished, FSH values are usually elevated; when the number of spermatogonia is normal, but maturation arrest exists at the spermatocyte or spermatid level, FSH values are within the normal range. However, for an individual patient, FSH levels do not accurately predict the spermatogenesis status because men with maturation arrest histology could have normal FSH and normal testis volume and still be azoospermic [13, 14].

5.1.2.3 Testicular biopsy
Testicular biopsy can be part of intracytoplasmic sperm injection (ICSI) treatment in patients with clinical

evidence of NOA. Testicular sperm extraction (TESE) is the technique of choice. Spermatogenesis may be focal, which means that in about 50% of men with NOA, spermatozoa can be found and used for ICSI. Most authors therefore recommend taking several testicular samples. There is a good correlation between the histology found upon diagnostic biopsy and the likelihood of finding mature sperm cells during testicular sperm retrieval and ICSI [15-17]. However, no threshold value has been found for FSH, inhibin B, or testicular volume and successful sperm harvesting. When there are complete AZFa and AZFb microdeletions, the likelihood of sperm retrieval is virtually zero and therefore TESE procedures are contraindicated. Microsurgical TESE increases retrieval rates vs. conventional TESE, and should be preferred in severe cases of non-obstructive azoospermia [18-21]. Positive retrievals are reported even in conditions such as Sertoli cell only syndrome type II [12].

The results of ICSI are worse when using sperm retrieved from men with NOA compared to sperm from ejaculated semen and from men with obstructive azoospermia (OA) [22-26]. Birth rates are lower in NOA vs. OA (19% vs 28%) [27, 28].
• ICSI results in significantly lower fertilisation and implantation rates.
• In longitudinal studies including patients with NOA as defined by testicular histopathology, only one out of seven NOA patients embarking for TESE and eventually ICSI will father their genetically-own child [29]
• Neonatal health in terms of birth parameters, major anomalies and chromosomal aberrations in a large cohort of children born after use of non-ejaculated sperm are comparable to the outcome of children born after use of ejaculated sperm [30]

5.1.3   Summary of evidence and recommendations

Summary of evidence	LE
The WHO laboratory manual proposes reference values based on fertility hence, these reference values do not allow classifying a man as being infertile.	2a
Impaired spermatogenesis is often associated with elevated FSH concentration.	3
For patients with NOA who have spermatozoa in their testicular biopsy, ICSI with fresh or cryopreserved spermatozoa is the only therapeutic option. Spermatozoa are found by a TESE procedure in about 50% of patients with NOA.	2a
Pregnancies and live births are eventually obtained in 30-50% of couples with NOA, when spermatozoa have been found in the testicular biopsy.	3

Recommendations	GR
For men who are candidates for sperm retrieval, give appropriate genetic counselling - also when testing for genetic abnormalities was negative.	A
In men with NOA, perform simultaneous testicular biopsy with multiple TESE (or micro TESE) to define spermatogenesis and diagnose ITGCNU.	A

ICSI = intracytoplasmic sperm injection; ITGCNU = intratubular germ cell neoplasma of unclassified type; TESE = testicular sperm extraction; NOA = non-obstructive azoospermia.

5.2   Genetic disorders in infertility
All urologists working in andrology must have an understanding of genetic abnormalities associated with infertility, so that they can provide correct advice to couples seeking fertility treatment. Men with very low sperm counts can be offered a reasonable chance of paternity, using in vitro fertilisation (IVF), ICSI and sperm harvesting from the testes in case of azoospermia. However, the spermatozoa of infertile men show an increased rate of aneuploidy, structural chromosomal abnormalities, and DNA damage, carrying the risk of passing genetic abnormalities to the next generation. Current routine clinical practice is based on the screening of genomic DNA from peripheral blood samples, however, screening of chromosomal anomalies in spermatozoa is also feasible and can be performed in selected cases [31].

5.2.1   Chromosomal abnormalities
Chromosome abnormalities can be numerical (e.g. trisomy) or structural (e.g. inversions or translocations). In a survey of pooled data from 11 publications, including 9,766 infertile men, the incidence of chromosomal abnormalities was 5.8% [32]. Of these, sex chromosome abnormalities accounted for 4.2% and autosomal abnormalities for 1.5%. In comparison, the incidence of abnormalities was 0.38% in pooled data from three series, with a total of 94,465 newborn male infants, of which 131 (0.14%) were sex chromosome abnormalities and 232 (0.25%) autosomal abnormalities [32]. The frequency of chromosomal abnormalities increases as

testicular deficiency becomes more severe. Patients with a spermatozoa count < 5 million/mL already show a 10-fold higher incidence (4%) of mainly autosomal structural abnormalities compared with the general population [33, 34]. Men with NOA are at highest risk, especially for sex chromosomal anomalies.

Based on the frequencies of chromosomal aberrations in patients with different sperm concentration, karyotype analysis is indicated in men with azoospermia or oligozoospermia (spermatozoa < 10 million/mL) [34]. A recent study proposes to restrict karyotype to NOA men with the purpose to prevent adverse pregnancy outcomes [35]. If there is a family history of recurrent spontaneous abortions, malformations or mental retardation, karyotype analysis should be requested, regardless of the sperm concentration.

5.2.1.1 Sex chromosome abnormalities (Klinefelter's syndrome and variants [47,XXY; 46,XY/47, XXY mosaicism])

Klinefelter's syndrome is the most common sex chromosome abnormality [36]. Adult men with Klinefelter's syndrome have small firm testicles, devoid of germ cells. The phenotype varies from a normally virilised man to one with the stigmata of androgen deficiency, including female hair distribution, scant body hair, and long arms and legs due to late epiphyseal closure. Leydig cell function is commonly impaired in men with Klinefelter's syndrome [37]. Testosterone levels may be normal or low, oestradiol levels normal or elevated, and FSH levels increased. Libido is often normal despite low testosterone levels, but androgen replacement may be needed as the patient ages.

Germ cell presence and sperm production are variable in men with Klinefelter's mosaicism, 46,XY/47,XXY. Based on sperm fluorescence *in situ* hybridisation (FISH) studies showing an increased frequency of sex chromosomal abnormalities and increased incidence of autosomal aneuploidy (disomy for chromosomes 13, 18 and 21), concerns have been raised about the chromosomal normality of the embryos generated through ICSI [38].

The production of 24,XY sperm has been reported in 0.9% and 7.0% of men with Klinefelter's mosaicism [39, 40] and in 1.36-25% of men with somatic karyotype 47,XXY [41-44]. In patients with azoospermia, TESE or (micro-TESE) can be proposed as a therapeutic option since spermatozoa can be recovered in about 30% of cases. There is growing evidence that TESE or micro TESE yields higher sperm recovery rates when done at younger age. Numerous healthy children have been born using ICSI without pre-implantation genetic diagnosis (PGD) and the conception of one 47,XXY foetus has been reported [36]. However, a study of ICSI combined with PGD in 113 embryos reported a significant fall in the rate of normal embryos for couples with Klinefelter's syndrome with respect to controls (54% vs. 77.2%) [44]. Due to the significant increase of sex chromosomal and autosomal abnormalities in the embryos of Klinefelter's patients, PGD or amniocentesis analysis should be considered.

Follow-up (possibly every year) of men with Klinefelter's syndrome is required and androgen replacement therapy should be started after fertility issues have been addressed and when testosterone level is in the range of hypoandrogenism.

TESE in peripubertal or pre-pubertal Klinefelter boys aiming at cryopreservation of testicular spermatogonial stem cells is to be considered experimental and should only be performed within a research protocol [45].

5.2.1.2 Autosomal abnormalities

Genetic counselling should be offered to all couples seeking fertility treatment (including IVF/ICSI) when the male partner has an autosomal karyotype abnormality. The most common autosomal karyotype abnormalities are Robertsonian translocations, reciprocal translocations, paracentric inversions, and marker chromosomes. It is important to look for these structural chromosomal anomalies because there is an increased associated risk of aneuploidy or unbalanced chromosomal complements in the foetus. As with Klinefelter's syndrome, sperm FISH analysis provides a more accurate risk estimation of affected offspring, however, the diffusion of this genetic test is largely limited by the availability of laboratories able to perform this analysis. When IVF/ICSI is carried out for men with translocations, PGD or amniocentesis should be performed.

5.2.1.3 Sperm chromosomal abnormalities

Sperm can be examined for their chromosomal constitution using multicolour FISH both in men with normal karyotype and with anomalies. Aneuploidy in sperm, particularly sex chromosome aneuploidy, is associated with severe damage to spermatogenesis [32, 46-48] and with translocations [49]. Florescence *in situ* hybridisation analysis of spermatozoa is only indicated for specific andrology conditions e.g. macrocephalia [48].

5.2.2 Genetic defects

5.2.2.1 X-linked genetic disorders and male fertility
Each man has only one X-chromosome. An X-linked recessive disorder manifests in males. The defect will be transmitted to daughters, but not to sons.

5.2.2.2 Kallmann syndrome
Patients with Kallmann syndrome have hypogonadotropic hypogonadism and anosmia, but may also have other clinical features, including facial asymmetry, cleft palate, colour blindness, deafness, maldescended testes, and unilateral renal aplasia. This syndrome can be due to mutation in the Kalig-1 gene [on the X-chromosome] or in several other autosomal genes and should be tested [48, 49].

Spermatogenesis can be relatively easily induced by hormonal treatment [50], therefore, genetic screening prior to therapy is advisable although it is limited by the rarity of specialised genetic laboratories that can offer this genetic test. Treatment with gonadotropins allows natural conception in most cases, even for men with a relatively low sperm count. Thus, identification of the involved gene (X-linked, autosomal dominant or recessive) can help to provide more accurate genetic counselling, that is, risk estimation for transmission to the offspring.

5.2.2.3 Mild androgen insensitivity syndrome
The AR gene is located on the long arm of the X-chromosome. Mutations in the AR gene may result in mild to complete androgen insensitivity. The phenotypic features of complete androgen insensitivity syndrome are female external genitalia and absence of pubic hair (Morris syndrome). In partial androgen insensitivity syndrome, phenotypes range from predominantly female phenotype through ambiguous genitalia, to predominantly male phenotype with micropenis, perineal hypospadias, and cryptorchidism. The latter phenotype is also termed Reifenstein syndrome. In the forementioned severe forms of androgen resistance, there is no risk of transmission because affected men cannot generate their own biological children using the current technologies. Patients with mild androgen insensitivity syndrome have male infertility as their primary or even sole symptom. Disorders of the androgen receptor causing infertility in the absence of any genital abnormality are rare, and only a few mutations have been reported in infertile [51-54] or fertile [55] men.

