

# Guidelines on **Male Infertility**

A. Jungwirth, T. Diemer, G.R. Dohle, A. Giwercman,  
Z. Kopa, C. Krausz, H. Tournaye

# TABLE OF CONTENTS

# PAGE

1.	METHODOLOGY	6
1.1	Introduction	6
1.2	Data identification	6
1.3	Level of evidence and grade of recommendation	6
1.4	Publication history	7
1.5	Definition	7
1.6	Epidemiology and aetiology	7
1.7	Prognostic factors	8
1.8	Recommendations on epidemiology and aetiology	8
1.9	References	8
2.	INVESTIGATIONS	9
2.1	Semen analysis	9
2.1.1	Frequency of semen analysis	9
2.2	Recommendations for investigations in male infertility	10
2.3	References	10
3.	TESTICULAR DEFICIENCY (SPERMATOGENIC FAILURE)	10
3.1	Definition	10
3.2	Aetiology	10
3.3	Medical history and physical examination	11
3.4	Investigations	11
3.4.1	Semen analysis	11
3.4.2	Hormonal determinations	11
3.4.3	Testicular biopsy	11
3.5	Conclusions and recommendations for testicular deficiency	12
3.6	References	12
4.	GENETIC DISORDERS IN INFERTILITY	14
4.1	Introduction	14
4.2	Chromosomal abnormalities	14
4.2.1	Sperm chromosomal abnormalities	14
4.2.2	Sex chromosome abnormalities	14
4.2.3	Autosomal abnormalities	15
4.3	Genetic defects	15
4.3.1	X-linked genetic disorders and male fertility	15
4.3.2	Kallmann syndrome	15
4.3.3	Mild androgen insensitivity syndrome	15
4.3.4	Other X-disorders	15
4.4	Y chromosome and male infertility	15
4.4.1	Introduction	15
4.4.2	Clinical implications of Y microdeletions	16
4.4.2.1	Testing for Y microdeletions	17
4.4.2.2	Y chromosome: 'gr/gr' deletion	17
4.4.2.3	Conclusions	17
4.4.3	Autosomal defects with severe phenotypic abnormalities and infertility	17
4.5	Cystic fibrosis mutations and male infertility	18
4.6	Unilateral or bilateral absence/abnormality of the vas and renal anomalies	18
4.7	Unknown genetic disorders	19
4.8	DNA fragmentation in spermatozoa	19
4.9	Genetic counselling and ICSI	19
4.10	Conclusions and recommendations for genetic disorders in male infertility	19
4.11	References	20
5.	OBSTRUCTIVE AZOOSPERMIA	24
5.1	Definition	24
5.2	Classification	25
5.2.1	Intratesticular obstruction	25

5.2.2	Epididymal obstruction	25
5.2.3	Vas deferens obstruction	25
5.2.4	Ejaculatory duct obstruction	25
5.2.5	Functional obstruction of the distal seminal ducts	25
5.3	Diagnosis	25
5.3.1	Clinical history	25
5.3.2	Clinical examination	26
5.3.3	Semen analysis	26
5.3.4	Hormone levels	26
5.3.5	Ultrasonography	26
5.3.6	Testicular biopsy	26
5.4	Treatment	27
5.4.1	Intratesticular obstruction	27
5.4.2	Epididymal obstruction	27
5.4.3	Proximal vas obstruction	27
5.4.4	Distal vas deferens obstruction	27
5.4.5	Ejaculatory duct obstruction	27
5.5	Conclusions and recommendation for obstructive azoospermia	28
5.6	References	28
6.	VARICOCELE	30
6.1	Introduction	30
6.2	Classification	30
6.3	Diagnosis	30
6.4	Basic considerations	30
6.4.1	Varicocele and fertility	30
6.4.2	Varicocelectomy	31
6.5	Treatment	31
6.6	Conclusions and recommendations for varicocele	32
6.7	References	32
7.	HYPOGONADISM	33
7.1	Introduction	33
7.2	Hypogonadotrophic hypogonadism	35
7.3	Hypergonadotrophic hypogonadism	35
7.4	Conclusion and recommendation for hypogonadism	36
7.5	References	36
8.	CRYPTORCHIDISM	37
8.1	Introduction	37
8.2	Incidence of cryptorchidism	37
8.3	Testicular descent and maldescent	37
8.4	Hormonal control of testicular descent	37
8.5	Pathophysiological effects in maldescended testes	37
8.5.1	Degeneration of germ cells	37
8.5.2	Relationship with fertility	37
8.5.3	Germ cell tumours	38
8.6	Treatment of undescended testes	38
8.6.1	Hormonal treatment	38
8.6.2	Surgical treatment	38
8.7	Conclusions and recommendations for cryptorchidism	38
8.8	References	39
9.	IDIOPATHIC MALE INFERTILITY	40
9.1	Introduction	40
9.2	Empirical treatments	40
9.3	References	40

10.	MALE CONTRACEPTION	41
10.1	Introduction	41
10.2	Vasectomy	41
10.2.1	Surgical techniques	41
10.2.2	Complications	41
10.2.3	Vasectomy failure	41
10.2.4	Counselling	42
10.3	Vasectomy reversal	42
10.3.1	Length of time since vasectomy	42
10.3.2	Epididymo-vasostomy	42
10.3.3	Microsurgical vasectomy reversal versus epididymal or testicular sperm retrieval and ICSI	42
10.4	Conclusions and recommendations for male contraception	42
10.5	References	43
11.	MALE ACCESSORY GLAND INFECTIONS	44
11.1	Introduction	44
11.2	Urethritis	44
11.2.1	Diagnosis and treatment	44
11.3	Prostatitis	44
11.3.1	Microbiology	45
11.3.2	Diagnosis	45
11.3.3	Ejaculate analysis	45
11.3.4	Microbiological findings	45
11.3.5	White blood cells	46
11.3.6	Sperm quality	46
11.3.7	Seminal plasma alterations	46
11.3.8	Glandular secretory dysfunction	46
11.3.9	Sperm antibodies	46
11.3.10	Reactive oxygen species	46
11.3.11	Therapy	46
11.4	Orchitis and epididymo-orchitis	46
11.4.1	Introduction	46
11.4.2	Diagnosis	47
11.4.3	Ejaculate analysis	47
11.4.4	Therapy	47
11.5	Epididymitis	47
11.5.1	Introduction	47
11.5.2	Diagnosis	47
11.5.3	Ejaculate analysis	47
11.5.4	Treatment	48
11.6	Conclusions and recommendations for male accessory gland infections	48
11.7	References	48
12.	GERM CELL MALIGNANCY AND TESTICULAR MICROCALCIFICATION	52
12.1	Germ cell malignancy and male infertility	52
12.2	Testicular germ cell cancer and reproductive function	52
12.3	Testicular microlithiasis	52
12.4	Recommendations for germ cell malignancy and testicular microcalcification	53
12.5	References	53
13.	DISORDERS OF EJACULATION	54
13.1	Definition	54
13.2	Classification and aetiology	54
13.2.1	Anejaculation	54
13.2.2	Anorgasmia	55
13.2.3	Delayed ejaculation	55
13.2.4	Retrograde ejaculation	55
13.2.5	Asthenic ejaculation	55
13.2.6	Premature ejaculation	56

	13.2.7	Painful ejaculation	56
13.3		Diagnosis	56
	13.3.1	Clinical history	56
	13.3.2	Physical examination	56
	13.3.3	Post-ejaculatory urinalysis	56
	13.3.4	Microbiological examination	56
13.4		Treatment	56
13.5		Aetiological treatment	57
13.6		Symptomatic treatment	57
	13.6.1	Premature ejaculation (PE)	57
	13.6.2	Retrograde ejaculation	57
	13.6.3	Anejaculation	57
13.7		Conclusion and recommendations for disorders of ejaculation	58
13.8		References	58
14.		SEMEN CRYOPRESERVATION	59
	14.1	Definition	59
	14.2	Introduction	59
	14.3	Indications for storage	59
	14.4	Precautions and techniques	60
	14.4.1	Freezing and thawing process	60
	14.4.2	Cryopreservation of very small numbers of sperm	60
	14.4.3	Testing for infections and preventing cross-contamination	60
	14.4.4	Fail-safe precautions to prevent loss of stored materials	61
	14.4.5	Orphan samples	61
	14.5	Biological aspects	61
	14.6	Conclusions and recommendations for semen cryopreservation	61
	14.7	References	62
15.		ABBREVIATIONS USED IN THE TEXT	64

# 1. METHODOLOGY

## 1.1 Introduction

The European Association of Urology (EAU) Guideline Panel on Male Infertility has prepared these guidelines to assist urologists and healthcare professionals from related specialities in the treatment of male infertility.

Urologists are usually the specialists who are initially responsible for assessing the male partner when male infertility is suspected. However, infertility can be a multifactorial condition requiring multidisciplinary involvement. The Male Infertility Guidelines Panel consists of urologists and endocrinologists with special training in andrology and experience in the diagnosis and treatment of male infertility.

## 1.2 Data identification

The recommendations provided in the current guidelines are based on a systemic literature search performed by the panel members. MedLine, Embase, and Cochrane databases were searched to identify original and review articles. The controlled vocabulary of the Medical Subject Headings (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'.

All articles published between January 2010 (previous update) and November 2011 were considered for review. The expert panel reviewed these records and selected articles with the highest evidence.

## 1.3 Level of evidence and grade of recommendation

References in the text have been assessed according to their level of scientific evidence (Table 1), and guideline recommendations have been graded (Table 2) according to the Oxford Centre for Evidence-based Medicine Levels of Evidence (1). Grading aims to provide transparency between the underlying evidence and the recommendation given.

**Table 1: Level of evidence (LE)\***

Level	Type of evidence
1a	Evidence obtained from meta-analysis of randomised trials
1b	Evidence obtained from at least one randomised trial
2a	Evidence obtained from one well-designed controlled study without randomisation
2b	Evidence obtained from at least one other type of well-designed quasi-experimental study
3	Evidence obtained from well-designed non-experimental studies, such as comparative studies, correlation studies and case reports
4	Evidence obtained from expert committee reports or opinions or clinical experience of respected authorities

\*Modified from Sackett *et al.* (1).

When recommendations are graded, the link between the level of evidence and grade of recommendation is not directly linear. Availability of RCTs may not translate into a grade A recommendation when there are methodological limitations or disparity in published results.

Absence of high-level evidence does not necessarily preclude a grade A recommendation, if there is overwhelming clinical experience and consensus. There may be exceptions where corroborating studies cannot be performed, perhaps for ethical or other reasons, and unequivocal recommendations are considered helpful. Whenever this occurs, it is indicated in the text as "upgraded based on panel consensus". The quality of the underlying scientific evidence must be balanced against benefits and burdens, values and preferences and cost when a grade is assigned (2-4).

The EAU Guidelines Office does not perform cost assessments, nor can it address local/national preferences systematically. The expert panels include this information whenever it is available.

**Table 2: Grade of recommendation (GR)\***

Grade	Nature of recommendations
A	Based on clinical studies of good quality and consistency addressing the specific recommendations and including at least one randomised trial
B	Based on well-conducted clinical studies, but without randomised clinical trials
C	Made despite the absence of directly applicable clinical studies of good quality

\*Modified from Sackett et al. (1).

#### 1.4 Publication history

The EAU Male infertility Guidelines were first published in 2001, followed by full text updates in 2004, 2007 and 2010. For this 2012 publication all sections have been revised and limited changes were implemented. Starting in 2012, the expert panel instigate start a new updating cycle. A quick reference guide presenting the main findings of the Male Infertility Guidelines is also available as well as a number of scientific publications in the EAU journal European Urology. All texts can be viewed and downloaded for personal use at the society website: <http://www.uroweb.org/guidelines/online-guidelines/>.

This document was peer-reviewed prior to publication.