5.2.2.4 Other X-disorders
An unexpectedly high number of genes with a testis-specific or enriched expression pattern have been identified on the X-chromosome, and in particular, premeiotic genes are over-represented on the X-chromosome compared with autosomal chromosomes [56]. Nevertheless, to date only a few genes have been screened in relatively small populations and none of them appear relevant for male infertility [57, 58]. On the other hand, two recent independent studies showed a significantly higher deletion load on the X-chromosome in men with spermatogenic failure with respect to normozoospermic controls [59, 60].

5.2.3 Y-chromosome and male infertility
Microdeletions on the Y-chromosome are termed AZFa, AZFb and AZFc [61]. Clinically relevant deletions remove partially, or in most cases completely, one or more of the AZF regions, and are the most frequent molecular genetic cause of severe oligozoospermia and azoospermia [62]. In each AZF region, there are several spermatogenesis candidate genes [63]. Deletions occur en bloc (i.e. removing more than one gene), thus, it is not possible to determine the role of a single AZF gene from the AZF deletion phenotype and it is unclear if they all participate in spermatogenesis. Gene-specific deletions, which remove a single gene, have been reported only in the AZFa region and concern the USP9Y gene. These studies have suggested that USP9Y is most likely to be a "fine tuner" of sperm production, and its specific screening is not advised [64].

5.2.3.1 Clinical implications of Y microdeletions
The clinical significance of Yq microdeletions can be summarised as follows:
- They are not found in normozoospermic men, proving there is a clear cut cause-and-effect relationship between Y-deletions and spermatogenic failure [65].
- The highest frequency of Y-deletions is found in azoospermic men (8-12%), followed by oligozoospermic (3-7%) men.
- Deletions are extremely rare with a sperm concentration > 5 million/mL (~0.7%).
- AZFc deletions are most common (65-70%), followed by Y-deletions of the AZFb and AZFb+c or AZFa+b+c regions (25-30%). AZFa region deletions are rare (5%).
- Complete removal of the AZFa region is associated with severe testicular phenotype (Sertoli cell only syndrome), while complete removal of the AZFb region is associated with spermatogenic rest. Complete removal of the AZFc region causes a variable phenotype ranging from azoospermia to oligozoospermia.

- Classical (complete) AZF deletions do not confer a risk for cryptorchidism or testicular cancer [62].

The specificity and genotype/phenotype correlation reported above means that Y-deletion analysis has both a diagnostic and prognostic value for testicular sperm retrieval [62].

5.2.3.1.1 Testing for Y microdeletions
Indications for AZF deletion screening are based on sperm count and include azoospermia and severe oligozoospermia (spermatozoa count < 5 million/mL). Thanks to the European Academy of Andrology (EAA) guidelines [66] and the EAA/EMQN (European Molecular Genetics Quality Network) external quality control programme (http://www.emqn.org/emqn/), Yq testing has become more reliable in different routine genetic laboratories. The EAA guidelines provide a set of primers capable of detecting > 95% of clinically relevant deletions [66].

5.2.3.1.2 Genetic counselling for AZF deletions
After conception, any Y-deletions are transmitted obligatorily to the male offspring, and genetic counselling is therefore mandatory. In most cases, father and son have the same microdeletion [66], but occasionally the son has a larger one [67]. The extent of spermatogenic failure (still in the range of azoo-/oligozoospermia) cannot be predicted entirely in the son, due to the different genetic background and the presence or absence of environmental factors with potential toxicity for reproductive function. A significant proportion of spermatozoa from men with complete AZFc deletion are nullisomic for sex chromosomes [68, 69], 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 [70]. Despite this theoretical risk, babies born from fathers affected by Yq microdeletions are phenotypically normal [62, 66]. This could be due to the reduced implantation rate and a likely higher risk of spontaneous abortion of embryos bearing a 45,X0 karyotype. When ICSI is used in the presence of a Y microdeletion, long-term follow-up of any male children is needed with respect to their fertility status, and cryopreservation of spermatozoa at a young age can be considered.

5.2.3.1.3 Y-chromosome: 'gr/gr' deletion
A new type of Yq deletion, known as the gr/gr deletion, has been described in the AZFc region [71]. 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 2.5-8 fold increased risk for oligozoospermia [66, 72-74]. The frequency of gr/gr deletion in oligozoospermic patients is ~4%.

According to four meta-analyses, gr/gr deletion is a significant risk factor for impaired sperm production [73, 74]. It is worth noting that both the frequency of gr/gr deletion and its phenotypic expression vary between different ethnic groups, depending on the Y-chromosome background. For example, in some Y haplogroups, 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 multicentre study has shown that gr/gr deletion is a potential risk factor for testicular germ cell tumours [75]. However, these data need further confirmation in an ethnically and geographically matched case-control study setting. For genetic counselling it is worth noticing that partial AZFc deletions (gr/gr and b2/b3) may predispose to complete AZFc deletion in the next generation [76, 77].

5.2.3.1.4 Autosomal defects with severe phenotypic abnormalities and infertility
Several inherited disorders are associated with severe or considerable generalised abnormalities and infertility. Among them, Prader-Willy Syndrome, Bardet-Biedl Syndrome, Noonan's Syndrome, Myotonic dystrophy, dominant polycystic kidney disease, 5 α-reductase deficiency, etc. Patients with these defects will be well known to doctors, often from childhood. A fertility problem must be managed in the context of the care of the man as a whole and considering the couple's ability to care for a child.

5.2.4 *Cystic fibrosis mutations and male infertility*
Cystic fibrosis (CF) is a fatal autosomal-recessive disorder. It is the most common genetic disease of Caucasians; 4% are carriers of gene mutations involving the CF transmembrane conductance regulator (CFTR) gene located on chromosome 7p. It encodes a membrane protein that functions as an ion channel and influences the formation of the ejaculatory duct, seminal vesicle, vas deferens and distal two-thirds of the epididymis.

Congenital bilateral absence of the vas deferens (CBAVD) is associated with CFTR gene mutations and was found in ~2% of men with OA attending a clinic in Edinburgh, UK [78]. The incidence in men with OA varies

between different countries. The clinical diagnosis of absent vasa is easy to miss and all men with azoospermia should be very carefully examined to exclude CBAVD, particularly those with a semen volume < 1.5 mL and pH < 7.0. Approximately 1,500 mutations are listed on the CFTR database http://www.genetsickkids.on.ca/cftr/). The most frequently found mutations are the F508, R117H and W1282X, but their frequency and the presence of other mutations largely depend on the ethnicity of the patient [79, 80]. Given the functional relevance of a DNA variant (the 5T allele) in a non-coding region of CFTR [75], it is now considered a mild CFTR mutation rather than a polymorphism and it should be analysed in each CBAVD patient. As more mutations are defined and tested for, almost all men with CBAVD will probably be found to have mutations. It is not practical to test for all known mutations, because many have a very low prevalence in a particular population. Routine testing is usually restricted to the most common mutations in a particular community through the analysis of a mutation panel. Given that this is a recessive disease if a second mutation is not found with the routine panel, a second step analysis is advised which comprises the direct sequencing of the entire gene. Men with CBAVD often have mild clinical stigmata of CF (e.g., history of chest infections). When a man has CBAVD, it is important to test also his partner for CF mutations. If the female partner is found to be a carrier of CFTR mutations, the couple must consider very carefully whether to proceed with ICSI using the male's sperm, as the risk of having a child with CF or CBAVD will be 50%, depending on the type of mutations carried by the parents. If the female partner is negative for known mutations, the risk of being a carrier of unknown mutations is ~0.4%.

5.2.4.1   Unilateral or bilateral absence/abnormality of the vas and renal anomalies
Unilateral absence of the vas deferens is usually associated with ipsilateral absence of the kidney and probably has a different genetic causation [81]. Consequently, in these subjects CFTR mutation screening is not indicated. Men with unilateral absence of the vas deferens are usually fertile, and the condition is most commonly encountered as an incidental finding in the vasectomy clinic. CFTR gene mutation screening is indicated in men with unilateral absence of the vas deferens with normal kidneys. An abdominal ultrasound should be undertaken both in unilateral and bilateral absence of vas deferens. Findings may range from unilateral absence of the vas with ipsilateral absence of the kidney, to bilateral vessel abnormalities and renal abnormalities, such as pelvic kidney [82].

5.2.4.2   Unknown genetic disorders
Considering the high predicted number of genes involved in male gametogenesis, it is likely that most idiopathic forms of spermatogenic disturbances are caused by mutations or polymorphisms in spermatogenesis candidate genes [57]. However, despite an intensive search for new genetic factors, no clinically relevant gene mutations or polymorphisms (except those related to the Y-chromosome) have so far been identified [57, 83, 84], and references therein. The introduction of new analytical approaches provided evidence for the importance of CNVs [59, 60] and further advances are expected with Next Generation Sequencing. Intracytoplasmic sperm injection is used to enable men with severely damaged spermatogenesis to father children in situations formerly considered hopeless and where very few spermatozoa can be obtained. This has led to concern that children may be born with a foetal abnormality, because ICSI may enable defective sperm to bypass the selective processes of the female genital tract and egg covering.

Intracytoplasmic sperm injection babies have a higher risk of de novo sex chromosomal aberrations (about a threefold increase compared with natural conceptions) and paternally inherited structural abnormalities. Treatment with assisted reproductive technology was associated with increased risk of cardiovascular, musculoskeletal, urogenital, and gastrointestinal defects and cerebral palsy [85-87].

5.2.4.3   DNA fragmentation in spermatozoa
There is increased DNA damage in spermatozoa from men with oligozoospermia. This increase is associated with reduced chances of natural conception and an increased chance of early pregnancy loss [88].

5.2.4.4   Genetic counselling and ICSI
Initially, the couple should be given full information about the risks to the child in order to help them decide whether to proceed with ICSI. Where there is conflict between the wishes of the couple and the interests of the future child, it may be ethically correct to withhold therapy. When both partners are known to carry defects (e.g., CFTR mutations), there is up to a 50% chance of the child developing a clinical condition. Many clinicians and infertility clinic personnel may consider it unethical to proceed because their duty of care to the future child and the interests of society outweigh the wishes of the individual couple. If there is a conflict that cannot be resolved by agreement, the interests of a future child probably take precedence over the interests of a couple. The couple also needs to give consideration to preimplantation diagnosis.

Summary of evidence	LE
In men with spermatogenic damage there is a higher prevalence of chromosome abnormalities reaching the highest frequency in NOA men.	1b
AZF deletions are clear-cut causes of spermatogenic impairments with diagnostic and prognostic value for TESE.	1a
AZF deletion will be obligatorily transmitted to the son.	1a
gr/gr deletion has been confirmed as a significant risk factor for impaired sperm production, whereas further evidence of the prognostic significance of gr/gr and development of a testicular germ cell tumour is needed.	2b

Recommendations	GR
Obtain standard karyotype analysis in all men with damaged spermatogenesis (spermatozoa < 10 million/mL) who are seeking fertility treatment by IVF.	B
Provide genetic counselling in all couples with a genetic abnormality found in clinical or genetic investigation and in patients who carry a (potential) inheritable disease.	A
For all men with Klinefelter's syndrome, provide long-term endocrine follow-up and androgen replacement therapy, if necessary.	A
Do not test for microdeletions in men with obstructive azoospermia (OA) when ICSI is used because spermatogenesis should be normal.	A
Inform men with Yq microdeletion and their partners who wish to proceed with ICSI that microdeletions will be passed to sons, but not to daughters.	A
In men with structural abnormalities of the vas deferens (unilateral or bilateral absence), test the man and his partner for CFTR gene mutations.	A

CFTR = transmembrane conductance regulator; IVF = in vitro fertilisation; OA = obstructive azoospermia;
FSH = follicle-stimulating hormone; ICSI = intracytoplasmic sperm injection; TESE = testicular sperm extraction;
CF = cystic fibrosis.

5.3 Obstructive azoospermia

Obstructive azoospermia (OA) is the absence of spermatozoa and spermatogenetic cells in semen and post-ejaculate urine due to obstruction. OA is less common than NOA and occurs in 15-20% of men with azoospermia. Men with OA present with normal FSH, normal size testes, and epididymal enlargement. Sometimes, the vas deferens is absent (CBAVD or Congenital Unilateral Absence of the Vas Deferens (CUAVD)). Obstruction in primary infertile men is frequently present at the epididymal level.

5.3.1 ***Classification***
5.3.1.1 *Intratesticular obstruction*
Intratesticular obstruction occurs in 15% of men with OA [89]. Congenital forms are less common than acquired forms (post-inflammatory or post-traumatic).

5.3.1.2 *Epididymal obstruction*
Epididymal obstruction is the most common cause of OA, affecting 30-67% of azoospermic men [89-93]. Congenital epididymal obstruction usually manifests as CBAVD, which is associated with at least one mutation of the CF gene in 82% of cases [92]. Congenital forms of epididymal obstruction include chronic sinopulmonary infections (Young's syndrome) [94]. Acquired forms secondary to acute (e.g., gonococcal) and subclinical (e.g., chlamydial) epididymitis are most common [95, 96]. Other causes may be trauma or surgical intervention [97, 98].

5.3.1.3 *Vas deferens obstruction*
Vas deferens obstruction is the most common cause of acquired obstruction following vasectomy [99]. Approximately 2-6% of these men request vasectomy reversal (see Chapter 5.6). Vasal obstruction may also occur after hernia repair [1, 100]. The most common congenital vasal obstruction is CBAVD, often accompanied by CF. Unilateral agenesis or a partial defect is associated with contralateral seminal duct anomalies or renal agenesis in 80% and 26% of cases, respectively [101] (see Chapter 5.2).

5.3.1.4 *Ejaculatory duct obstruction*
Ejaculatory duct obstruction is found in 1-3% of cases of OA [89] and is classified as either cystic or post-

inflammatory. Cystic obstructions are usually congenital (i.e., Mullerian duct cyst or urogenital sinus/ejaculatory duct cysts) and are typically midline. In urogenital sinus abnormalities, one or both ejaculatory ducts empty into the cyst [102], while in Mullerian duct anomalies, the ejaculatory ducts are laterally displaced and compressed by the cyst [103]. Paramedian or lateral intraprostatic cysts are rare [104]. Post-inflammatory obstructions of the ejaculatory duct are usually secondary to urethroprostatitis [105]. Congenital or acquired complete obstructions of the ejaculatory ducts are commonly associated with low semen volume, decreased or absent seminal fructose, and acid pH. The seminal vesicles are usually dilated (anterior-posterior diameter > 15 mm) [105, 106].

5.3.1.5 Functional obstruction of the distal seminal ducts
Functional obstruction of the distal seminal ducts might be attributed to local neuropathy [107]. This abnormality is often associated with urodynamic dysfunction. Impaired sperm transport may be idiopathic or associated with SSRI medication as well.

5.3.2 Diagnostic evaluation
5.3.2.1 Clinical history
Clinical history taking should follow the suggestions for the diagnostic evaluation of infertile men (4.2).

5.3.2.2 Clinical examination
Clinical examination should follow suggestions for the diagnostic evaluation of infertile men. The following findings indicate OA:
- At least one testis with a volume > 15 mL, although a smaller volume may be found in some patients with OA and concomitant partial testicular failure.
- Enlarged and hardened epididymis.
- Nodules in the epididymis or vas deferens.
- Absence or partial atresia of the vas.

5.3.2.3 Semen analysis
At least two examinations must be carried out at an interval of 2-3 months, according to the WHO (see Chapter 3A.2). Azoospermia means the inability to detect spermatozoa after centrifugation at ×400 magnification. When semen volume is low, a search must be made for spermatozoa in urine after ejaculation. Absence of spermatozoa and immature germ cells in semen smears suggest complete seminal duct obstruction.

5.3.2.4 Hormone levels
Serum FSH levels may be normal, but do not exclude a testicular cause of azoospermia. FSH level is normal in 40% of men with primary spermatogenic failure. Inhibin B seems to have a higher predictive value for normal spermatogenesis [93].

5.3.2.5 Ultrasonography
In addition to physical examination, a scrotal ultrasound may be helpful in finding signs of obstruction (e.g., dilatation of rete testis, enlarged epididymis with cystic lesions, or absent vas deferens) and may demonstrate signs of testicular dysgenesis (e.g., non-homogeneous testicular architecture and microcalcifications) and testis tumours. For patients with a low seminal volume and in whom distal obstruction is suspected, transrectal ultrasound (TRUS) is essential. Invasive diagnosis, including testicular biopsy, scrotal exploration, and distal seminal duct evaluation, are indicated in patients with OA in whom an acquired obstruction of the seminal ducts is suspected. Explorative and recanalisation surgery should be carried out simultaneously.

5.3.2.6 Testicular biopsy
In selected cases, testicular biopsy is indicated to exclude spermatogenic failure. Testicular biopsy should be combined with extraction of testicular spermatozoa (i.e., TESE) for cryopreservation.

5.3.3 Disease management
5.3.3.1 Intratesticular obstruction
Only TESE allows sperm retrieval in these patients and is therefore recommended.

5.3.3.2 Epididymal obstruction
Microsurgical epididymal sperm aspiration (MESA) [108] is indicated in men with CBAVD. TESE and PESA are also viable options [109]. Usually, one MESA procedure provides sufficient material for several ICSI cycles [110] and it produces high pregnancy and fertilisation rates [111]. In patients with azoospermia due to acquired epididymal obstruction, microsurgical reconstruction is recommended, with the preferred technique

being microsurgical intussusception tubulovasectomy [112]. Anatomical recanalisation following surgery may require 3-18 months. Before microsurgery (and in all cases where recanalisation is impossible), epididymal spermatozoa should be aspirated and cryopreserved for use in ICSI [110]. Patency rates range between 60% and 87% [98, 113] and cumulative pregnancy rates between 10% and 43%. Recanalisation success rates may be adversely affected by preoperative and intraoperative findings.

5.3.3.3 Proximal vas obstruction
Proximal vas obstruction after vasectomy requires microsurgical vasectomy reversal (see Chapter 3G). Vasovasostomy is also required in rare cases of proximal vasal obstructions. The absence of spermatozoa in the intraoperative vas deferens fluid suggests the presence of a secondary epididymal obstruction; especially if the seminal fluid of the proximal vas has a thick "toothpaste" appearance. Microsurgical tubulovasostomy is then indicated.

5.3.3.4 Distal vas deferens obstruction
It is usually impossible to correct large bilateral vas deferens defects, resulting from involuntary excision of the vasa deferentia during hernia surgery in early childhood or previous orchidopexy [114]. In these cases TESE/ MESA or proximal vas deferens sperm aspiration [115] can be used for cryopreservation for future ICSI.

5.3.3.5 Ejaculatory duct obstruction
The treatment of ejaculatory duct obstruction depends on its aetiology. Transurethral resection of the ejaculatory ducts (TURED) [105] can be used in large postinflammatory obstruction and when one or both ejaculatory ducts empty into an intraprostatic midline cyst. Resection may remove part of the verumontanum. In cases of obstruction due to a midline intraprostatic cyst, incision or unroofing of the cyst is required [105]. Intraoperative TRUS makes this procedure safer. If distal seminal tract evaluation is carried out at the time of the procedure, installation of methylene blue dye into the vas deferens can help to document opening of the ducts. The limited success rate of surgical treatment of ejaculatory duct obstruction in terms of spontaneous pregnancies should be weighed against sperm aspiration and ICSI. Complications following TURED include retrograde ejaculation due to bladder neck injury and urine reflux into the ejaculatory ducts, seminal vesicles, and vasa. The alternatives to TURED are MESA, TESE, proximal vas deferens sperm aspiration, seminal vesicle ultrasonically guided aspiration, and direct cyst aspiration. Spermatozoa can then be retrieved by antegrade seminal tract washout [116].

5.3.4 Summary of evidence and recommendations for obstructive azoospermia

Summary of evidence	LE
Obstructive lesions of the seminal tract are frequent in azoospermic or severely oligozoospermic patients with normal-sized testes and normal reproductive hormones.	3

Recommendations	GR
For azoospermia caused by vasal or epididymal obstruction, perform microsurgical vasovasostomy or tubulovasostomy.	B
Use sperm retrieval techniques, such as MESA, TESE, and PESA only when cryostorage of the material obtained is available.	B

5.4 Varicocele
Varicocele is a common abnormality which may be associated with the following andrological conditions:
- Failure of ipsilateral testicular growth and development.
- Symptoms of pain and discomfort.
- Male subfertility.
- Hypogonadism.