#### 1.5 Definition

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

#### 1.6 Epidemiology and aetiology

About 15% of couples do not achieve pregnancy within 1 year and seek medical treatment for infertility. Eventually, 5% remain unwillingly childless. 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 becomes manifest if both partners have reduced fertility (5). Male fertility can be reduced as a result of (5):

- congenital or acquired urogenital abnormalities;
- 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 fertility problems and have normal findings on physical examination and endocrine laboratory testing. However, semen analysis reveals a decreased number of spermatozoa (oligozoospermia), decreased sperm motility (asthenozoospermia), and many abnormal forms of sperm (teratozoospermia). These sperm abnormalities usually occur together and are called oligo-astheno-teratozoospermia (OAT) syndrome. Table 3 summarises the main male infertility-associated factors. Idiopathic male infertility may be explained by several factors, including endocrine disruption as a result of environmental pollution, reactive oxygen species, or genetic abnormalities.

**Table 3: Male infertility associated factors and percentage of distribution in 10,469 patients**

Male infertility associated factor	Distribution %
Idiopathic male infertility	31
Maldescended testes	7.8
Urogenital infection	8.0
Disturbances of semen deposition and sexual factors	5.9
General and systemic disease	3.1
Varicocele	15.6
(Endocrine) Hypogonadism	8.9
Immunological factors	4.5
Obstructions	1.7
Other abnormalities	5.5

### 1.7 Prognostic factors

Prognostic factors for male infertility are:

- duration of infertility;
- primary or secondary infertility;
- results of semen analysis;
- age and fertility status of female partner.

The cumulative pregnancy rate in infertile couples with 2 years of follow-up and oligozoospermia as the primary cause of infertility is 27% (7). Female age is the most important single variable influencing outcome in assisted reproduction (8). 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.

### 1.8 Recommendations on epidemiology and aetiology

Recommendations	GR
To categorise infertility, both partners should be investigated simultaneously.	C
In the diagnosis and management of male subfertility, the fertility status of the female partner must also be considered, as this might determine the final outcome (8).	B
The urologist/andrologist should examine any male with fertility problems for urogenital abnormalities. This applies to all males diagnosed with reduced sperm quality. A diagnosis is mandatory to start appropriate therapy (drugs, surgery, assisted reproduction) (5).	C

### 1.9 References

1. Oxford Centre for Evidence-based Medicine Levels of Evidence (May 2001). Produced by Bob Phillips, Chris Ball, Dave Sackett, Doug Badenoch, Sharon Straus, Brian Haynes, Martin Dawes since November 1998. Produced by Updated by Jeremy Howick March 2009. <http://www.cebm.net/index.aspx?o=1025> [Access date January 2012]
2. Atkins D, Best D, Briss PA, et al; GRADE Working Group. Grading quality of evidence and strength of recommendations. *BMJ* 2004 Jun 19;328(7454):1490. <http://www.ncbi.nlm.nih.gov/pubmed/15205295>
3. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008;336(7650):924-6. <http://www.ncbi.nlm.nih.gov/pubmed/18436948>
4. Guyatt GH, Oxman AD, Kunz R, et al; GRADE Working Group. Going from evidence to recommendations. *BMJ* 2008 May 10;336(7652):1049-51. <http://www.bmj.com/content/336/7652/1049.long>
5. World Health Organization. WHO Manual for the Standardised Investigation and Diagnosis of the Infertile Couple. Cambridge: Cambridge University Press, 2000.



6. Andrology. In: Nieschlag E, Behre HM (eds). *Male reproductive health and dysfunction*. 2nd edn. Berlin: Springer Verlag, 1997, Chapter 5, pp. 83-7.
7. Snick HK, Snick TS, Evers JL, et al. The spontaneous pregnancy prognosis in untreated subfertile couples: the Walcheren primary care study. *Hum Reprod* 1997 Jul;12(7):1582-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/9262301>
8. Rowe T. Fertility and a woman's age. *J Reprod Med* 2006 Mar;51(3):157-63.  
<http://www.ncbi.nlm.nih.gov/pubmed/16674009>

## 2. INVESTIGATIONS

### 2.1 Semen analysis

A medical history and physical examination are standard assessments in all men, including semen analysis. A comprehensive andrological examination is indicated if semen analysis shows abnormalities compared with reference values (Table 4). As important treatment decisions are based on the results of semen analysis, 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.) (1). It is the consensus that modern spermatology must follow these guidelines.

**Table 4: Lower reference limits (5th centiles and their 95% confidence intervals) for semen characteristics**

Parameter	Lower reference limit (range)
Semen volume (mL)	1.5 (1.4-1.7)
Total sperm number (10 <sup>6</sup> per ejaculate)	39 (33-46)
Sperm concentration (10 <sup>6</sup> per 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 <sup>6</sup> per mL)	< 1.0
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

*PR = progressive; NP = non-progressive; MAR = Mixed antiglobulin reaction.*

#### 2.1.1 Frequency of semen analysis

If the results of semen analysis are normal according to WHO criteria, one test should be 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% motile spermatozoa
- teratozoospermia: < 4% normal forms.

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

## 2.2 Recommendations for investigations in male infertility

Recommendations	GR
According to WHO criteria, andrological investigations are indicated if semen analysis is abnormal in at least two tests.	C
Assessment of andrological status must consider the suggestions made by WHO for the standardised investigation, diagnosis, and management of the infertile couple; this will result in implementation of evidence-based medicine in this interdisciplinary field of reproductive medicine (2).	C
Semen analysis must follow the guidelines of the <i>WHO Laboratory Manual for the Examination and Processing</i> (5th edn) (1).	B

## 2.3 References

1. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th edn. WHO, 2010.  
<http://www.who.int/reproductivehealth/publications/infertility/9789241547789/en/index.html>
2. World Health Organization. WHO Manual for the Standardised Investigation and Diagnosis of the Infertile Couple. Cambridge: Cambridge University Press, 2000.

# 3. TESTICULAR DEFICIENCY (SPERMATOGENIC FAILURE)

## 3.1 Definition

Testicular deficiency as a consequence of spermatogenic failure is caused by conditions other than hypothalamic-pituitary disease and obstructions of the male genital tract. It is the commonest form of reduced male fertility. Testicular deficiency may have different aetiologies and present clinically as severe OAT or non-obstructive azoospermia (NOA) (1).

## 3.2 Aetiology

The causes of testicular deficiency are summarised in Table 5.

**Table 5: Causes of testicular deficiency**

Factors	Causes
Congenital	Anorchia
	Testicular dysgenesis/cryptorchidism
	Genetic abnormalities (karyotype, Y chromosome deletions)
	Germ cell aplasia, resulting in Sertoli cell only syndrome
	Spermatogenic arrest (maturation arrest)
Acquired	Trauma
	Testicular torsion
	Post-inflammatory forms, particularly mumps orchitis
	Exogenous factors (medications, cytotoxic drugs, irradiation, heat)
	Systemic diseases (liver cirrhosis, renal failure)
	Testicular tumour
	Varicocele
	Surgery that may compromise vascularisation of the testes and subsequently testicular atrophy
Idiopathic	Unknown aetiology
	Unknown pathogenesis

### 3.3 Medical history and physical examination

Typical findings from the history and physical examination of a patient with testicular deficiency are:

- cryptorchidism;
- testicular torsion;
- genitourinary infection;
- testicular trauma;
- exposure to environmental toxin(s);
- gonadotoxic medication;
- exposure to radiation or chemical(s);
- testicular cancer;
- absence of testes;
- abnormal secondary sexual characteristics;
- gynaecomastia;
- abnormal testicular volume and/or consistency;
- varicocele.

### 3.4 Investigations

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

#### 3.4.1 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 minutes and a thorough microscopic examination by phase contrast optics at x200 magnification of the pellet. All samples can be stained and re-examined microscopically (2).

#### 3.4.2 Hormonal determinations

In men with testicular deficiency hypergonadotrophic 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 spermatocyte or spermatid blockage is complete. FSH values are within normal range.

However, for an individual patient, FSH levels do not accurately predict the spermatogenesis status (3-5). Preliminary data indicate a stronger correlation between low inhibin B level and spermatogenic damage (6).

#### 3.4.3 Testicular biopsy

Testicular biopsy can be part of an intracytoplasmic sperm injection (ICSI) treatment in patients with clinical evidence of NOA. Testicular sperm extraction (TESE) is the technique of choice and shows excellent repeatability (7-9). Spermatogenesis may be focal, which means that in about 50-60% of men with NOA, spermatozoa can be found and used for ICSI. Most authors therefore recommend taking several testicular samples (10,11). 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 (12,13). However, no clear relationship has been found between 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.

Microsurgical testicular sperm extraction may increase retrieval rates, even though comparative studies are not yet available (14-16). After opening the testis, tubules exhibiting larger diameter are excised using micro-scissors or forceps. Then, tubules are minced using mechanical or enzymatic digestion to facilitate sperm search (17). Positive retrievals are reported even in conditions, such as Sertoli cell only syndrome type II (14). Percutaneous Epididymal Sperm Aspiration (PESA) results in lower retrieval rates and does not allow histological examination to detect for instance carcinoma in situ (CIS) and testicular malignancies (18,19). PESA may also result in more tubular and vascular damage than TESE (20).

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) (21-24):

- Birth rates are lower in NOA versus OA (19% vs 28%) (25).
- Fertilisation and implantation rates are significantly lower (26).
- Miscarriage rates are higher in NOA versus OA (11.5% vs 2.5%) (27).

In OA, there were no significant differences in ICSI results between testicular and epididymal sperm (24). Also,

no significant differences have been reported in ICSI results between the use of fresh and frozen-thawed sperm (22,26-28).

### 3.5 Conclusions and recommendations for testicular deficiency

Conclusions
Impaired spermatogenesis is often associated with elevated FSH concentration.
Testicular biopsy is the best procedure to define the histological diagnosis and the possibility of finding sperm. Spermatozoa should be cryopreserved for use in ICSI.
Spermatozoa are found in about 60% of patients with non-obstructive azoospermia (NOA).
Men who are candidates for sperm retrieval must receive appropriate genetic advice.
For patients with NOA, who have spermatozoa in their testicular biopsy, ICSI with fresh or cryopreserved spermatozoa is the only therapeutic option.
Pregnancies and live births are achieved in 30-50% of couples with NOA, when spermatozoa has been found in the testicular biopsy.

Recommendations	GR
Men with non-obstructive azoospermia (NOA) can be offered a testicular sperm extraction with cryopreservation of the spermatozoa to be used for intracytoplasmic sperm injection (28).	B
To increase the chances of positive sperm retrievals in men with NOA, testicular sperm extraction (single, multiple or microsurgical) should be used rather than PESA.	B

### 3.6 References

1. World Health Organization. WHO Manual for the Standardised Investigation, Diagnosis and Management of the Infertile Male. Cambridge: Cambridge University Press, 2000.
2. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th edn. WHO, 2010.  
<http://www.who.int/reproductivehealth/publications/infertility/9789241547789/en/index.html>
3. Hauser R, Temple-Smith PD, Southwick GJ, et al. Fertility in cases of hypergonadotropic azoospermia. *Fertil Steril* 1995 Mar;63(3):631-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/7851598>
4. Martin-du Pan RC, Bischof P. Increased follicle stimulating hormone in infertile men. Is increased plasma FSH always due to damaged germinal epithelium? *Hum Reprod* 1995 Aug;10(8):1940-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/8567817>
5. De Kretser DM, Burger HG, Hudson B. The relationship between germinal cells and serum FSH in males with infertility. *J Clin Endocrinol Metab* 1974 May;38(5):787-93.  
<http://www.ncbi.nlm.nih.gov/pubmed/4823921>
6. Pierik FH, Vreeburg JT, Stijnen T, et al. Serum inhibin B as a marker of spermatogenesis. *J Clin Endocrinol Metab* 1998 Sep;83(9):3110-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/9745412>
7. Amer M, Hagggar SE, Moustafa T, et al. Testicular sperm extraction: impact to testicular histology on outcome, number of biopsies to be performed and optional time for repetition. *Hum Reprod* 1999 Dec;14(12):3030-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/10601092>
8. Colpi GM, Piediferro G, Nerva F, et al. Sperm retrieval for intra-cytoplasmic sperm injection in nonobstructive azoospermia. *Minerva Urol Nefrol* 2005 Jun;57(2):99-107.  
<http://www.ncbi.nlm.nih.gov/pubmed/15951734>
9. Vernaev V, Verheyen G, Goossens A, et al. How successful is repeat testicular sperm extraction in patients with azoospermia? *Hum Reprod* 2006 Jun;21(6):1551-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/16473930>
10. Gottschalk-Sabag S, Weiss DB, Folb-Zacharow N, et al. Is one testicular specimen sufficient for quantitative evaluation of spermatogenesis? *Fertil Steril* 1995 Aug;64(2):399-402.  
<http://www.ncbi.nlm.nih.gov/pubmed/7615120>
11. Turek PJ, Cha I, Ljung BM. Systematic fine-needle aspiration of the testis: correlation to biopsy and results of organ 'mapping' for mature sperm in azoospermic men. *Urology* 1997 May;49(5):743-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/9145981>