5.4.1 Classification
The following classification of varicocele [12] is useful in clinical practice:
- Subclinical: not palpable or visible at rest or during Valsava manoeuvre, but can be shown by special tests (Doppler ultrasound studies).
- Grade 1: palpable during Valsava manoeuvre, but not otherwise.
- Grade 2: palpable at rest, but not visible.
- Grade 3: visible and palpable at rest.

5.4.2　　Diagnostic evaluation
The diagnosis of varicocele is made by clinical examination and should be confirmed by ultrasound investigation and colour Duplex analysis [12]. In centres where treatment is carried out by antegrade or retrograde sclerotherapy or embolisation, diagnosis is additionally confirmed by X-ray.

5.4.3　　Basic considerations
5.4.3.1　　Varicocele and fertility
Varicocele is a physical abnormality present in 11.7% of adult men and in 25.4% of men with abnormal semen analysis [117]. The exact association between reduced male fertility and varicocele is unknown, but a recent meta-analysis showed that semen improvement is usually observed after surgical correction [118]. Varicocelectomy can reverse sperm DNA damage [119].

5.4.3.2　　Varicocelectomy
Varicocele repair has been a subject of debate for several decades. A meta-analysis of randomised controlled trials and observational studies showed that surgical varicocelctomy significantly improves semen parameters in men with abnormal semen, but only in men with a clinical varicoceles [118].

In randomised controlled studies varicocele repair in men with a subclinical varicocele was found to be ineffective in increasing the chance of spontaneous pregnancies [120]. Also, in randomized studies that included mainly men with normal semen parameters no benefit was found in favour of treatment over observation [121]. A Cochrane review from 2013 concluded that there is evidence suggesting that treatment of a varicocele in men from couples with otherwise unexplained subfertility may improve a couple's chance of pregnancy. In a subgroup analyses of five RCT`s comparing treatment to observation in men with a clinical varicocele, oligospermia and otherwise unexplained infertility the analyses favoured treatment, with a combined OR 2.39 (95% CI 1.56 to 3.66) [122].

5.4.3.3　　Prophylactic Varicocelectomy
In adolescents with a varicocle there is a significant risk of overtreatment since most adolescents with a varicocele will have no problem achieving pregnancy later in life [123]. Prophylactic treatment is only advised in case of documented growth deterioration of the testis documented by serial clinical examinations and in case of impaired semen quality.

5.4.4　　Disease management
Several treatments are available for varicocele (Table 4). Current evidence indicates that microsurgical varicocelectomy is the most effective method among the different varicocelectomy techniques [123]. Microsurgical repair has resulted in fewer complications and lower recurrence rates compared to the other techniques. This procedure, however, requires microsurgical training. The various other techniques are still considered viable options, although recurrences and hydrocele formation are more likely to occur.

Table 4: Recurrence and complication rates associated with treatments for varicocele

Treatment	Ref.	Recurrence/ persistence %	Complication rates
Antegrade sclerotherapy	[124]	9	Complication rate 0.3-2.2%: testicular atrophy, scrotal haematoma, epididymitis, left-flank erythema
Retrograde sclerotherapy	[125]	9.8	Adverse reaction to contrast medium, flank pain, persistent thrombophlebitis, vascular perforation
Retrograde embolisation	[126, 127]	3.8-10	Pain due to thrombophlebitis, bleeding haematoma, infection, venous perforation, hydrocele, radiological complication (e.g., reaction to contrast media), misplacement or migration of coils, retroperitoneal haemorrhage, fibrosis, ureteric obstruction
Open operation			
Scrotal operation		-	Testicular atrophy, arterial damage with risk of devascularisation and testicular gangrene, scrotal haematoma, post-operative hydrocele
Inguinal approach	[128]	13.3	Possibility of missing out a branch of testicular vein
High ligation	[129]	29	5-10% incidence of hydrocele (< 1%)
Microsurgical inguinal or subinguinal	[130, 131]	0.8-4	Post-operative hydrocele arterial injury, scrotal haematoma
Laparoscopy	[132, 133]	3-7	Injury to testicular artery and lymph vessels; intestinal, vascular and nerve damage; pulmonary embolism; peritonitis; bleeding; postoperative pain in right shoulder (due to diaphragmatic stretching during pneumoperitoneum); pneumoscrotum: wound infection

5.4.5   *Summary of evidence and recommendations for varicocele*

Summary of evidence	LE
The presence of varicocele in some men is associated with progressive testicular damage from adolescence onwards and a consequent reduction in fertility.	2a
Although the treatment of varicocele in adolescents may be effective, there is a significant risk of overtreatment: the majority of boys with a varicocele will have no fertility problems later in life.	3
Varicocele repair was shown to be effective in men with oligospermia, a clinical varicocele and otherwise unexplained infertility.	1a

Recommendations	GR
Treat varicoceles in adolescents with progressive failure of testicular development documented by serial clinical examination.	B
Do not treat varicoceles in infertile men who have normal semen analysis and in men with a subclinical varicocele.	A
Treat varicoceles in men with a clinical varicocele, oligospermia and otherwise unexplained infertility in the couple.	A

5.5   Hypogonadism

Hypogonadism is characterised by impaired testicular function, which may affect spermatogenesis and/or testosterone synthesis. The symptoms of hypogonadism depend on the degree of androgen deficiency and if the condition develops before or after pubertal development of the secondary sex characteristics.

5.5.1   *Epidemiology and aetiology*
The aetiological and pathogenetic mechanisms of male hypogonadism can be divided into three main categories:
• Primary (hypergonadotropic) hypogonadism due to testicular failure.
• Secondary (hypogonadotropic) hypogonadism caused by insufficient gonadotropin-releasing hormone (GnRH) and/or gonadotropin (FSH, LH) secretion.

- Androgen insensitivity (end-organ resistance).

The most common conditions within these three categories are given in Table 5 (see also Chapter 3.3).

Table 5: Disorders associated with male hypogonadism*

Primary (Hypergonadotropic) hypogonadism (testicular failure)*
Anorchia
Maldescended testes
Klinefelter's syndrome
Y-chromosome microdeletions
Numerical and structural chromosomal anomalies
Trauma, testicular torsion, orchitis
Iatrogenic (surgery, medications, irradiation, or cytostatic drugs)
Exogenous factors (toxins, heat, or occupational hazards)
Systemic diseases (liver cirrhosis, or renal failure)
Testicular tumour
Varicocele
Idiopathic (e.g., late-onset hypogonadism)
Secondary (hypogonadotropic) hypogonadism (secondary testicular failure)
Congenital
Idiopathic hypogonadotropic hypogonadism
Normosmic
Hiposmic/anosmic (Kallmann syndrome)
Acquired (tumours in the following regions)
Diencephalon (craniopharyngioma or meningioma)
Hypothalamus or pituitary
Empty sella syndrome
Granulomatous illnesses
Fractures of the skull base
Ischaemic or haemorrhagic lesions in hypothalamic area
Hyperprolactinaemia
Drugs/anabolic steroids, radiotherapy
Target organ resistance to androgens
Testicular feminisation
Reifenstein syndrome

Modified from Nieschlag et al. [7].

5.5.2 Idiopathic hypogonadotropic hypogonadism: aetiology, diagnosis and therapeutic management

Idiopathic hypogonadotropic hypogonadism is characterised by low levels of gonadotropins and sex steroid in the absence of anatomical or functional abnormalities of the hypothalamic-pituitary-gonadal axis [134]. Idiopathic hypogonadotropic hypogonadism may be an isolated condition or may be associated with anosmia/hyposmia (Kallmann syndrome). Genetic factors causing a deficit of gonadotropins may act at the hypothalamic or pituitary level. Mutations in candidate genes (X-linked or autosomal) can be found in ~30% of congenital cases [134] and should be screened prior to assisted reproduction [135]. Acquired hypogonadotropic hypogonadism can be caused by some drugs, hormones, anabolic steroids, or tumours.

A suspected tumour requires imaging [computed tomography (CT) or magnetic resonance imaging (MRI)] of the sella region and a complete endocrine work-up. Normal androgen levels and subsequent development of secondary sex characteristics (in cases of onset of hypogonadism before puberty) and a eugonadal state can be achieved by androgen replacement alone. However, stimulation of sperm production requires treatment with human chorionic gonadotropin (hCG) combined with recombinant FSH or urinary FSH or human menopausal gonadotropins (HMGs) [136]. If hypogonadotropic hypogonadism is hypothalamic in origin, an alternative to hCG treatment is pulsatile GnRH [137]. In patients who have developed hypogonadism before puberty and have not been treated with gonadotropins or GnRH, 1-2 years of therapy may be needed to achieve sperm production.

*5.5.3	**Hypergonadotropic hypogonadism: aetiology, diagnosis and therapeutic management***
Many conditions in men with testicular failure are associated with Hypergonadotropic hypogonadism (Table 5, see also Chapter 5.2). Most conditions listed in Table 5 only affect the reproductive function of the testes so that only FSH level is elevated. However, it has been reported that men with infertility are at higher risk for developing impaired Leydig cell function [138], while men with Klinefelter's syndrome often show high LH values and develop hypoandrogenism with ageing [139]. A decrease in testosterone blood concentrations after extensive testicular biopsy in the context of TESE/ICSI has been observed, raising questions about the need for long-term endocrine follow-up of these patients [140]. Laboratory diagnosis of Hypergonadotropic hypogonadism is based on a high level of FSH, decreased serum testosterone, and increased LH levels [135]. Testosterone levels should be evaluated in view of the serum concentration of sex hormone binding globulin (SHBG). Based on levels of total testosterone, albumin and SHBG, free and bioavailable testosterone can be calculated. Due to diurnal variation, blood samples for testosterone assessment should be taken before 10.00 am.

Generally, androgen replacement should not be given to men who are considering parenthood or in case of male infertility. Testosterone suppresses pituitary production of LH and FSH, therefore, replacement therapy should not be given for infertility . In obese men, low levels of testosterone may exist due to the conversion of testosterone in oestradiol by the enzyme aromatase [141]. Anti-oestrogens and aromatase inhibitors may help in these patients elevating FSH and LH and potentially increase sperm quality, next to weight reduction. Injectable, oral and transdermal testosterone preparations are available for clinical use [135]. See also EAU Guidelines on Male Hypogonadism [142].