12. Abdel-Meguid TA. Predictors of sperm recovery and azoospermia relapse in men with nonobstructive azoospermia after varicocele repair. *J Urol* 2012 Jan;187(1):222-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/22100001>
13. Kim ED, Gilbaugh JH 3rd, Patel VR, et al. Testis biopsies frequently demonstrate sperm in men with azoospermia and significantly elevated follicle-stimulating hormone levels. *J Urol* 1997 Jan;157(1):144-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/8976237>
14. Colpi GM, Piediferro G, Nerva F, et al. Sperm retrieval for intra-cytoplasmic sperm injection in nonobstructive azoospermia. *Minerva Urol Nefrol* 2005 Jun;57(2):99-107.  
<http://www.ncbi.nlm.nih.gov/pubmed/15951734>
15. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 1999 Jan;14(1):131-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/10374109>
16. Okada H, Dobashi M, Yamazaki T, et al. Conventional versus microdissection testicular sperm extraction for non obstructive azoospermia. *J Urol* 2002 Sep;168(3):1063-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/12187223>
17. Esteves SC, Miyaoka R, Agarwal A. Sperm retrieval techniques for assisted reproduction. *Int Braz J Urol* 2011 Sep-Oct;37(5): 570-83  
<http://www.ncbi.nlm.nih.gov/pubmed/22099268>
18. Monzó A, Kondylis F, Lynch D, et al. Outcome of intracytoplasmic sperm injection in azoospermic patients: stressing the liaison between the urologist and reproductive medicine specialist. *Urology* 2001 Jul;58(1):69-75.  
<http://www.ncbi.nlm.nih.gov/pubmed/11445482>
19. Vernaev V, Tournaye H, Osmanagaoglu K, et al. Intracytoplasmic sperm injection with testicular spermatozoa is less successful in men with nonobstructive azoospermia than in men with obstructive azoospermia. *Fertil Steril* 2003 Mar;79(3):529-33.  
<http://www.ncbi.nlm.nih.gov/pubmed/12620435>
20. Silber S, Munné S. Chromosomal abnormalities in embryos derived from testicular sperm extraction (tese) in men with non-obstructive azoospermia. In: *Proceedings of EAA International Symposium. Genetics of male infertility: from research to clinic*. October 2-4, 2003, Florence, Italy.
21. Schwarzer J, Fiedler K, Hertwig I, et al. Sperm retrieval procedures and intracytoplasmic spermatozoa injection with epididymal and testicular sperms. *Urol Int* 2003;70(2):119-23.  
<http://www.ncbi.nlm.nih.gov/pubmed/12592040>
22. Ghanem M, Bakr NI, Elgayaar MA, et al. Comparison of the outcome of intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia in the first cycle: a report of case series and meta-analysis. *Int J Androl* 2005 Feb;28(1):16-21.  
<http://www.ncbi.nlm.nih.gov/pubmed/15679616>
23. Borges E Jr, Rossi-Ferragut LM, Pasqualotto FF, et al. Testicular sperm results in elevated miscarriage rates compared to epididymal sperm in azoospermic patients. *Sao Paulo Med J* 2002 Jul;120(4):122-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/12436160>
24. Gil Salóm M. [Spermatic recovery techniques for intracytoplasmic spermatozoid injection (ICSI) in male infertility.] *Arch Esp Urol* 2004 Nov;57(9):1035-46. [Article in Spanish]  
<http://www.ncbi.nlm.nih.gov/pubmed/15624403>
25. Ben-Yosef D, Yogev L, Hauser R, et al. Testicular sperm retrieval and cryopreservation prior to initiating ovarian stimulation as the first line approach in patients with non-obstructive azoospermia. *Hum Reprod* 1999 Jul;14(7):1794-801.  
<http://www.ncbi.nlm.nih.gov/pubmed/10402392>
26. Gil-Salóm M, Romero J, Rubio C, et al. Intracytoplasmic sperm injection with cryopreserved testicular spermatozoa. *Mol Cell Endocrinol* 2000 Nov;169(1-2):15-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/11155947>
27. Sousa M, Cremades N, Silva J, et al. Predictive value of testicular histology in secretory azoospermic subgroups and clinical outcomes after microinjection of fresh and frozen-thawed sperm and spermatids. *Hum Reprod* 2002 Jul;17(7):1800-10.  
<http://www.ncbi.nlm.nih.gov/pubmed/12093843>
28. Hauser R, Yogev L, Amit A, et al. Severe hypospermatogenesis in cases of nonobstructive azoospermia: should we use fresh or frozen testicular spermatozoa? *J Androl* 2005 Nov-Dec;26(6):772-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/16291973>

## 4. GENETIC DISORDERS IN INFERTILITY

### 4.1 Introduction

All urologists working in andrology must have an understanding of genetic abnormalities in infertility, so that they can provide correct advice to couples seeking fertility treatment. Men with very low sperm counts can be given a reasonable chance of paternity, using in vitro fertilisation (IVF), ICSI and sperm harvesting from the epididymis or the testis in case of azoospermia. However, the sperm of infertile men show an increase in aneuploidy, other genetic abnormalities and DNA damage and carry the risk of passing genetic abnormalities to the next generation. Although there are prospects for screening of sperm (1,2), current routine clinical practice is based on screening peripheral blood samples.

### 4.2 Chromosomal abnormalities

Chromosome abnormalities can be numerical (e.g. trisomy) or structural (e.g. inversions or translocations) (3,4). In a survey of pooled data from 11 publications, including 9,766 infertile men, the incidence of chromosomal abnormalities was 5.8% (3). Of these, sex chromosome abnormalities accounted for 4.2% and autosomal abnormalities for 1.5%. For 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 (4). The frequency of chromosomal abnormalities increases as the testicular deficiency becomes more severe. Patients with < 5 million spermatozoa/mL already show a 10-fold higher incidence (4%) of mainly autosomal structural abnormalities compared with the general population (5). At highest risk are secretory azoospermic men.

Based on the frequencies of chromosomal aberrations in patients with different sperm concentration, karyotype analysis is indicated in azoospermic men and in oligozoospermic men with < 5 million spermatozoa/mL (5). If there is a family history of recurrent abortions, malformations or mental retardation, karyotype analysis should be requested, regardless of the sperm concentration.

#### 4.2.1 Sperm chromosomal abnormalities

Sperm can be examined for chromosomal normality using multicolour fluorescent *in situ* hybridisation (FISH). Aneuploidy in sperm, particularly sex chromosome aneuploidy, is associated with severe damage to spermatogenesis (3,6-10) and is also seen in men with translocations (11).

FISH analysis of spermatozoa is a research investigation. It should be used to assess spermatozoa from men with defined andrological conditions (6). Techniques are needed to separate populations of genetically abnormal sperm from normal sperm or to safely screen individual spermatozoa before IVF and ICSI.

#### 4.2.2 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 (3,12). Adult men with Klinefelter's syndrome have small firm testicles devoid of germ cells. The phenotype varies from a normally virilised man to a man 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 (13). 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. There is one case report of declining spermatogenesis in a man with Klinefelter's syndrome, with the recommendation that early sperm retrieval sperm should be considered (14). Based on sperm FISH studies showing an increased frequency of sex chromosomal abnormalities and increased incidence of autosomal aneuploidies (disomy for chromosomes 13, 18 and 21), concerns have been raised about the chromosomal normality of the embryos generated through ICSI (15).

The production of 24,XY sperm has been reported in 0.9% and 7.0% of men with Klinefelter's mosaicism (16-18) and in 1.36-25% of men with somatic karyotype 47,XXY (19-22). In azoospermic patients, TESE or (MicroTESE) can be proposed as a therapeutic option since spermatozoa can be recovered in about 30% of cases. To date, 49 healthy children have been born using ICSI without preimplantation genetic diagnosis (PDG) and the conception of one 47,XXY fetus has been reported (12). However, a study of ICSI combined with PDG in 113 embryos reported a significant fall in the rate of normal embryos for couples with Klinefelter's syndrome in respect to controls (54% vs 77.2%) (15). Due to the significant increase of sex chromosomal and autosomal abnormalities in the embryos of Klinefelter's patients, pre-implantation diagnosis or amniocentesis and karyotype analysis should be strongly advised.

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



with Klinefelter's syndrome who undergo testicular biopsy procedures for sperm retrieval need long-term endocrine follow-up.

#### **4.2.3 Autosomal abnormalities**

Genetic counselling should be offered to all couples seeking fertility treatment (including IVF/ICSI) when the male partner is known or found to have 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 fetus. As with Klinefelter's syndrome, sperm FISH analysis provides a more accurate risk estimation of affected offspring.

When IVF/ICSI is carried out for men with translocations, preimplantation genetic diagnosis or amniocentesis and karyotype analysis should be used. Embryos with known unbalanced translocation should probably not be implanted.

### **4.3 Genetic defects**

#### **4.3.1 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.

#### **4.3.2 Kallmann syndrome**

The most common X-linked disorder in infertility practice is Kallmann syndrome. The predominant form is an X-linked recessive disorder caused by a mutation in the KALIG-1 gene on Xp22.3 (23). A number of newly identified autosomal gene mutations can also cause Kallmann syndrome (24). 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 renal abnormalities.

Since spermatogenesis can be relatively easily induced by hormonal treatment (25), genetic screening prior to therapy is strongly advised. Treatment with gonadotrophins allows natural conception in most cases, even in 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 i.e. risk estimation for transmission to the offspring.

#### **4.3.3 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 (26). The phenotypic features of complete androgen insensitivity syndrome (CAIS) are female external genitalia and absence of pubic hair (Morris syndrome). In partial androgen insensitivity syndrome, several different phenotypes are evident, ranging from predominantly female phenotype through ambiguous genitalia, to predominantly male phenotype with micropenis, perineal hypospadias, and cryptorchidism. The later phenotype is also termed Reifenstein syndrome. In the above mentioned severe forms of androgen resistances there is no risk of transmission since affected men cannot generate their own biological children using the current technologies. Patients with mild AIS 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, only a few mutations have been reported in infertile men (26-30).

#### **4.3.4 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 especially pre-meiotic genes are over-represented on the X chromosome compared with autosomal chromosomes (31,32). Nevertheless, up to now only two genes, USP26 and TAF7L, have been screened in relatively small study populations and neither of them appear relevant for male infertility (33,34).

### **4.4 Y chromosome and male infertility**

#### **4.4.1 Introduction**

The first association between azoospermia and microscopically detectable deletions of the long arm of the Y chromosome was demonstrated by Tiepolo and Zuffardi in 1976 (35). The first cases of Y microdeletions and male infertility were reported in 1992 (36), and many case series have subsequently been published. Microdeletions have been found in three non-overlapping regions, AZFa+b+c, of the Y chromosome (37). Several years after the discovery of the three AZF regions and with knowledge of the precise structure of the Y chromosome in Yq11, it was realised that the AZFb and AZFc regions overlap and that there was no AZFd region (38). 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 (39). In each AFZ region, there are a number of candidate genes, but their function in spermatogenesis remains largely unknown (40).

Since deletions occur in block (i.e. removing more than one gene), it is not possible to determine the role of a single AZF gene from the AZF deletion phenotype and thus it is unclear if they are all participating in spermatogenesis. Gene-specific deletions, which remove a single gene, have been reported only in the AZFa region. These studies suggested that the USP9Y gene is not essential for spermatogenesis and is most likely to be a 'fine tuner' of sperm production (41).