*5.5.4	**Recommendations for hypogonadism***

Recommendations	GR
Provide testosterone replacement therapy for symptomatic patients with primary and secondary hypogonadism who are not considering parenthood.	A
In men with hypogonadotropic hypogonadism, induce spermatogenesis by an effective drug therapy (hCG/hMG/rFSH).	A*
Do not use testosterone replacement for the treatment of male infertility.	A*

*Upgraded following panel consensus.
FSH = follicle-stimulating hormone; LH = luteinising hormone.

## 5.6	Cryptorchidism
Cryptorchidism is the most common congenital abnormality of the male genitalia, at one year of age nearly 1% of all full-term male infants have cryptorchidism [143]. Approximately 30% of undescended testes are non-palpable and may be located within the abdominal cavity. This guideline only deals with the management in adults.

*5.6.1	**Aetiology and pathophysiology***
It has been postulated that cryptorchidism may be a part of the so-called testicular dysgenesis syndrome (TDS), which is a developmental disorder of the gonads caused by environmental and/or genetic influences early in pregnancy. Besides cryptorchidism, TDS includes hypospadias, reduced fertility, increased risk of malignancy, and Leydig cell dysfunction [144].

*5.6.1.1	Pathophysiological effects in maldescended testes*
5.6.1.1.1	Degeneration of germ cells
The degeneration of germ cells in maldescended testes is apparent after the first year of life and vary, depending on the position of the testis [145]. During the second year, the number of germ cells declines. Early treatment is therefore recommended (after the age of six months surgery should be performed within the subsequent year with age 18 months the latest) to conserve spermatogenesis and hormone production, as well as to decrease the risk for tumours [146]. Surgical treatment is the most effective. Medical treatment with GnRH may be beneficial but long-term follow-up data are awaited. It has been reported that hCG treatment may be harmful to future spermatogenesis therefore, the Nordic Consensus Statement on treatment of undescended testes does not recommend it on a routine basis [147].

5.6.1.1.2	Relationship with fertility
Semen parameters are often impaired in men with a history of cryptorchidism [148]. Early surgical treatment may have a positive effect on subsequent fertility [149]. In men with a history of unilateral cryptorchidism,

paternity is almost equal (89.7%) to that in men without cryptorchidism (93.7%). In men with bilateral cryptorchidism, oligozoospermia can be found in 31% and azoospermia in 42%. In cases of bilateral cryptorchidism, the rate of paternity is only 35-53%.

5.6.1.1.3 Germ cell tumours
As a component of the Testicular Dysgenesis Syndrome Cryptorchidism is a risk factor for testicular cancer and is associated with testicular microcalcification and intratubular germ cell neoplasia of unclassified type (ITGCNU); formerly carcinoma *in situ* (CIS) of the testes. In 5-10% of testicular cancers, there is a history of cryptorchidism [150]. The risk of a germ cell tumour (GCT) is 3.6-7.4 times higher than in the general population and 2-6% of men with a history of cryptorchidism will develop a testicular tumour [150]. Orchidopexy performed before the age of puberty has been reported to decrease the risk of testicular cancer [151].

5.6.2 Disease management
5.6.2.1 Hormonal treatment
Human chorionic gonadotropin or GnRH is not recommended for the treatment of cryptorchidism in adulthood.

5.6.2.2 Surgical treatment
In adolescence removal of intra-abdominal testis (with a normal contralateral testis) can be recommended, because of the theoretical risk of later malignancy [152]. In adulthood, palpable undescended testis should not be removed because it still produces testosterone. Furthermore, as indicated above, correction of bilateral cryptorchidism, even in adulthood, can lead to sperm production in previously azoospermic men [153]. Vascular damage is the most severe complication of orchidopexy and can cause testicular atrophy in 1-2% of cases. In men with non-palpable testes, the postoperative atrophy rate was 12% in those cases with long vascular pedicles that enabled scrotal positioning. Postoperative atrophy in staged orchidopexy has been reported in up to 40% of patients [154]. At the time of orchidopexy performed in adulthood testicular biopsy for detection of ITGCNU (formerly CIS) is recommended. At the time of orchiectomy in the treatment of germ cell tumours biopsy of the contralateral testis should be offered to patients at high risk for ITGCNU (i.e. history of cryptorchidism, < 12 ml. testicular volume, poor spermatogenesis [155]),

5.6.3 Summary of evidence recommendations for cryptorchidism

Summary of evidence	LE
Cryptorchidism is multifactorial in origin and can be caused by genetic factors and endocrine disruption early in pregnancy.	2a
Cryptorchidism is often associated with testicular dysgenesis and is a risk factor for infertility and germ cell tumours.	2b
Paternity in men with unilateral cryptorchidism is almost equal to that in men without cryptorchidism.	3
Bilateral cryptorchidism significantly reduces the likelihood of paternity.	3

Recommendations	GR
Do not use hormonal treatment of cryptorchidism in adults.	A
If undescended testes are corrected in adulthood, perform simultaneous testicular biopsy for detection of ITGCNU (formerly CIS).	B

ITGCNU = intratubular germ cell neoplasia of unclassified type.

5.7 Idiopathic male infertility
No demonstrable cause of infertility is found in at least 44% of infertile men [156].

5.7.1 Disease management
5.7.1.1 Empirical treatments
A wide variety of empirical drug treatments of idiopathic male infertility have been used. However, there is little scientific evidence for an empirical approach [157]. Clomiphen citrate and tamoxifen have been widely used in idiopathic OAT: a recent meta-analysis reported some improvement in sperm quality and spontaneous pregnancy rates [158]. Androgens, bromocriptine, alpha-blockers, systemic corticosteroids and magnesium supplementation are not effective in the treatment of OAT syndrome. Gonadotrophins (hCG/HMG/rFSH) might be beneficial in regards to pregnancy rates and live birth in idiopathic male factor subfertility [159]. Men taking oral antioxidants had an associated significant increase in sperm parameters [160] and in live birth rates in IVF patients in a Cochrane analysis [161]. Concerning natural conception the role of antioxidants needs further investigations [162].

5.7.2 Recommendation for idiopathic male infertility

Recommendation	GR
Use medical treatment of male infertility only for cases of hypogonadotropic hypogonadism.	A
No recommendation can be made for treatment with gonadotropins, anti-oestrogens and antioxidants even for a subset of patients.	B

5.8 Male contraception

Development of male contraceptive methods is important because up to 40% of women have an unmet need for family planning, with approximately 80 million women every year having unintended or unwanted pregnancies [163]. Three of the four methods of male contraception have been in use for hundreds of years (i.e., condoms, periodic abstinence, and withdrawal). The typical first-year failure rates of traditional male methods are high (withdrawal 19%, periodic abstinence 20%, and condoms 3-14%) compared to the failure rates of 0.1-3% for modern reversible female methods [164]. For men, male contraceptive methods must be acceptable, cheap, reversible, and effective. The method nearest to being generally available clinically is hormonal male contraception, which is based on the suppression of gonadotropins and testosterone substitution to maintain male sexual function and bone mineralisation, and to prevent muscle wasting [165]. Various contraceptive regimens have been developed and tested, including testosterone monotherapy, androgen/progestin combinations, testosterone with GnRH analogues, and selective androgen- and progestin-receptor modulators [166]. There are racial differences in the response to androgens alone. However, a combination of testosterone with progestin results in complete suppression of spermatogenesis in all races, and provides contraceptive efficacy equivalent to female hormonal methods [167].

5.8.1 Vasectomy

Vasectomy is an effective method of permanent male surgical sterilisation [168]. Extensive guidelines on vasectomy were published by the EAU in 2012 [3]. Before vasectomy, the couple should be fully informed about the benefits and risks, especially as an Australian telephone survey found that 9.2% of respondents regretted having a vasectomy [169].

5.8.1.1 Surgical techniques

Various techniques are available for vasectomy. The least invasive approach is no-scalpel vasectomy which is also associated with a low rate of complications [170, 171]. The most effective occlusion technique is cauterisation of the lumen of the vas deferens and fascial interposition [172-174]. Most techniques can be carried out safely under local anaesthesia in an outpatient clinic.

5.8.1.1.1 Complications

Vasectomy does not significantly alter spermatogenesis and Leydig cell function. The volume of ejaculate remains unchanged. Potential systemic effects of vasectomy, including atherosclerosis, have not been proven, and there is no evidence of a significant increase in any systemic disease after vasectomy. An increased rate of prostate cancer in men who underwent vasectomy has not been detected [168, 175]. Acute local complications associated with vasectomy include haematoma, wound infection, and epididymitis in up to 5% of cases [168]. The potential long-term complications (e.g., chronic testicular pain) [176] must be discussed with the patient before the procedure.

5.8.1.1.2 Vasectomy failure

If an effective occlusion technique is used, the risk of recanalisation after vasectomy should be < 1% [177]. However, patients should be informed preoperatively that, although rare, long-term recanalisation might occur [178]. No motile spermatozoa should be detected 3 months after vasectomy. Persistent motility is a sign of vasectomy failure, and the procedure will need to be repeated. A "special clearance" given by the urologist with non-motile spermatozoa < 100,000/mL is still under discussion [179].

5.8.2 Counselling

Counselling with regard to vasectomy must address the following aspects:
• Vasectomy should be considered irreversible.
• Vasectomy is associated with a low complication rate; however, because it is an elective operation, even small risks must be explained, because men (and their partners) might wish to consider these before giving consent.
• Vasectomy can fail, although the failure rate is low.
• Couples should be advised to continue with other effective contraception until clearance is confirmed.
• All available data indicate that vasectomy is not associated with any serious, long-term, side-effects [180].

- Vasectomy involving cauterisation and fascial interposition appears to be the most effective technique in the prevention of early recanalization [172, 177, 181].

5.8.3 Vasectomy reversal

A wide range of surgical success rates have been published for vasectomy reversal (up to 90%), depending on the time between vasectomy and re-fertilisation, type of vasectomy (e.g., open-ended or sealed), type of reversal (vasovasostomy or vasoepididymostomy), and whether reversal was unilateral or bilateral. Microsurgical techniques should be used [182].

5.8.3.1 Length of time since vasectomy

Vasovasostomy results have shown patency rates up to 90%. The longer the interval is from vasectomy to reversal, the lower is the pregnancy rate. In a study of 1,469 men who had undergone microsurgical vasectomy reversal, patency and pregnancy rates were 97% and 76%, respectively, for an interval up to 3 years after vasectomy; 88% and 53% for 3-8 years, 79% and 44% for 9-14 years, and 71% and 30% for > 15 years [183].

5.8.3.2 Tubulovasostomy

The chance of secondary epididymal obstruction after vasectomy increases with time. After an interval of 10 years, 25% of men appear to have epididymal blockage. If secondary epididymal obstruction occurs, tubulovasostomy is needed to reverse the vasectomy (see Chapter 3.4) [184].

5.8.3.3 Microsurgical vasectomy reversal vs. epididymal or testicular sperm retrieval and ICSI

According to the calculations of cost per delivery for vasectomy reversal vs. sperm retrieval/ICSI, under a wide variety of initial assumptions, it is clear that vasectomy reversal is associated with a considerably lower cost per delivery and higher delivery rates [76, 109, 185, 186]. Sperm retrieval and ICSI must yield an 81% pregnancy rate per cycle to achieve equal costs to vasectomy reversal.

5.8.4 Summary of evidence and recommendations for male contraception

Summary of evidence	LE
Vasectomy meets best the criteria for the male contribution to contraception, with regard to efficacy, safety and side effects.	1a
All available data indicate that vasectomy is not associated with any serious, long-term side-effects.	1b
Microsurgical vasectomy reversal is a low-risk and (cost-) effective method of restoring fertility.	1a
Methods of male contraception other than vasectomy are associated with high failure rates or are still experimental (e.g., hormonal approach).	3

Recommendations	GR
Vasectomy is the gold standard for the male contribution to permanent contraception.	A
Cauterisation and fascial interposition are the most effective techniques for the prevention of early recanalisation.	A
Inform patients seeking vasectomy about the surgical method, risk of failure, potential irreversibility, the need for post-procedure contraception until clearance, and the risk of complications.	A*
To achieve pregnancy, MESA/PESA/TESE - together with ICSI is a second-line option for men who decline a vasectomy reversal and those with failed vasectomy reversal surgery.	B

*Upgraded following panel consensus
MESA = microsurgical epididymal sperm aspiration; PESA = percutaneous epididymal sperm aspiration;
TESE = testicular sperm extraction; ICSI = intracytoplasmic sperm injection.

5.9 Male accessory gland infections and infertility

5.9.1 Introduction

Infections of the male urogenital tract are potentially curable causes of male infertility [12, 187, 188]. The WHO considers urethritis, prostatitis, orchitis and epididymitis to be male accessory gland infections (MAGIs) [12]. However, specific data are not available to confirm that these diseases have a negative influence on sperm quality and male fertility in general.

5.9.2 Diagnostic evaluation

5.9.2.1 Ejaculate analysis

Ejaculate analysis (see Chapter 4.2) clarifies whether the prostate is involved as part of a generalised MAGI

and provides information about sperm quality. In addition, leukocyte analysis allows differentiation between inflammatory and non-inflammatory chronic pelvic pain syndrome (CPPS) (NIH IIa vs. NIH 3B).

5.9.2.2 Microbiological findings

After exclusion of urethritis and bladder infection, $>10^6$ peroxidase-positive white blood cells (WBCs) per millilitre of ejaculate indicate an inflammatory process. In this case, a culture should be performed for common urinary tract pathogens. A concentration of $>10^3$ cfu/mL urinary tract pathogens in the ejaculate is indicative of significant bacteriospermia. The sampling time can influence the positive rate of microorganisms in semen and the frequency of isolation of different strains [189]. The ideal diagnostic test for Chlamydia trachomatis in semen has not yet been established [190]. In contrast to serological findings in women, antibody tests for C. trachomatis in seminal plasma are not indicative if no type-specific methods are used [190]. Ureaplasma urealyticum is pathogenic only in high concentrations ($>10^3$ cfu/mL ejaculate). No more than 10% of samples analysed for ureaplasma exceed this concentration [191]. Normal colonisation of the urethra hampers the clarification of mycoplasma-associated urogenital infections, using samples such as the ejaculate [192].

5.9.2.3 White blood cells

The clinical significance of an increased concentration of leukocytes in the ejaculate is controversial [193]. Infection is indicated only by an increased level of leukocytes. Although leukocytospermia is a sign of inflammation, it is not necessarily associated with bacterial or viral infections [194]. According to WHO classification, leukocytospermia is defined as $>10^6$ WBCs/mL. Only two studies have analysed alterations of WBCs in the ejaculate of patients with proven prostatitis [195, 196]. Both studies found more leukocytes in men with prostatitis compared to those without inflammation (CPPS, type NIH 3B).

5.9.2.4 Sperm quality

The deleterious effects of chronic prostatitis on sperm density, motility and morphology are under debate [188]. All investigations have given contradictory results, and have not confirmed that chronic prostatitis has a decisive role in altering conventional semen parameters [197-199].

5.9.2.5 Seminal plasma alterations

Seminal plasma elastase is a biochemical indicator of polymorphonuclear lymphocyte activity in the ejaculate [188, 200, 201], with a suggested cut-off level of approximately 600 ng/mL [186]. Various cytokines are involved in inflammation and can influence sperm function. Several studies have investigated the association between interleukin (IL) concentration, leukocytes, and sperm function [202-204], but no correlations have been found. The prostate is the main site of origin of IL-6 and IL-8 in the seminal plasma. Cytokines, especially IL-6, play an important role in the male accessory gland inflammatory process [205]. However, elevated cytokine levels do not depend on the number of leukocytes in expressed prostatic secretion (EPS) [206].

5.9.2.6 Glandular secretory dysfunction

Infections of the sex glands can impair their excretory function. Decreased quantities of citric acid, phosphatase, fructose, zinc, and α-glutamyl-transferase activity are indicators of disturbed prostatic secretory parameters [188]. Reduced fructose concentration indicates impaired vesicular function [191, 207].

5.9.2.7 Reactive oxygen species

Reactive oxygen species might be increased in chronic urogenital infections associated with increased leukocyte numbers [208]. However, their biological significance in prostatitis remains unclear [188].

5.9.2.8 Disease management

Treatment of chronic prostatitis is usually targeted at relieving symptoms [209, 210]. The aims of therapy for altered semen composition in male adnexitis are:
*   reduction or eradication of microorganisms in prostatic secretions and semen;
*   normalisation of inflammatory (e.g., leukocytes) and secretory parameters;
*   improvement of sperm parameters to counteract fertility impairment [211].

Only antibiotic therapy of chronic bacterial prostatitis (NIH II) has provided symptomatic relief, eradication of microorganisms, and a decrease in cellular and humoral inflammatory parameters in urogenital secretions. Although antibiotics might improve sperm quality [211], there is no evidence that treatment of chronic prostatitis increases the probability of conception [188, 212].

5.9.3 Epididymitis

Inflammation of the epididymis causes unilateral pain and swelling, usually with acute onset. Among sexually

active men < 35 years of age, epididymitis is most often caused by *C. trachomatis* or *Neisseria gonorrhoea* [213, 214]. Sexually transmitted epididymitis is usually accompanied by urethritis. Non-sexually transmitted epididymitis is associated with urinary tract infection and occurs more often in men aged > 35 years [215].

5.9.3.1   Diagnostic evaluation
5.9.3.1.1   Ejaculate analysis
Ejaculate analysis according to WHO criteria, might indicate persistent inflammatory activity. Transiently decreased sperm counts and forward motility are observed [213, 216, 217]. Semen culture might help to identify pathogenic microorganisms. Development of stenosis in the epididymal duct, reduction of sperm count, and azoospermia are more important in the follow-up of bilateral epididymitis (see Chapter 5.3).

5.9.3.1.2   Disease management
Antibiotic therapy is indicated before culture results are available.

Treatment of epididymitis results in:
• microbiological cure of infection;
• improvement of clinical signs and symptoms;
• prevention of potential testicular damage;
• prevention of transmission;
• decrease of potential complications (e.g., infertility or chronic pain).

Patients with epididymitis known or suspected to be caused by *N. gonorrhoeae* or *C. trachomatis* must be told to refer their sexual partners for evaluation and treatment [218].

5.9.4   Summary of evidence and recommendation for male accessory gland infections

Summary of evidence	LE
Urethritis and prostatitis are not clearly associated with male infertility.	3
Antibiotic treatment often only eradicates microorganisms; it has no positive effect on inflammatory alterations and cannot reverse functional deficits and anatomical dysfunction.	2a
Although antibiotic treatment for MAGI might provide improvement in sperm quality, it does not necessarily enhance the probability of conception.	2a

Recommendation	GR
Instruct patients with epididymitis that is known or suspected to be caused by *N. gonorrhoeae* or *C. trachomatis* to refer their sexual partners for evaluation and treatment.	B

5.10   Germ cell malignancy and testicular microcalcification
5.10.1   Germ cell malignancy and male infertility
Testicular germ cell tumour (TGCT) is the most common malignancy in Caucasian men aged 15-40 years, and affects approximately 1% of subfertile men. The lifetime risk of TGCT varies between ethnic groups and countries. The highest annual incidence of TGCT occurs in Caucasians, and varies from 10/100,000 (e.g., in Denmark and Norway) to 2/100,000 (e.g., in Finland and the Baltic countries). Generally, seminomas and non-seminomas are preceded by CIS, and untreated ITGCNU will eventually progress to invasive cancer [219, 220]. The most convincing evidence for a general decline in male reproductive health is the increase in testicular cancer seen in western countries [221, 222]. In almost all countries with reliable cancer registers, the incidence of testicular cancer has increased [62, 223]. Cryptorchidism and hypospadias are associated with an increased risk of testicular cancer; men with cryptorchidism and/or hypospadias are over-represented among patients with testicular cancer. Men with dysgenic testes have an increased risk of developing testicular cancer in adulthood. These cancers arise from premalignant gonocytes or CIS cells [224]. Testicular microlithiasis (TM), seen on ultrasound, can be associated with GCT and CIS of the testes.