A new type of Yq deletions, known as 'gr/gr deletion' has been described in the AZFc region (42). This deletion removes half of the AZFc region gene content and affects the dosage of multicopy genes mapping inside this region (e.g. DAZ, CDY1, BPY2).

#### 4.4.2 **Clinical implications of Y microdeletions**

The clinical significance of Yq deletions have been debated for a long time because of the large variability found in deletion frequencies and reports of Yq deletions in 'fertile' men. More than 10 years of clinical research has found the following about Y deletions:

- They are not found in normospermic men, proving there is clearly a cause-and-effect relationship between Y deletions and spermatogenic failure (43).
- The highest frequency of Y deletions is found in azospermic men (8-12%), followed by oligozoospermic (3-7%) men.
- Deletions are extremely rare with a sperm concentration > 5 million of spermatozoa/mL (approximately 0.7%).
- AZFc deletions are most common (approximately 65-70%), followed by deletions of the AZFb and AZFb+c or AZFa+b+c regions (25-30%). AZFa region deletions are extremely 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 arrest. Complete removal of the AZFc region causes a variable phenotype ranging from azoospermia to oligozoospermia.
- Classical AZF deletions do not confer a risk for cryptorchidism or testicular cancer (39).

The specificity and genotype/phenotype correlation reported above means that Y deletion analysis has both a diagnostic and prognostic value for testicular sperm retrieval (39). In the case of gr/gr deletion, there is no such strict genotype/phenotype correlation. This type of partial AZFc deletion can also be found in normozoospermic men, although at a significantly lower frequency (0.5-1%) than in men with abnormal spermatogenesis (3-5%). In the largest Caucasian study population (> 1000 men), gr/gr deletion carriers were 7-fold more likely to develop oligozoospermia (44). The phenotypic expression may vary in different ethnic groups, depending on the Y chromosome background (45,46). An overall risk of 2.4-fold for reduced sperm production in gr/gr deletion carriers has recently been reported by a meta-analysis that included only studies free from methodological and selection bias (47). There has also been a report of gr/gr deletion as a potential risk factor for testicular germ cell tumours (48). However, this data needs further confirmation in an ethnically and geographically matched case-control study setting.

After conception, any Y deletions are transmitted automatically to a male offspring, and genetic counselling is therefore mandatory. In most cases, father and son have the same microdeletion (49-52), but occasionally the son has a larger microdeletion (53). It has been proposed that partial AZFc deletions (gr/gr and b2/b3) may predispose to complete AZFc deletion in the next generation (54). There is a substantial variation in the son's phenotype and the extent of spermatogenic failure (still in the range of azoo/oligozoospermia) cannot be predicted entirely, 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 chromosome (55,56), 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.

The screening for Y chromosome microdeletions in patients bearing a mosaic 46,XY/45,X0 karyotype with sexual ambiguity and/or Turner stigmata has shown a relatively high incidence of AZFc deletions (33%) (57). There is data to support the association of Yq microdeletions with an overall Y chromosomal instability, which leads to the formation of 45,X0 cell lines (58,59). Despite this theoretical risk, babies born from fathers affected by Yq microdeletions are phenotypically normal (39,60). This could be due to the reduced implantation rate and a likely higher risk of spontaneous abortions 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 cryoconservation of spermatozoa at a young age can be considered. However, there has only been a single report (48) of an enhanced risk for testicular germ cell



tumours in carriers of gr/gr deletion. Thus, it is only necessary to consider introducing preventive measures (e.g. testis ultrasound) in the sons of gr/gr deletion carriers if confirmatory studies are published.

#### 4.4.2.1 Testing for Y microdeletions

Indications for AZF deletions screening are based on sperm count and include azoospermia and severe oligozoospermia (< 5 million spermatozoa/mL). Thanks to the European Academy of Andrology (EAA) guidelines (60) and EAA/EMQN (European Molecular Genetics Quality Network) external quality control programme (<http://www.emqn.org/emqn/>), Yq testing has become more homogeneous and reliable in different routine genetic laboratories. The EAA guidelines provide a set of primers capable of detecting > 95% of clinically relevant deletions (60). The primers consist of two markers for each region and control markers from the Yp and X chromosome. The initial reports of large variability of deletion frequencies are more likely to have been caused by technical problems and unreliable markers rather than be an expression of true ethnic differences.

#### 4.4.2.2 Y chromosome: 'gr/gr' deletion

A new type of Yq deletions, known as the gr/gr deletion, has been described in the AZFc region (42). This deletion removes half of the gene content of the AZFc region, affecting the dosage of multicopy genes mapping inside this region. There is an almost 8-fold higher risk of developing oligozoospermia (OR = 7.9, 95% CI: 1.8-33.8; p < 0.001) in gr/gr deletion carriers in the largest Caucasian study population published to date (43). The frequency of gr/gr deletion in oligozoospermic patients is about 4%. According to four meta-analyses, gr/gr deletion is a significant risk factor for impaired sperm production (61,62).

However, 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). The routine screening for gr/gr deletion is still a debated issue, especially in those laboratories serving diverse ethnic and geographic populations.

#### 4.4.2.3 Conclusions

Testing for microdeletions is not necessary in men with obstructive azoospermia when ICSI is used because spermatogenesis should be normal.
Men with severely damaged spermatogenesis (with < 5 million spermatozoa/mL) should be advised to undergo Yq microdeletion testing for both diagnostic and prognostic purposes. Yq microdeletion also has important implications for genetic counselling (see below).
If complete AZFa or AZFb microdeletions are detected, microtesticular sperm extraction is not worth doing because it is extremely unlikely that any sperm will be found.
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 TCGTs is needed.
If a man with microdeletion and his partner wish to proceed with ICSI, they should be advised that microdeletions will be passed to sons, but not to daughters.
A son who inherits a microdeletion will have abnormal spermatogenesis because complete AZF deletions are not reported in normozoospermic men.

#### 4.4.3 Autosomal defects with severe phenotypic abnormalities and infertility

Several inherited disorders are associated with severe or considerable generalised abnormalities and infertility (Table 6). 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.

**Table 6: Less common inherited disorders associated with infertility and other alterations to phenotype**

Disorder	Phenotype	Genetic basis
Prader-Willi syndrome	Obesity, mental retardation	Deletion of 15q12 on paternally inherited chromosome
Bardet-Biedle syndrome	Obesity, mental retardation, retinitis pigmentosa, polydactyly	Autosomal recessive 16q21
Cerebellar ataxia and hyogonadotrophic hypogonadism	Eunuchoidism, disturbances of gait and speech	Autosomal recessive
Noonan's syndrome	Short stature, webbed neck, cardiac and pulmonary abnormalities, cryptorchidism	Autosomal dominant
Myotonic dystrophy	Muscle wasting, cataract, testicular atrophy	Autosomal dominant 19q13.3
Dominant polycystic kidney disease	Renal cysts, obstruction from epididymal cysts	Autosomal dominant 16p13.3 and 4q
5-alpha reductase deficiency	Perineal or scrotal hypospadias, vaginal pouch, immature female phenotype	Autosomal recessive

#### 4.5 Cystic fibrosis mutations and male infertility

Cystic fibrosis 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. This gene is located on the short arm of chromosome 7. 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 approximately 2% of men with OA attending a clinic in Edinburgh (63). 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 of < 1.5 mL and pH less than 7.0.

Approximately 1,500 mutations are listed on the CFTR database (<http://www.genet.sickkids.on.ca/cftr/>). Many series of men with CBAVD tested for varying numbers of mutations have been published. In general, the more mutations tested for, the higher the percentage of men found to have them. In a review of published series of 449 men with CBAVD, the Delta F508 mutation was detected in 244 men, the R117H mutation in 54 men and the W1282X mutation in 37; 63 other mutations were found in 1 to 9 men, but not all mutations were tested for in all case series (64).

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, as many have a very low prevalence in a particular population. Testing is usually restricted to the most common mutations in a particular community.

Mutations may be found in both copies of the CFTR gene; however, in most men with CBAVD, mutation is found in only one copy. In some of these supposedly heterozygous cases, there may be an unknown second mutation, but there is also another mechanism. In two-thirds of men with CBAVD, a DNA variant (the fifth allele) can be detected in a non-coding region of CFTR (65). Consequently, since the 5T-tract variant is now considered a mild CFTR mutation rather than a polymorphism, it should be analysed in each CAVD patient.

Men with CBAVD often have mild clinical stigmata of CF (e.g. history of chest infections). Children born after ICSI, where the father has CBAVD and is either hetero- or homozygous, must be followed up.

When a man has CBAVD, it is important to test him and his partner for CF mutations. If the female partner is found to be a carrier of CFTR, the couple must consider very carefully whether to proceed with ICSI using the husband's sperm, as the risk of a having a baby with CF will be 25% if the man is heterozygous and 50% if the man is homozygous. If the female partner is negative for known mutations, the risk of being a carrier of unknown mutations is about 0.4%.

#### 4.6 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 (66) and probably has a different genetic causation. 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. Nevertheless, men with unilateral absence of the vas deferens and CF mutations may have the same underlying genetic diseases as men with true CBAVD. Men with bilateral absence of vas deferens and renal abnormalities do not have CFTR gene abnormalities (67).

Men who have unilateral absence of the vas and normal kidneys or bilateral absence or bilateral abnormality, should be tested for CF mutations. If the results are negative and renal anatomy has not been defined, an abdominal ultrasound should be undertaken. 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.

#### 4.7 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 (34). 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 (34, 68, 69, and references therein). The introduction of new analytical approaches is likely to provide major advancement in this field (70,71).

ICSI 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 fetal abnormality, because ICSI may enable defective sperm to bypass the selective processes of the female genital tract and egg covering. Alternatively, eggs may be fertilised that would otherwise not be fertilised. However, fetal abnormality statistics from ICSI centres do not indicate any increase in congenital malformations compared with the general population.

On the other hand, ICSI babies have a higher risk of *de novo* sex chromosomal aberrations (about a 3-fold increase compared with natural conceptions) and paternally inherited structural abnormalities (72-74).

Indications for ICSI are constantly being extended to include fertilisation with immature sperm forms, and it is therefore particularly important to continue to monitor fetal abnormality rates, using detailed subgroup analysis according to the father's clinical and molecular diagnosis.

#### 4.8 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, to a lesser extent, conception after IVF/ICSI, and with an increase in early pregnancy loss (75,76). DNA damage may improve after varicocele ligation (77,78).

#### 4.9 Genetic counselling and ICSI

The best management is to agree treatment with the couple and provide them with full information on the genetic risks. Initially, the couple should be given full information about the risks to the child 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. CF mutations), there is up to a 50% chance of the child developing a clinical condition and dying early after a number of years of morbidity. Many clinicians and infertility clinic personnel may consider it is 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 need to give consideration to preimplantation diagnosis and replacement only of normal embryos.

#### 4.10 Conclusions and recommendations for genetic disorders in male infertility

Conclusions
New insights into the genetic basis of infertility and the advent of ICSI require a good understanding of genetics by clinicians and the general public.
Diagnostic advances will allow us to identify the genetic basis of more disorders and diagnose known disorders at a lower cost. For some of these disorders, gene therapy might be practical.