5.10.2   Testicular germ cell cancer and reproductive function
Men with TGCT have decreased semen quality, even before cancer is diagnosed [225]. Orchidectomy implies a risk of azoospermia in these men, with sperm found in the ejaculate before the tumour-bearing testis has been removed. Semen cryopreservation before orchidectomy should therefore be considered (see Chapter 5.12). Treatment of TGCT can result in additional impairment of semen quality [226]. In addition to spermatogenic failure, patients with TGCT have Leydig cell dysfunction, even in the contralateral testis [227]. The risk of

hypogonadism may therefore be increased in men treated for TGCT. The measurement of pre-treatment levels of testosterone, SHBG, LH and oestradiol might help to anticipate post-treatment hypogonadism. Men who have had TGCT and have low normal androgen levels should receive long-term follow-up because they are at risk of developing hypogonadism as a result of an age-related decrease in testosterone production [228]. The risk of hypogonadism is most pronounced in TGCT patients treated with ≥ 3 cycles of chemotherapy or irradiation of retroperitoneal lymph nodes. However, this risk is greatest at 6-12 months post-treatment. This suggests there may be some improvement in Leydig cell function, and why it is reasonable to expect initiation of androgen replacement, until the patient shows continuous signs of testosterone deficiency, even at 2 years follow-up [219]. The risk of low libido and erectile dysfunction is also increased in TGCT patients [229]. In case of azoospermia, testicular sperm may be recovered to safeguard patient's fertility (Onco-TESE) [230].

5.10.3 *Testicular microlithiasis*
Microcalcification inside the testicular parenchyma can be found in 0.6-9% of men referred for testicular ultrasound [231-233]. Although the true incidence of microcalcification in the general population is unknown, it is probably rare. However, ultrasound findings of TM are common in men with TGCT, cryptorchidism, testicular dysgenesis, infertility, testicular torsion and atrophy, Klinefelter's syndrome, hypogonadism, male pseudoherma-phroditism, varicocele, epididymal cysts, pulmonary microlithiasis, and non-Hodgkin's lymphoma. The incidence reported seems to be higher with high-frequency ultrasound machines [234]. The relationship between TM and infertility is unclear, but probably relates to dysgenesis of the testes, with degenerate cells being sloughed inside an obstructed seminiferous tubule and failure of the Sertoli cells to phagocytose the debris. Subsequently, calcification occurs. Testicular microlithiasis is found in testes at risk of malignant development. The reported incidence of TM in men with TGCT is 6-46% [235-237]. TM should therefore be considered premalignant. Testicular biopsies from men with TM have found a higher prevalence of CIS, especially in those with bilateral microlithiasis [238]. However, TM is found most often in men with a benign testicular condition and the microcalcification itself is not malignant. Further investigation of the association between TM and CIS will require testicular biopsies in large series of men without signs of TGCT. However, available data indicate that men in whom TM is found by ultrasound, and who have an increased risk of TGCT, should be offered testicular biopsy for detection of CIS. The list of high-risk patients includes men with infertility and bilateral TM, atrophic testes, undescended testes, a history of TGCT, and contralateral TM [222].

5.10.4 *Recommendations for germ cell malignancy and testicular microcalcification*

Recommendations	GR
As for all men, encourage patients with TM and without special risk factors (see below) to perform self-examination because this might result in early detection of TGCT.	B
Do not perform testicular biopsy, follow-up scrotal ultrasound, routine use of biochemical tumour markers, or abdominal or pelvic CT, in men with isolated TM without associated risk factors (e.g. infertility, cryptorchidism, testicular cancer, and atrophic testis).	B
Perform testicular biopsy for men with TM, who belong to one of the following high-risk groups: infertility and bilateral TM, atrophic testes, undescended testes, a history of TGCT.	B
If there are suspicious findings on physical examination or ultrasound in patients with TM and associated lesions, perform surgical exploration with testicular biopsy or orchidectomy.	B
Follow men with TGCT because they are at increased risk of developing hypogonadism and sexual dysfunction.	B

TM = testicular microlithiasis; TGCT = testicular germ cell tumour; CT = computed tomography.

5.11 Disorders of ejaculation
Disorders of ejaculation are uncommon, but important causes of male infertility.

5.11.1 *Classification and aetiology*
5.11.1.1 *Anejaculation*
Anejaculation involves complete absence of antegrade or retrograde ejaculation. It is caused by failure of semen emission from the seminal vesicles, prostate and ejaculatory ducts into the urethra [239]. True anejaculation is usually associated with a normal orgasmic sensation. True anejaculation is always associated with central or peripheral nervous system dysfunction or with drugs [240] (Table 6).

5.11.1.2 *Anorgasmia*
Anorgasmia is the inability to reach orgasm and can give rise to anejaculation. Anorgasmia is often a primary condition and its cause is usually psychological.

5.11.1.3 Delayed ejaculation

In delayed ejaculation, abnormal stimulation of the erect penis is needed to achieve orgasm with ejaculation [239]. Delayed ejaculation can be considered a mild form of anorgasmia. The causes of delayed ejaculation can be psychological, organic (e.g. incomplete spinal cord lesion [241] or iatrogenic penile nerve damage [242]), or pharmacological (e.g. selective serotonin re-uptake inhibitors (SSRIs), antihypertensives, or antipsychotics) [243].

5.11.1.4 Retrograde ejaculation

Retrograde ejaculation is the total, or sometimes partial, absence of antegrade ejaculation as a result of semen passing backwards through the bladder neck into the bladder. Patients experience a normal or decreased orgasmic sensation. The causes of retrograde ejaculation can be divided into neurogenic, pharmacological, urethral, or bladder neck incompetence (Table 6).

Table 6: Aetiology of anejaculation and retrograde ejaculation

Neurogenic	Pharmacological
Spinal cord injury	Antihypertensives
Cauda equina lesions	α1-adrenoceptor antagonists
Multiple sclerosis	Antipsychotics and antidepressants
Autonomic neuropathy (diabetes mellitus)	Alcohol
Retroperitoneal lymphadenectomy	
Sympathectomy or aortoiliac surgery	
Colorectal and anal surgery	
Parkinson´s disease	
Urethral	**Bladder neck incompetence**
Ectopic ureterocele	Congenital defects/dysfunction of hemitrigone
Urethral stricture	Bladder extrophy
Urethral valves or verumontaneum hyperplasia prostate)	Bladder neck resection (transurethral resection of the
Congenital dopamine β-hydroxylase deficiency	Prostatectomy

5.11.1.5 Asthenic ejaculation

Asthenic ejaculation is characterised by an altered propulsive phase, with a normal emission phase [243]. The orgasmic sensation is reduced and the typically rhythmical contractions associated with ejaculation are missing. Asthenic ejaculation does not usually affect semen quality.

5.11.1.6 Premature ejaculation

The International Society for Sexual Medicine (ISSM) has adopted the first evidence-based definition of lifelong premature ejaculation (PE): "Premature ejaculation is a male sexual dysfunction characterised by ejaculation which always or nearly always occurs prior to or within about one minute of vaginal penetration; and inability to delay ejaculation on all or nearly all vaginal penetrations; and negative personal consequences, such as distress, bother, frustration and/or the avoidance of sexual intimacy". Premature ejaculation may be strictly organic (e.g., prostatitis-related) or psychogenic, partner-related or non-selective, and can be associated with erectile dysfunction. It does not impair fertility, provided intravaginal ejaculation occurs.

5.11.2 Diagnostic evaluation

Diagnostic management includes the following recommended procedures.

5.11.2.1 Clinical history

The patient must be carefully checked for diabetes, neuropathy, trauma, urogenital infection, previous surgery, and medication. Particular attention must be paid to the characteristics of micturition and ejaculation (presence of nocturnal emission, ejaculatory ability in given circumstances, and primary or acquired disorder), as well as to psychosexual aspects.

5.11.2.2 Physical examination

Genital and rectal examinations are conducted, including evaluation of the prostate, bulbocavernosus reflex and anal sphincter tone.

5.11.2.3   Post-ejaculatory urinalysis
Post-ejaculatory urinalysis of centrifuged urine can be used to determine if there is total or partial retrograde ejaculation.

5.11.2.4   Microbiological examination
Initial, mid-stream urine, EPS, and/or urine after prostatic massage are cultured for evidence of prostatic infection. In cases of increased leukocytes in semen, semen culture or biochemical infection marker tests are also suggested [244].

5.11.2.5   Optional diagnostic work-up
This diagnostic work-up can include:
*   neurophysiological tests (bulbocavernosus evoked response and dorsal nerve somatosensory evoked potentials);
*   tests for autonomic neuropathy;
*   psychosexual evaluation;
*   videocystometry;
*   cystoscopy;
*   transrectal ultrasonography;
*   uroflowmetry;
*   vibratory stimulation of the penis.

*5.11.3   **Disease management***
Infertility caused by disorders of ejaculation is seldom treated on the basis of aetiology. Treatment usually involves retrieval of spermatozoa for use in assisted reproduction techniques (ARTs). The following aspects must be considered when selecting treatment:
*   Age of patient and his partner.
*   Psychological problems of the patient and his partner.
*   Couple's willingness and acceptance of different fertility procedures.
*   Associated pathology.
*   Psychosexual counselling.

5.11.3.1   Aetiological treatment
If possible, any pharmacological treatment that is interfering with ejaculation should be stopped. In painful ejaculation, tamsulosin can be administered during antidepressant treatment [245]. Treatment should be given for urogenital infections (i.e., in case of painful ejaculation) [244]. Dapoxetin is an SSRI that has been introduced for the therapy of PE [246], because it appears that PE is related to serotonin levels. Psychotherapy is usually not very effective.

5.11.3.2   Symptomatic treatment
5.11.3.2.1 Premature ejaculation
Premature ejaculation can be treated with the SSRI dapoxetine or topical anaesthetic agents to increase intravaginal ejaculation latency time, behavioural therapy, and/or psychotherapy.

5.11.3.2.2 Retrograde ejaculation
In the absence of spinal cord injury, anatomical anomalies of the urethra, or pharmacological agents, drug treatment must be used to induce antegrade ejaculation (Table 7). Alternatively, the patient can be encouraged to ejaculate when his bladder is full to increase bladder neck closure [247].

Table 7: Drug therapy for retrograde ejaculation

Drug	Dosage regimen	Ref.
Ephedrine sulphate	10-15 mg four times daily	[248]
Midodrine	5 mg three times daily	[249]
Brompheniramine maleate	8 mg twice daily	[250]
Imipramine	25-75 mg three times daily	[251]
Desipramine	50 mg every second day	[252]

Sperm collection from post-orgasmic urine for use in ART is recommended if:
*   drug treatment is ineffective or intolerable as a result of side-effects;

- the patient has a spinal cord injury;
- drug therapy inducing retrograde ejaculation cannot be interrupted.

If the biological sperm preparation is not of sufficient quality for intrauterine insemination, the couple must undergo in vitro reproductive procedures (e.g. ICSI). In the case of insufficient drug therapy, testicular (TESE or PESA) or epididymal (MESA) sperm retrieval techniques can be used for assisted reproduction.

5.11.3.2.3 Anejaculation
Drug treatment for anejaculation caused by lymphadenectomy and neuropathy, or psychosexual therapy for anorgasmia is not very effective. In all these cases, and in men who have a spinal cord injury, vibrostimulation (i.e., application of a vibrator to the penis) is first-line therapy. In anejaculation, vibrostimulation evokes the ejaculation reflex [253], which requires an intact lumbosacral spinal cord segment. If the quality of semen is poor, or ejaculation is retrograde, the couple may enter an IVF programme. If vibrostimulation has failed, electro-ejaculation can be the therapy of choice [254]. When electro-ejaculation fails or cannot be carried out, sperm can be retrieved from the seminal ducts by aspiration from the vas deferens [255] (see Chapter 3D) or seminal tract washout [256]. TESE can then be used [244, 257]. Anejaculation following either surgery for testicular cancer or total mesorectal excision can be prevented using monolateral lymphadenectomy or autonomic nerve preservation [257], respectively.

5.11.4   *Summary of evidence and recommendations for disorders of ejaculation*

Summary of evidence	LE
Ejaculation disorders can be treated using a wide range of drugs and physical stimulation (eg vibratory stimulation), with a high level of efficacy.	3
Pharmacotherapy includes either dapoxetine on demand (a short-acting SSRI that is the only approved pharmacological treatment for premature ejaculation) or other off-label antidepressants, i.e. daily SSRIs and clomipramine, that are not amenable to on-demand dosing. Alternatively use topical anesthetics (LE: 1b) or tramadol (LE: 2a).	1a
In men with spinal cord injury, vibrostimulation and/or electro-ejaculation are effective methods of sperm retrieval.	2

Recommendations	GR
Offer aetiological treatments for ejaculatory disorders before performing sperm collection and ART.	B
To treat disorders of ejaculation, offer pharmacological treatment of either dapoxetine on demand (a short-acting SSRI that is the only approved pharmacological treatment for premature ejaculation) or other off-label antidepressants, i.e. daily SSRIs and clomipramine, that are not amenable to on-demand dosing.	A
Alternatively offer topical anaesthetics or tramadol.	A

ART = assisted reproduction technique; SSRIs = selective serotonin reuptake inhibitors.

5.12   Semen cryopreservation
Cryopreservation is the storage of biological material at sub-zero temperatures [e.g., - 80 or -196°C (the boiling point of liquid nitrogen)], at which biochemical processes of cell metabolism are slowed or interrupted. At -196°C, the biochemical reactions that lead to cell death are stopped.

5.12.1   *Indications for storage*
Storage of sperm is available in many clinics for the following indications:
- Before potentially sterilising chemotherapy or radiotherapy for cancer or for non-malignant diseases [258].
- Before surgery that might interfere with fertility (e.g. bladder neck surgery in a younger man or removal of a testicle in a man with testicular malignancy, or before vasectomy or transgender surgery).
- For men with progressive decrease in semen quality as a result of diseases that have an associated risk of subsequent azoospermia (i.e., pituitary macroadenoma, craniopharyngioma, empty sella syndrome, chronic nephropathy, uncontrolled diabetes mellitus, and multiple sclerosis).
- For men with paraplegia when sperm have been obtained by electro-ejaculation or obtained by penile vibratory stimulation.
- For men with psychogenic anejaculation, after sperm have been obtained either by electro-ejaculation or a sperm retrieval procedure.
- After gonadotropin treatment has induced spermatogenesis in men with hypogonadotropic hypogonadism.

- For men with NOA, the chance of finding sperm using micro-TESE is ~50%.

Cryopreservation can be used for sperm collected through TESE, avoiding repeated sperm retrieval procedures and unnecessary hyperstimulation of the female partner:
- In any situation in which sperm have been obtained by a sperm retrieval procedure (e.g., after failed vasectomy reversal, or in some cases of epididymal obstruction not amenable to surgery).
- For storage of donor sperm, because cryopreservation reduces the risk of transmission of infection from sperm donors. According to the European directives 2004/23 EC and 2006/17 EC fresh sperm are no longer to be used for non-partner donations.

5.12.2 Precautions and techniques

5.12.2.1 Freezing and thawing process

The cryopreservation techniques currently used are not yet optimal because damage occurs to cells during cryopreservation and prolonged storage. Most damage occurs during freezing and thawing. Major causes of damage during freezing are ice crystal formation and cell dehydration, which disrupt the cell wall and intracellular organelles. Sperm morphology, motility and vitality decrease significantly after thawing, and cryopreservation increases the damage done to sperm DNA [259-262]. Further damage can be caused by contamination of samples with microorganisms and high levels of superoxide radicals [263, 264]. To reduce ice crystal formation, a cryopreservation solution is added before freezing. Various cryopreservation solutions are available commercially, most of which contain varying proportions of glycerol and albumin. After freezing, the samples are immersed in liquid nitrogen.

Several techniques have been developed to try to reduce damage caused by freezing and thawing, including:
- One-step freezing method [265, 266]: sample is held in the vapour phase for 10 min before being plunged into liquid nitrogen.
- Slow or multi-step method [267]: sample is gradually cooled in the vapour phase for approximately 40 min. A programmable automatic freezing machine, which is preset to cool at a rate of 1-10°C/min is used.

The method available depends on the resources of the laboratory. Whichever freezing technique is used, it should be tested using donor sperm and post-thaw examination, and should regularly undergo a quality-control programme. The likelihood of sperm survival decreases with repeated freezing and thawing. The maximum viable storage time for human sperm is not known.

5.12.2.2 Cryopreservation of small numbers of sperm

Standard cryopreservation in straws is an efficient way of storing large numbers of sperm (e.g., for a donor insemination programme). However, in micro-TESE, few sperm might be obtained, and the choice is either to freeze testicular tissue and find sperm after thawing the tissue, or to freeze small numbers of sperm. If sperm are frozen in straws, it can be difficult to find any sperm after thawing. Instead, the sperm should be frozen in a pellet [268] or in a container [269].

5.12.2.3 Testing for infections and preventing cross-contamination

Sperm storage in straws is used extensively. Large numbers of straws are stored in canisters, with the straws being bathed in a pool of liquid nitrogen. Microbial contamination of the pool of liquid nitrogen results in contamination of the outside of all the straws [270]. The most widely used safeguard is to use so-called high security closed straws. According to the European directives 2004/23 and 2006/17, samples should be tested for hepatitis B and C and human immunodeficiency virus (HIV). In case of non-partner donation, samples are also tested for C. Trachomatis (by Nucleic Acid Testing [NAT]) and syphilis, as well as genetics, that is, karyotype and most prevalent genetic disorders in the population to which the non-partner donor belongs. Until the test results are known, samples must be stored in an individual quarantine vessel (separate storage). If open straws are used (e.g., for vitrification purposes) some laboratories use the additional safeguard of double-wrapping the straws before freezing, although this is more costly. Some centres carry out cytomegalovirus testing and store negative and positive samples separately. Considerable ethical issues surround the storage of samples before cancer chemotherapy in men who are hepatitis-virus- or HIV-positive. Few clinics have separate storage facilities for HIV-positive samples. However, the success of antiretroviral treatment is increasing the number of HIV-positive men who may wish to store sperm. There is also concern about HIV transmission to children conceived using HIV-positive sperm, because sperm-washing techniques fail in ~5% of cases.

5.12.2.4 Fail-safe precautions to prevent loss of stored materials

Any laboratory that undertakes long-term storage of human biological materials should have procedures that

guard against accidental loss of material caused by storage vessel failure. This is particularly important for sperm stored before potentially sterilising cancer chemotherapy, because these patients may not be able to obtain further sperm.

5.12.2.5   Orphan samples
In malignancy and some other situations, several years might pass before stored samples are required. Inevitably, during this time, the owners of some samples might disappear or die, leaving behind orphan samples for which the owner is no longer contactable. The duty of the laboratory and the legal ownership of these samples can create considerable problems.

*5.12.3   **Biological aspects***
Cryopreservation induces deterioration of semen quality. After the sample has been thawed, motility [271] and morphology [272, 273] are worsened, including mitochondrial acrosomal and sperm tail damage [262]. Sperm freezing decreases motility by 31% and mitochondrial activity by 36%, and causes morphological disruption in 37% of sperm [266]. Motility is correlated best with IVF capacity of the thawed sample. Further improvement can be achieved by selecting the subpopulation of sperm with the best motility and DNA integrity and freezing these sperm in seminal plasma [268].

*5.12.4   **Summary of evidence and recommendations for semen cryopreservation***

Summary of evidence	LE
The purpose of sperm cryopreservation is to enable future assisted reproduction techniques procedures.	1b
Cryopreservation techniques are not optimal, and future efforts are needed to improve the outcome of sperm banking.	3

Recommendations	GR
Offer cryopreservation of semen to all men who are candidates for chemotherapy, radiation or surgical interventions that might interfere with spermatogenesis or cause ejaculatory disorders.	A
Offer simultaneous sperm cryopreservation if testicular biopsies will be performed for fertility diagnosis.	A
If cryopreservation is not available locally, inform patients about the possibility of visiting, or transferring to a cryopreservation unit before therapy starts.	C
Take precautions to prevent transmission of viral, sexually transmitted or any other infection by cryostored materials from donor to recipient, and to prevent contamination of stored samples. These precautions include testing of the patient and the use of rapid testing and quarantine of samples until test results are known. Do not store samples from men who are positive for hepatitis virus or HIV. It must not be stored in the same container as samples from men who have been tested and are free from infection.	C

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7.   CONFLICT OF INTEREST

All members of the EAU Male Infertility Guidelines Panel have provided disclosure statements of all relationships that they have that might be perceived as a potential source of a conflict of interest. This information is publicly accessible through the European Association of Urology website. This document was developed with the financial support of the European Association of Urology. No external sources of funding and support have been involved. The EAU is a non-profit organisation and funding is limited to administrative assistance and travel and meeting expenses. No honoraria or other reimbursements have been provided.