Recommendations	GR
Standard karyotype analysis should be offered to all men with damaged spermatogenesis (< 10 million spermatozoa/mL) who are seeking fertility treatment by in vitro fertilisation/intracytoplasmic sperm injection (ICSI) (2).	B
Men with Klinefelter's syndrome might require androgen replacement therapy as they get older.	B
All men with Klinefelter's syndrome who undergo testicular biopsy procedures for sperm retrieval need long-term endocrine follow-up.	B
For men with severely damaged spermatogenesis (< 5 million spermatozoa/mL), testing for Yq microdeletions is strongly advised (39,60).	B
When a man has structural abnormalities of the vas deferens (bilateral absence of vas deferens, unilateral absence of the vas), it is important to test him and his partner for CF gene mutations (64).	A
Genetic counselling is mandatory in couples with a genetic abnormality found in clinical or genetic investigation and in patients who carry a (potential) inheritable disease (1).	A

#### 4.11 References

- Griffin DK, Finch KA. The genetic and cytogenetic basis of male infertility. *Human Fertil Mar* 2005;8(1):19-26.  
<http://www.ncbi.nlm.nih.gov/pubmed/15823847>
- Carrell DT. The clinical implementation of sperm chromosome aneuploidy testing: pitfalls and promises. *J Androl* 2008 Mar-Apr;29(2):124-33.  
<http://www.ncbi.nlm.nih.gov/pubmed/17881765>
- Johnson MD. Genetic risks of intracytoplasmic sperm injection in the treatment of male infertility: recommendations for genetic counseling and screening. *Fertil Steril* 1998 Sep;70(3):397-411.  
<http://www.ncbi.nlm.nih.gov/pubmed/9757865>
- van Assche EV, Bonduelle M, Tournaye H, et al. Cytogenetics of infertile men. *Hum Reprod* 1996 Dec;11(Suppl 4):1-24; discussion 25-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/9147109>
- Vincent MC, Daudin M, De MP, et al. Cytogenetic investigations of infertile men with low sperm counts: a 25-year experience. *J Androl* 2002 Jan-Feb;23(1):18-22.  
<http://www.ncbi.nlm.nih.gov/pubmed/11780918>
- Tempest HG, Martin RH. Cytogenetic risks in chromosomally normal infertile men. *Curr Opin Obstet Gynecol* 2009 Jun;21(3):223-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/19424064>
- Clementini E, Palka C, Iezzi I, et al. Prevalence of chromosomal abnormalities in 2078 infertile couples referred for assisted reproduction techniques. *Hum Reprod* 2005 Feb;20(2):437-42.  
<http://www.ncbi.nlm.nih.gov/pubmed/15567875>
- Gianaroli L, Magli MC, Cavallini G, et al. Frequency of aneuploidy in sperm from patients with extremely severe male factor infertility. *Hum Reprod* 2005 Aug;20(8):2140-52.  
<http://www.ncbi.nlm.nih.gov/pubmed/15845594>
- Pang MG, Kim YJ, Lee SH, et al. The high incidence of meiotic errors increases with decreased sperm count in severe male factor infertilities. *Hum Reprod* 2005 Jun;20(6):1688-94.  
<http://www.ncbi.nlm.nih.gov/pubmed/15734753>
- Machev N, Gosset P, Viville S. Chromosome abnormalities in sperm from infertile men with normal somatic karyotypes: teratozoospermia. *Cytogenet Genome Res* 2005;111(3-4):352-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/16192715>
- Baccetti B, Collodel G, Marzella R, et al. Ultrastructural studies of spermatozoa from infertile males with Robertsonian translocations and 18, X, Y aneuploidies. *Hum Reprod* 2005 Aug;20(8):2295-300.  
<http://www.ncbi.nlm.nih.gov/pubmed/15878922>
- Lanfranco F, Kamischke A, Zitzmann M, et al. Klinefelter's syndrome. *Lancet* 2004 Jul 17-23;364(9430):273-83.  
<http://www.ncbi.nlm.nih.gov/pubmed/15262106>
- Wang C, Baker HW, Burger HG, et al. Hormonal studies in men with Klinefelter's syndrome. *Clin Endocrinol (Oxf)* 1975 Jul;4(4):399-411.  
<http://www.ncbi.nlm.nih.gov/pubmed/1157343>
- Ichioka K, Utsunomiya N, Kohei N, et al. Adult onset of declining spermatogenesis in a man with nonmosaic Klinefelter's syndrome. *Fertil Steril* 2006 May;85(5):1511.e1-2.  
<http://www.ncbi.nlm.nih.gov/pubmed/16616747>

15. Staessen C, Tournaye H, Van Assche E, et al. PGD in 47,XXY Klinefelter's syndrome patients. *Hum Reprod Update* 2003 Jul-Aug;9(4):319-30.  
<http://www.ncbi.nlm.nih.gov/pubmed/12926526>
16. Chevret E, Rousseaux S, Monteil M, et al. Increased incidence of hyperhaploid 24 XY spermatozoa detected by three-colour FISH in a 46,XY/47,XXY male. *Hum Genet* 1996 Feb;97(2):171-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/8566948>
17. Martini E, Geraedts JP, Liebaers I, et al. Constitution of semen samples from XYY and XXY males as analysed by in-situ hybridization. *Hum Reprod* 1996 Aug;11(8):1638-43.  
<http://www.ncbi.nlm.nih.gov/pubmed/8921108>
18. Lenz P, Luetjens CM, Kamischke A, et al. Mosaic status in lymphocytes of infertile men with or without Klinefelter syndrome. *Hum Reprod* 2005 May;20(5):1248-55.  
<http://www.ncbi.nlm.nih.gov/pubmed/15665007>
19. Cozzi J, Chevret E, Rousseaux S, et al. Achievement of meiosis in XXY germ cells: study of 543 sperm karyotypes from an XY/XXY mosaic patient. *Hum Genet* 1994 Jan;93(1):32-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/8270252>
20. Guttenbach M, Michelmann HW, Hinney B, et al. Segregation of sex chromosomes into sperm nuclei in a man with 47,XXY Klinefelter's karyotype: a FISH analysis. *Hum Genet* 1997 Apr;99(4):474-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/9099836>
21. Estop AM, Munné S, Ciepły KM, et al. Meiotic products of a Klinefelter 47,XXY male as determined by sperm fluorescence in-situ hybridization analysis. *Hum Reprod* 1998 Jan;13(1):124-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/9512242>
22. Foresta C, Galeazzi C, Bettella A, et al. High incidence of sperm sex chromosomes aneuploidies in two patients with Klinefelter's syndrome. *J Clin Endocrinol Metab* 1998 Jan;83(1):203-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/9435442>
23. Franco B, Guioli S, Pragliola A, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature* 1991 Oct;353(6344):529-36.  
<http://www.ncbi.nlm.nih.gov/pubmed/1922361>
24. Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nat Rev Endocrinol* 2009 Oct;5(10):569-76.  
<http://www.ncbi.nlm.nih.gov/pubmed/19707180>
25. Miyagawa Y, Tsujimura A, Matsumiya K, et al. Outcome of gonadotropin therapy for male hypogonadotropic hypogonadism at university affiliated male infertility centers: a 30-year retrospective study. *J Urol* 2005 Jun;173(6):2072-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/15879837>
26. Gottlieb B, Beitel LK, Wu JH, et al. The androgen receptor gene mutations database (ARDB): 2004 update. *Hum Mutat* 2004 Jun;23(6):527-33.  
<http://www.ncbi.nlm.nih.gov/pubmed/15146455>
27. Tincello DG, Saunders PT, Hargreave TB. Preliminary investigations on androgen receptor gene mutations in infertile men. *Mol Hum Reprod* 1997 Nov;3(11):941-3.  
<http://www.ncbi.nlm.nih.gov/pubmed/9433918>
28. Gottlieb B, Lombroso R, Beitel LK, et al. Molecular pathology of the androgen receptor in male (in) fertility. *Reprod Biomed Online* 2005 Jan;10(1):42-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/15705293>
29. Ferlin A, Vinanzi C, Garolla A, et al. Male infertility and androgen receptor gene mutations: clinical features and identification of seven novel mutations. *Clin Endocrinol (Oxf)* 2006;65(5):606-10.  
<http://www.ncbi.nlm.nih.gov/pubmed/17054461>
30. Rajender S, Singh L, Thangaraj K. Phenotypic heterogeneity of mutations in androgen receptor gene. *Asian J Androl* 2007 Mar;9(2):147-79.  
<http://www.ncbi.nlm.nih.gov/pubmed/17334586>
31. Wang PJ, McCarrey JR, Yang F, et al. An abundance of X-linked genes expressed in spermatogonia. *Nat Genet* 2001 Apr;27(4):422-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/11279525>
32. Wang PJ. X chromosomes, retrogenes and their role in male reproduction. *Trends Endocrinol Metab* 2004 Mar;15(2):79-83.  
<http://www.ncbi.nlm.nih.gov/pubmed/15036254>
33. Stouffs K, Tournaye H, Liebaers I, et al. Male infertility and the involvement of the X chromosome. *Hum Reprod Update* 2009 Nov-Dec;15(6):623-37.  
<http://www.ncbi.nlm.nih.gov/pubmed/19515807>

34. Nuti F, Krausz C. Gene polymorphisms/mutations relevant to abnormal spermatogenesis. *Reprod Biomed Online* 2008 Apr;16(4):504-13.  
<http://www.ncbi.nlm.nih.gov/pubmed/18413059>
35. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet* 1976 Oct 28;34(2):119-24.  
<http://www.ncbi.nlm.nih.gov/pubmed/1002136>
36. Ma K, Sharkey, A, Kirsch S, et al. Towards the molecular localisation of the AZF locus: mapping of microdeletions in azoospermic men within 14 subintervals of interval 6 of the human Y chromosome. *Hum Mol Genet* 1992 Apr;1(1):29-33.  
<http://www.ncbi.nlm.nih.gov/pubmed/1301132>
37. Vogt P, Edelmann A, Kirsch S, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet* 1996 Jul;5(7):933-43.  
<http://www.ncbi.nlm.nih.gov/pubmed/8817327>
38. Repping S, Skaletsky H, Lange J, et al. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am J Hum Genet* 2002 Oct;71: 906-922.  
<http://www.ncbi.nlm.nih.gov/pubmed/12297986>
39. Krausz C, Degl'Innocenti S. Y chromosome and male infertility: update, 2006. *Front Biosci* 2006 Sep;11:3049-61.  
<http://www.ncbi.nlm.nih.gov/pubmed/16720375>
40. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 2003 Jun;423(6942):825-37.  
<http://www.ncbi.nlm.nih.gov/pubmed/12815422>
41. Tyler-Smith C, Krausz C. The will-o'-the-wisp of genetics--hunting for the azoospermia factor gene. *N Engl J Med* 2009 Feb;360(9):925-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/19246366>
42. Repping S, Skaletsky H, Brown L, et al. Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. *Nat Genet* 2003 Nov;35(3):247-51.  
<http://www.ncbi.nlm.nih.gov/pubmed/14528305>
43. Krausz C, Forti G, McElreavey K. The Y chromosome and male fertility and infertility. *Int J Androl* 2003 Apr;26(2):70-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/12641824>
44. Giachini C, Laface I, Guarducci E, et al. Partial AZFc deletions and duplications: clinical correlates in the Italian population. *Hum Genet* 2008 Nov;124(4):399-410.  
<http://www.ncbi.nlm.nih.gov/pubmed/18807255>
45. Vogt PH. AZF deletions and Y chromosomal haplogroups: history and update based on sequence. *Hum Reprod Update* 2005 Jul-Aug;11(4):319-36.  
<http://www.ncbi.nlm.nih.gov/pubmed/15890785>
46. Krausz C, Giachini C, Xue Y, et al. Phenotypic variation within European carriers of the Y-chromosomal gr/gr deletion is independent of Y-chromosomal background. *J Med Genet* 2009 Jan;46(1):21-31.  
<http://www.ncbi.nlm.nih.gov/pubmed/18782837>
47. Visser L, Westerveld GH, Korver CM, et al. Y chromosome gr/gr deletions are a risk factor for low semen quality. *Hum Reprod* 2009 Oct;24(10):2667-73.  
<http://www.ncbi.nlm.nih.gov/pubmed/19602516>
48. Nathanson KL, Kanetsky PA, Hawes R, et al. The Y deletion gr/gr and susceptibility to testicular germ cell tumor. *Am J Hum Genet* 2005 Dec;77(6):1034-43.  
<http://www.ncbi.nlm.nih.gov/pubmed/16380914>
49. Mulhall JP, Reijo R, Alagappan R, et al. Azoospermic men with deletion of the DAZ gene cluster are capable of completing spermatogenesis: fertilization, normal embryonic development and pregnancy occur when retrieved testicular spermatozoa are used for intracytoplasmic sperm injection. *Hum Reprod* 1997 Mar;12(3):503-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/9130751>
50. Silber SJ, Alagappan R, Brown LG, et al. Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. *Hum Reprod* 1998 Dec;13(12):3332-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/9886509>



51. Kamischke A, Gromoll J, Simoni M, et al. Transmission of a Y chromosomal deletion involving the deleted in azoospermia (DAZ) and chromodomain (CDY1) genes from father to son through intracytoplasmic sperm injection: case report. *Hum Reprod* 1999 Sep;14(9):2320-2.  
<http://www.ncbi.nlm.nih.gov/pubmed/10469702>
52. Mau Kai C, Juul A, McElreavey K, et al. Sons conceived by assisted reproduction techniques inherit deletions in the azoospermia factor (AZF) region of the Y chromosome and the DAZ gene copy number. *Hum Reprod* 2008 Jul;23(7):1669-78.  
<http://www.ncbi.nlm.nih.gov/pubmed/18440997>
53. Stuppia L, Gatta V, Calabrese G, et al. A quarter of men with idiopathic oligo-azospermia display chromosomal abnormalities and microdeletions of different types in interval 6 of Yq11. *Hum Genet* 1998 May;102(5):566-70.  
<http://www.ncbi.nlm.nih.gov/pubmed/9654206>
54. Zhang F, Lu C, Li Z, et al. Partial deletions are associated with an increased risk of complete deletion in AZFc: a new insight into the role of partial AZFc deletions in male infertility. *J Med Genet* 2007 Jul;44(7):437-44.  
<http://www.ncbi.nlm.nih.gov/pubmed/17412880>
55. Siffroi JP, Le Bourhis C, Krausz C, et al. Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. *Hum Reprod* 2000 Dec;15(12):2559-62.  
<http://www.ncbi.nlm.nih.gov/pubmed/11098026>
56. Jaruzelska J, Korcz A, Wojda A, et al. Mosaicism for 45,X cell line may accentuate the severity of spermatogenic defects in men with AZFc deletion. *J Med Genet* 2001 Nov;38(11):798-802.  
<http://www.ncbi.nlm.nih.gov/pubmed/11732492>
57. Patsalis PC, Sismani C, Quintana-Murci L, et al. Effects of transmission of Y chromosome AZFc deletions. *Lancet* 2002 Oct;360(9341):1222-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/12401251>
58. Patsalis PC, Skordis N, Sismani C, et al. Identification of high frequency of Y chromosome deletions in patients with sex chromosome mosaicism and correlation with the clinical phenotype and Y-chromosome instability. *Am J Med Genet A* 2005 Jun;135(2):145-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/15880425>
59. Le Bourhis C, Siffroi JP, McElreavey K, et al. Y chromosome microdeletions and germinal mosaicism in infertile males. *Mol Hum Reprod* 2000 Aug;6(8):688-93.  
<http://www.ncbi.nlm.nih.gov/pubmed/10908277>
60. Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. *Int J Androl* 2004 Aug;27(4):240-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/15271204>
61. Stouffs K, Lissens W, Tournaye H, Haentjens P. What about gr/gr deletions and male infertility? Systematic review and meta-analysis. *Hum Reprod Update* 2011 Mar-Apr;17(2):197-209.  
<http://www.ncbi.nlm.nih.gov/pubmed/20959348>
62. Navarro-Costa P, Gonçalves J, Plancha CE. The AZFc region of the Y chromosome: at the crossroad between genetic diversity and male infertility. *Hum Reprod Update* 2010 Sep-Oct;16(5):525-42.  
<http://www.ncbi.nlm.nih.gov/pubmed/20304777>
63. Donat R, McNeill AS, Fitzpatrick DR, et al. The incidence of cystic fibrosis gene mutations in patients with congenital bilateral absence of the vas deferens in Scotland. *Br J Urol* 1997 Jan;79(1):74-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/9043501>
64. De Braekeleer M, Férec C. Mutations in the cystic fibrosis gene in men with congenital bilateral absence of the vas deferens. *Mol Hum Reprod* 1996 Sep;2(9):669-77.  
<http://www.ncbi.nlm.nih.gov/pubmed/9239681>
65. Chillón M, Casals T, Mercier B, et al. Mutations in cystic fibrosis gene in patients with congenital absence of the vas deferens. *New Engl J Med* 1995 Jun;332(22):1475-80.  
<http://www.ncbi.nlm.nih.gov/pubmed/7739684>
66. Drake MJ, Quinn FM. Absent vas deferens and ipsilateral multicystic dysplastic kidney in a child. *Br J Urol* 1996 May;77(5):756-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/8689131>
67. Augarten A, Yahav Y, Kerem BS, et al. Congenital bilateral absence of the vas deferens in the absence of cystic fibrosis. *Lancet* 1994 Nov 26;344(8935):1473-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/7968122>
68. Krausz C, Giachini C. Genetic risk factors in male infertility. *Arch Androl* 2007 May-Jun;53(3):125-33.  
<http://www.ncbi.nlm.nih.gov/pubmed/17612870>

69. Tüttelmann F, Rajpert-De Meyts E, Nieschlag E, et al. Gene polymorphisms and male infertility--a meta-analysis and literature review. *Reprod Biomed Online* 2007 Dec;15(6):643-58.  
<http://www.ncbi.nlm.nih.gov/pubmed/18062861>
70. Aston KI, Carrell DT. Genome-wide study of single-nucleotide polymorphisms associated with azoospermia and severe oligozoospermia. *J Androl* 2009 Nov-Dec;30(6):711-25.  
<http://www.ncbi.nlm.nih.gov/pubmed/19478329>
71. Carrell DT, De Jonge C, Lamb DJ. The genetics of male infertility: a field of study whose time is now. *Arch Androl* 2006 Jul-Aug;52(4):269-74.  
<http://www.ncbi.nlm.nih.gov/pubmed/16728342>
72. Van Steirteghem A, Bonduelle M, Devroey P, et al. Follow-up of children born after ICSI. *Hum Reprod Update* 2002 Mar-Apr;8(2):111-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/12099626>
73. Bonduelle M, Van Assche E, Joris H, et al. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum Reprod* 2002 Oct;17(10):2600-14.  
<http://www.ncbi.nlm.nih.gov/pubmed/12351536>
74. ESHRE Capri Workshop group Intracytoplasmic sperm injection (ICSI) in 2006: evidence and evolution. *Hum Reprod Update* 2007 Nov-Dec;13(6):515-26.  
<http://www.ncbi.nlm.nih.gov/pubmed/17630396>
75. Zini A, Meriano J, Kader K, et al. Potential adverse effect of sperm DNA damage on embryo quality after ICSI. *Hum Reprod* 2005 Dec;20(12):3476-80.  
<http://www.ncbi.nlm.nih.gov/pubmed/16123087>
76. Zini A, Sigman M. Are tests of sperm DNA damage clinically useful? Pros and cons. *J Androl* 2009 May-Jun;30(3):219-29.  
<http://www.ncbi.nlm.nih.gov/pubmed/19059901>
77. Zini A, Blumenfeld A, Libman J, et al. Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity. *Hum Reprod* 2005 Apr;20(4):1018-21.  
<http://www.ncbi.nlm.nih.gov/pubmed/15608026>
78. Smit M, Romijn JC, Wildhagen MF, et al. Decreased sperm DNA fragmentation after surgical varicocelectomy is associated with increased pregnancy rate. *J Urol* 2010 Jan;183(1):270-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/19913801>

## 5. OBSTRUCTIVE AZOOSPERMIA

### 5.1 Definition

Obstructive azoospermia (OA) is the inability to detect both spermatozoa and spermatogenic cells in semen and post-ejaculate urine due to bilateral obstruction of the seminal ducts. OA is less common than NOA and occurs in 15-20% of men with azoospermia. Common causes of OA are summarised in Table 7.

Men with OA present with normal FSH, normal size testes and epididymal enlargement. Sometimes, the vas deferens is absent due to congenital factors or previous inguinal or scrotal surgery. Obstruction in primary infertile men is often present at the epididymal level; other sites of obstruction are the ejaculatory ducts and the vas deferens. In 25% of men with a suspected obstruction, no spermatozoa are found in the epididymis during scrotal exploration, indicating an intratesticular obstruction.

**Table 7: Classification of OA, on the basis of ductal obstruction due to congenital and acquired causes**

Conditions	Congenital	Acquired
Epididymal obstruction	Idiopathic epididymal obstruction	Post-infective (epididymitis) Post-surgical (epididymal cysts)
Vas deferens obstruction	Congenital absence of vas deferens	Post-vasectomy Post-surgical (hernia, scrotal surgery)
Ejaculatory duct obstruction	Prostatic cysts (Müllerian cysts)	Post-surgical (bladder neck surgery) Post-infective



## 5.2 Classification

### 5.2.1 *Intratesticular obstruction*

Intratesticular obstruction occurs in 15% of OA (1). Congenital forms (dysjunction between rete testis and efferent ductules) are less common than acquired forms, i.e. post-inflammatory or post-traumatic obstructions. Acquired forms are often associated with an obstruction of epididymis and vas deferens.

### 5.2.2 *Epididymal obstruction*

Epididymal obstruction is the most common cause of OA, affecting 30-67% of azoospermic men with a serum FSH less than twice the upper limit of normal (1-4).

Congenital epididymal obstruction usually manifests as CBAVD, which is associated with at least one mutation of the CF gene in 82% of cases (5). This form is often accompanied by absence of the distal part of the epididymis and seminal vesicle agenesis (see above Chapter 4: Genetic disorders in infertility). Other congenital forms of obstruction are rare, e.g. disjunction between efferent ductules and the corpus epididymis, agenesis/atresia of a short part of the epididymis.

Congenital forms of epididymal obstruction include chronic sinopulmonary infections (Young's syndrome) (6), in which obstruction results from a mechanical blockage due to debris within the proximal epididymal lumen.

Acquired forms secondary to acute (e.g. gonococcal) and subclinical (e.g. chlamydial) epididymitis are most common (7,8) (see below Chapter 11: Male accessory gland infections). Acute or chronic traumas can result in epididymal damage (9).

Azoospermia caused by surgery may occur after epididymal surgery, e.g. cyst removal. Epididymal obstruction secondary to long-lasting distal obstruction must be considered when repairing seminal ducts (10).

### 5.2.3 *Vas deferens obstruction*

Vas deferens obstruction is the most common cause of acquired obstruction following vasectomy for sterilisation, with possible subsequent germ cell impairment and fibrosis (11,12). Approximately 2-6% of these men request vasectomy reversal. Of those undergoing vaso-vasostomy, 5-10% have epididymal blockage as a result of tubule rupture, making epididymo-vasostomy mandatory (see below Chapter 10: Male contraception). Vasal obstruction may also occur after herniotomy (13). Polypropylene mesh herniorrhaphy appears to be able to induce a fibroblastic response able to entrap or obliterate the vas deferens (14).

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 (15) (see above Chapter 4: Genetic disorders in infertility). Distal vas deferens obstruction includes CBAVD and accidental injury to the vas deferens during hernia surgery (16).

### 5.2.4 *Ejaculatory duct obstruction*

Ejaculatory duct obstruction is found in about 1-3% of OA (1) and is classified as either cystic or post-inflammatory.

Cystic obstructions are usually congenital (i.e. Müllerian duct cyst or urogenital sinus/ejaculatory duct cysts) and are medially located in the prostate between the ejaculatory ducts. In urogenital sinus abnormalities, one or both ejaculatory ducts empty into the cyst (17), while in Müllerian duct anomalies, ejaculatory ducts are laterally displaced and compressed by the cyst (18).

Paramedian or lateral intraprostatic cysts are Wolffian in origin and rare in clinical practice (19). Post-inflammatory obstructions of the ejaculatory duct are usually secondary to acute, non-acute, or chronic urethro-prostatitis (20).

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) (20,21).

### 5.2.5 *Functional obstruction of the distal seminal ducts*

Functional obstruction of the distal seminal ducts might be attributed to local neuropathy (22). This abnormality is often associated with urodynamic dysfunction because of the vasographic patterns of ampullo-vesicular atony or of ejaculatory duct hypertony. Functional obstruction of the distal seminal ducts has been reported in juvenile diabetes and polycystic kidney disease (23); however, no relevant pathology has been found in most cases. Results of semen analysis vary between azoospermia, cryptozoospermia and severe OAT syndrome.

## 5.3 Diagnosis

### 5.3.1 *Clinical history*

Clinical history taking should follow the suggestions for investigation of infertile men (see Chapter 2: Investigations).

Patients should be asked about:

- haemospermia;
- post-ejaculatory pain;
- previous or present urethritis or prostatitis;
- obstructive or irritative urinary symptoms;
- previous scrotal enlargement or pain or surgery;
- previous inguinal herniorrhaphy or traumas;
- chronic sinopulmonary infections.

### 5.3.2 **Clinical examination**

Clinical examination should follow suggestions for investigation of the infertile man. The following findings indicate OA:

- at least one testis with a volume > 15 ml, although a smaller testicular 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;
- signs of urethritis;
- prostatic abnormalities.

### 5.3.3 **Semen analysis**

At least two examinations must be carried out at an interval of 2-3 months, according to the WHO (*see above* Chapter 2: Investigations). Azoospermia means the inability to detect spermatozoa after centrifugation at x400 magnification. Careful repeat observation of several smears after semen liquefaction is needed. If no spermatozoa are found in a wet preparation, then aliquots or the whole semen sample should be centrifuged at 3000 G for 15 minutes. The pellet must be examined for spermatozoa.

Ejaculatory duct obstruction or CBAVD is suggested by a semen volume of less than 1.5 mL, acid pH and a low fructose level. When semen volume is low, a search must be made for spermatozoa in urine after ejaculation, as their presence confirms an ejaculatory disorder. Absence of spermatozoa and immature germ cells in semen smears suggest complete proximal or distal seminal duct obstruction.

### 5.3.4 **Hormone levels**

Serum FSH levels may be normal, but do not exclude a testicular cause of azoospermia (e.g. spermatogenic arrest). Follicle-stimulating hormone is normal in 40% of men with primary spermatogenic failure. Inhibin B seems to have a higher predictive value for normal spermatogenesis (4).

### 5.3.5 **Ultrasonography**

Scrotal ultrasound is helpful in finding signs of obstruction (e.g. dilatation of rete testis, enlarged epididymis with cystic lesions, absent vas deferens) and may demonstrate signs of testicular dysgenesis (e.g. non-homogenous testicular architecture and microcalcifications) and associated carcinoma *in situ* of the testis. For patients with a low seminal volume and in whom distal obstruction is suspected, transrectal ultrasound (TRUS) is essential. If possible, TRUS should be performed at high resolution and with high frequency (> 7 MHz) biplane transducers. Seminal vesicle enlargement (anterior-posterior diameter 15 mm) (21) and roundish, anechoic areas in the seminal vesicle (24) are TRUS anomalies more often associated with ejaculatory duct obstruction, especially when semen volume is < 1.5 mL. Müllerian duct cysts or urogenital sinus/ejaculatory duct cysts (20) and ejaculatory duct calcifications (25) are other known anomalies in obstructive azoospermia. Transrectal ultrasound may also be used to aspirate seminal vesicle fluid (26).

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 at the same time.

### 5.3.6 **Testicular biopsy**

In selected cases, testicular biopsy may be indicated to exclude spermatogenic failure. Testicular biopsy should be combined with extraction of testicular spermatozoa (i.e. TESE) for cryopreservation and subsequent ICSI, when surgical recanalisation cannot be carried out or has failed. A scoring system for testicular biopsies is given in Table 8 (27).

**Table 8: Scoring system for testicular biopsies (Johnsen score)\***

Score	Histological criteria
10	Full spermatogenesis
9	Slightly impaired spermatogenesis, many late spermatids, disorganised epithelium
8	< 5 spermatozoa per tubule, few late spermatids
7	No spermatozoa, no late spermatids, many early spermatids
6	No spermatozoa, no late spermatids, few early spermatids
5	No spermatozoa or spermatids, many spermatocytes
4	No spermatozoa or spermatids, few spermatocytes
3	Spermatogonia only
2	No germinal cells, Sertoli cells only
1	No seminiferous epithelium

\* From Johnsen, 1970 (27).

## 5.4 Treatment

### 5.4.1 Intratesticular obstruction

At this level seminal duct recanalisation is impossible. Both Testicular Sperm Extraction (TESE) or Microsurgical Epididymal Sperm Aspiration (MESA) allow sperm retrieval in nearly all OA patients. TESE and MESA are therefore recommended. The spermatozoa retrieved may be used immediately for ICSI, or may be cryopreserved.

### 5.4.2 Epididymal obstruction

Microsurgical epididymal sperm aspiration (MESA) (28) is indicated in men with CBAVD. TESA and PESA are also viable options for retrieving epididymal sperm from men with OA (29). Retrieved spermatozoa are used for ICSI. Usually, one MESA procedure provides sufficient material for several ICSI cycles (30) and it produces high pregnancy and fertilisation rates (31). In patients with azoospermia due to acquired epididymal obstruction, end-to-end or end-to-side microsurgical epididymo-vasostomy is recommended, with the preferred technique being microsurgical intussusception epididymo-vasostomy (32).

Reconstruction may be carried out unilaterally or bilaterally; patency and pregnancy rates are usually higher with bilateral reconstruction. Before microsurgery, it is important to check for full patency downstream of the epididymis. 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 in case of surgical failure (30).

Patency rates range between 60% and 87% (33-35) and cumulative pregnancy rates between 10% and 43%. Recanalisation success rates may be adversely affected by pre-operative and operative findings (e.g. concomitant abnormal testicular histology, absence of sperm in the spermatic fluid on sectioning the small epididymal tubules, wide fibrosis of the epididymis).

### 5.4.3 Proximal vas obstruction

Proximal vas obstruction after vasectomy requires microsurgical vasectomy reversal (see Chapter 10: Male contraception). Vaso-vasostomy is also required in rare cases of proximal vasal obstructions (iatrogenic, post-traumatic, post-inflammatory). The absence of spermatozoa in the intraoperative vas deferens fluid may suggest the presence of a secondary epididymal obstruction, especially if the seminal fluid of the proximal vas has a thick 'toothpaste' appearance. Microsurgical vaso-epididymostomy is then indicated.

### 5.4.4 Distal vas deferens obstruction

It is usually impossible to correct large bilateral vas deferens defects, resulting from involuntary excision of the vas deferences during hernia surgery in early childhood or previous orchidopexy (16). In these cases, proximal vas deferens sperm aspiration (37) or TESE/MESA can be used for cryopreservation for future ICSI. In large unilateral vas deferens defects associated with contralateral testicular atrophy, the vas deferens of the atrophic testis can be used for a cross-over vaso-vasostomy or vaso-epididymostomy.

### 5.4.5 Ejaculatory duct obstruction

The treatment of ejaculatory duct obstruction depends on its aetiology. Transurethral resection of the ejaculatory ducts (TURED) (20,38) can be used in large post-inflammatory 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 (20). Intra-operative 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 ducts, seminal vesicles and vasa (causing poor sperm motility, acid semen pH and epididymitis). The alternatives to TURED are MESA, TESE, proximal vas deferens sperm aspiration, seminal vesicle ultrasonically guided aspiration and direct cyst aspiration.

In cases of functional obstruction of the distal seminal ducts, TURED often fails to improve sperm output. Spermatozoa can then be retrieved by antegrade seminal tract washout (38). Spermatozoa retrieved by any of the aforementioned surgical techniques should always be cryopreserved for assisted reproductive procedures.

## 5.5 Conclusions and recommendation for obstructive azoospermia

Conclusions
Obstructive lesions of the seminal tract should be suspected in azoospermic or severely oligozoospermic patients with normal-sized testes and normal endocrine parameters.
Results of reconstructive microsurgery depend on the cause and location of the obstruction and the surgeon's expertise. Standardised procedures include vaso-vasostomy and epididymo-vasostomy.
Sperm retrieval techniques, such as MESA, TESE, and PESA can be used additionally. These methods should be used only when cryostorage of the material obtained is available.

Recommendation	GR
In azoospermia caused by epididymal obstruction, a scrotal exploration with microsurgical epididymal sperm aspiration and cryopreservation of the spermatozoa should be carried out, together with a microsurgical reconstruction (35).	B

## 5.6 References

- Hendry WF. Azoospermia and surgery for testicular obstruction. In: Hargreave TB (ed). *Male Infertility*. Berlin: Springer-Verlag, 1997, pp. 319-36.
- Hendry WF, Parslow JM, Stedronska J. Exploratory scrototomy in 168 azoospermic males. *Br J Urol* 1983 Dec;55(6):785-91.  
<http://www.ncbi.nlm.nih.gov/pubmed/6652453>
- Jequier AM. Obstructive azoospermia: a study of 102 patients. *Clin Reprod Fertil* 1985 Mar;3(1):21-36.  
<http://www.ncbi.nlm.nih.gov/pubmed/3978535>
- Pierik FH, Vreeburg JT, Stijnen T, et al. Serum inhibin B as a marker of spermatogenesis. *J Clin Endocrinol Metab* 1998 Sep;83(9):3110-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/9745412>
- Oates RD, Amos JA. The genetic basis of congenital bilateral absence of the vas deferens and cystic fibrosis. *J Androl* 1994 Jan-Feb;15(1):1-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/8188533>
- Handelsman DJ, Conway AJ, Boylan LM, et al. Young's syndrome: obstructive azoospermia and chronic sinopulmonary infections. *New Engl J Med* 1984 Jan;310(1):3-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/6689737>
- Silber SJ, Grotjan HE. Microscopic vasectomy reversal 30 years later: a summary of 4010 cases by the same surgeon. *J Androl* 2004 Nov-Dec;25(6):845-59.  
<http://www.ncbi.nlm.nih.gov/pubmed/15477352>
- Schoysman R. Vaso-epididymostomy - a survey of techniques and results with considerations of delay of appearance of spermatozoa after surgery. *Acta Eur Fertil* 1990 Sep-Oct;21(5):239-45.  
<http://www.ncbi.nlm.nih.gov/pubmed/2132475>
- Matthews GJ, Schlegel PN, Goldstein M. Patency following microsurgical vasoepididymostomy and vasovasostomy: temporal considerations. *J Urol* 1995 Dec;154(6):2070-3.  
<http://www.ncbi.nlm.nih.gov/pubmed/7500460>

10. Jarvi K, Zini A, Buckspan MB, et al. Adverse effects on vasoepididymostomy outcomes for men with concomitant abnormalities in the prostate and seminal vesicle. *J Urol* 1998 Oct;160(4):1410-2.  
<http://www.ncbi.nlm.nih.gov/pubmed/9751365>
11. Raleigh D, O'Donnell L, Southwick GJ, et al. Stereological analysis of the human testis after vasectomy indicates impairment of spermatogenic efficiency with increasing obstructive interval. *Fertil Steril* 2004 Jun;81(6):1595-603.  
<http://www.ncbi.nlm.nih.gov/pubmed/15193483>
12. McVicar CM, O'Neill DA, McClure N, et al. Effects of vasectomy on spermatogenesis and fertility outcome after testicular sperm extraction combined with ICSI. *Hum Reprod* 2005 Oct;20(10):2795-800.  
<http://www.ncbi.nlm.nih.gov/pubmed/15958397>
13. Sheynkin YR, Hendin BN, Schlegel PN, et al. Microsurgical repair of iatrogenic injury to the vas deferens. *J Urol* 1998 Jan;159(1):139-41.  
<http://www.ncbi.nlm.nih.gov/pubmed/9400456>
14. Shin D, Lipshultz LI, Goldstein M, et al. Herniorrhaphy with polypropylene mesh causing inguinal vassal obstruction: a preventable cause of obstructive azoospermia. *Ann Surg* 2005 Apr;241(4):553-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/15798455>
15. Schlegel PN, Shin D, Goldstein M. Urogenital anomalies in men with congenital absence of the vas deferens. *J Urol* 1996 May;155(5):1644-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/8627844>
16. Borovikov A. Treatment of large vasal defects. In: Goldstein M (ed). *Surgery of Male Infertility*. Philadelphia: WB Saunders, 1995, pp. 77-95.
17. Elder JS, Mostwin JL. Cyst of the ejaculatory duct/urogenital sinus. *J Urol* 1984 Oct;132(4):768-71.  
<http://www.ncbi.nlm.nih.gov/pubmed/6471229>
18. Schuhrke TD, Kaplan GW. Prostatic utricle cysts (müllerian duct cysts). *J Urol* 1978 Jun;119(6):765-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/26814>
19. Surya BV, Washecka R, Glasser J, et al. Cysts of the seminal vesicles: diagnosis and management. *Br J Urol* 1988 Nov;62(5):491-3.  
<http://www.ncbi.nlm.nih.gov/pubmed/3208033>
20. Schroeder-Printzen I, Ludwig M, Kohn F, et al. Surgical therapy in infertile men with ejaculatory duct obstruction: technique and outcome of a standardized surgical approach. *Hum Reprod* 2000 Jun;15(6):1364-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/10831570>
21. Kuligowska E, Baker CE, Oates RD. Male infertility: role of transrectal US in diagnosis and management. *Radiology* 1992 Nov;185(2):353-60.  
<http://www.ncbi.nlm.nih.gov/pubmed/1410338>
22. Colpi GM, Casella F, Zanollo A, et al. Functional voiding disturbances of the ampullo-vesicular seminal tract: a cause of male infertility. *Acta Eur Fertil* 1987 May-Jun;18(3):165-79.  
<http://www.ncbi.nlm.nih.gov/pubmed/3125711>
23. Hendry WF, Rickards D, Pryor JP, et al. Seminal megavesicles with adult polycystic kidney disease. *Hum Reprod* 1998 Jun;13(6):1567-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/9688393>
24. Colpi GM, Negri L, Nappi RE, et al. Is transrectal ultrasonography a reliable diagnostic approach in ejaculatory duct sub-obstruction? *Hum Reprod* 1997 Oct;12(10):2186-91.  
<http://www.ncbi.nlm.nih.gov/pubmed/9402280>
25. Meacham RB, Hellerstein DK, Lipshultz LI. Evaluation and treatment of ejaculatory duct obstruction in the infertile male. *Fertil Steril* 1993 Feb;59(2):393-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/8425637>
26. Jarow JP. Seminal vesicle aspiration of fertile men. *J Urol* 1996 Sep;156(3):1005-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/8709296>
27. Johnsen SG. Testicular biopsy score count—a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones* 1970;1(1):2-25.  
<http://www.ncbi.nlm.nih.gov/pubmed/5527187>
28. Silber SJ, Balmaceda J, Borrero C, et al. Pregnancy with sperm aspiration from the proximal head of the epididymis: a new treatment for congenital absence of the vas deferens. *Fertil Steril* 1988 Sep;50(3):525-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/3410105>
29. Esteves SC, Miyaoka R, Agarwal A. Sperm retrieval techniques for assisted reproduction. *Int Braz J Urol* 2011 Sep-Oct;37(5): 570-83  
<http://www.ncbi.nlm.nih.gov/pubmed/22099268>

30. Schroeder-Printzen I, Zumbé G, Bispink L, et al. Microsurgical epididymal sperm aspiration: aspirate analysis and straws available after cryopreservation in patients with non-reconstructable obstructive azoospermia. MESA/TESE Group Giessen. Hum Reprod 2000 Dec;15(12):2531-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/11098022>
31. Van Peperstraten A, Proctor ML, Johnson NP, et al. Techniques for surgical retrieval of sperm prior to ICSI for azoospermia. Cochrane Database Syst Rev 2006 Jul 19;3:CD002807.  
<http://www.mrw.interscience.wiley.com/cochrane/clsystrev/articles/CD002807/frame.html>
32. Chan PT, Brandell RA, Goldstein M. Prospective analysis of outcomes after microsurgical intussusception vasoepididymostomy. BJU Int 2005 Sep;96(4):598-601.  
<http://www.ncbi.nlm.nih.gov/pubmed/16104917>
33. Matthews GJ, Schlegel PN, Goldstein M. Patency following microsurgical vasoepididymostomy and vasovasostomy: temporal consideration. J Urol 1995 Dec;154(6):2070-3.  
<http://www.ncbi.nlm.nih.gov/pubmed/7500460>
34. Mangoli V, Dandekar S, Desai S, et al. The outcome of ART in males with impaired spermatogenesis. Hum Reprod Sci 2008 Jul;1(2):73-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/19562049>
35. Kim ED, Winkel E, Orejuela F, et al. Pathological epididymal obstruction unrelated to vasectomy: results with microsurgical reconstruction. J Urol 1998 Dec;160(6 Pt 1):2078-80.  
<http://www.ncbi.nlm.nih.gov/pubmed/9817328>
36. Kolettis PN, Thomas AJ Jr. Vasoepididymostomy for vasectomy reversal: a critical assessment in the era of intracytoplasmic sperm injection. J Urol 1997 Aug;158(2):467-70.  
<http://www.ncbi.nlm.nih.gov/pubmed/9224325>
37. Ruiz-Romero J, Sarquella J, Pomerol JM. A new device for microsurgical sperm aspiration. Andrologia 1994 Mar-Apr;26(2):119-20.  
<http://www.ncbi.nlm.nih.gov/pubmed/8042769>
38. Fisch H, Lambert SM, Goluboff ET. Management of ejaculatory duct obstruction: etiology, diagnosis, and treatment. World J Urol 2006 Dec;24(6):604-10.  
<http://www.ncbi.nlm.nih.gov/pubmed/17077974>

## 6. VARICOCELE

### 6.1 Introduction

Varicocele is a common abnormality (see Chapter 2: Investigations) with the following andrological implications:

- failure of ipsilateral testicular growth and development;
- symptoms of pain and discomfort;
- infertility.

### 6.2 Classification

The following classification of varicocele (1,2) is useful in clinical practice:

- subclinical: not palpable or visible at rest or during valsalva manoeuvre, but can be shown by special tests (Doppler ultrasound studies) (3);
- grade 1: palpable during valsalva manoeuvre, but not otherwise;
- grade 2: palpable at rest, but not visible;
- grade 3: visible and palpable at rest.

### 6.3 Diagnosis

The diagnosis of varicocele is made by clinical examination and can be confirmed by colour Doppler analysis (2). In centres where treatment is carried out by antegrade or retrograde sclerotherapy or embolisation, diagnosis is additionally confirmed by X-ray.

### 6.4 Basic considerations

#### 6.4.1 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 (4). 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 (5). Current information fits with the hypothesis that in some men the presence of varicocele is associated with progressive testicular damage from adolescence onwards, and consequent reduction in fertility. Varicocele is associated



with increased sperm DNA damage, and this sperm pathology may be secondary to varicocele-mediated oxidative stress. Varicocelectomy can reverse this sperm DNA damage, as shown in several studies (6).

#### 6.4.2 **Varicocelectomy**

Varicocele repair has been a subject of debate for decades: controversy exists as to whether varicocele repair results in more spontaneous pregnancies as compared to observation. The 2009 Cochrane Database review concluded that there is no evidence that treatment of the varicocele improves a couples' chance of conception (7). This meta-analysis was criticised for including several heterogenous studies, men with normal semen analysis and men with a subclinical varicocele (8). In 3 randomised controlled studies varicocele repair in men with a subclinical varicocele was found to be ineffective (9-11). Also, studies of men with a varicocele and normal semen analysis showed no clear benefit of treatment over observation (12,13).

The duration of the infertility also seems of importance: in a recent study it was shown that couples with an infertility duration of more than 2 years had a significant higher pregnancy rate compared to couples with an uncorrected varicocele. In couples with a shorter duration of infertility, such a difference was not observed (14).

In a recent meta-analysis of 4 RCTs on varicocelectomy in men with a clinical varicocele, oligospermia and otherwise unexplained infertility a trend in favour of surgical correction was observed (15). The combined odds ratio was 2.23 (95% confidence interval [CI], 0.86-5.78; p=0.091), indicating that varicocelectomy is moderately superior to observation, but the effect was not statistically significant.

There is a need for a large, properly conducted RCT of varicocele treatment in men with abnormal semen from couples with otherwise unexplained subfertility (16). While treatment of varicocele in infertile men may be effective, in adolescents there is a significant risk of overtreatment: most adolescents with a varicocele will have no problem achieving pregnancy later in life (17).

### 6.5 **Treatment**

Several treatments are available for varicocele (Table 9). The type of intervention chosen depends mainly on the experience of the therapist. Although laparoscopic varicocelectomy is feasible, it must be justified in terms of cost effectiveness.

**Table 9: Recurrence and complication rates associated with treatments for varicocele**

Treatment	Ref.	Recurrence/ persistence %	Complication rates
Antegrade sclerotherapy	18	9	Complication rate 0.3-2.2%: testicular atrophy, scrotal haematoma, epididymitis, left-flank erythema
Retrograde sclerotherapy	19	9.8	Adverse reaction to contrast medium, flank pain, persistent thrombophlebitis, vascular perforation
Retrograde embolisation	20,21	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
<i>Open operation</i>			
Scrotal operation		-	Testicular atrophy, arterial damage with risk of devascularisation and gangrene of testicle, scrotal haematoma, post-operative hydrocele
Inguinal approach	22	13.3	Possibility of missing out a branch of testicular vein
High ligation	23	29	5-10% incidence of hydrocele (< 1%)
Microsurgical inguinal or subinguinal	24,25	0.8-4	Post-operative hydrocele arterial injury, scrotal haematoma
Laparoscopy	26,27	3-7	Injury to testicular artery and lymph vessels, intestinal, vascular and nerve damage, pulmonary embolism, peritonitis, bleeding, post-operative pain in right shoulder (due to diaphragmatic stretching during pneumoperitoneum), pneumoscrotum, wound infection

## 6.6 Conclusions and recommendations for varicocele

Conclusions
Current information supports the hypothesis that the presence of varicocele in some men is associated with progressive testicular damage from adolescence onwards and a consequent reduction in fertility.
Although the treatment of varicocele in adolescents may be effective, there is a significant risk of over-treatment.
Varicocele repair may be effective in men with subnormal semen analysis, a clinical varicocele and otherwise unexplained infertility. Further RCTs are needed to confirm that this subgroup of infertile couples will benefit from treatment.

Recommendations	GR
Varicocele treatment is recommended for adolescents with progressive failure of testicular development documented by serial clinical examination (9,10).	B
No evidence indicates benefit from varicocele treatment in infertile men who have normal semen analysis or in men with subclinical varicocele. In this situation, varicocele treatment cannot be recommended (15-17).	A
Varicocele repair should be considered in case of a clinical varicocele, oligospermia, duration of infertility of at least 2 years and otherwise unexplained infertility in the couple.	B

## 6.7 References

- Hudson RW, Perez Marrero RA, Crawford VA, et al. Hormonal parameters in incidental varicoceles and those causing infertility. *Fertil Steril* 1986 May;45(5):692-700.  
<http://www.ncbi.nlm.nih.gov/pubmed/3084304>
- World Health Organization. WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. Cambridge: Cambridge University Press, 2000.
- Dhabuwala CB, Hamid S, Moghissi KS. Clinical versus subclinical varicocele: improvement in fertility after varicocelectomy. *Fertil Steril* 1992 Apr;57(4):854-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/1555699>
- No authors listed. The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. World Health Organisation. *Fertil Steril* 1992; 57(6):1289-93.
- Argawal A, Deepinder F, Cocuzza M, et al. Efficacy of varicocelectomy in improving semen parameters: new meta-analytical approach. *Urology* 2007 Sep;70(3):532-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/17905111>
- Zini A, Dohle G. Are varicoceles associated with increased deoxyribonucleic acid fragmentation? *Fertil Steril* 2011 Dec;96(6):1283-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/22035729>
- Evers JH, Collins J, Clarke J. Surgery or embolisation for varicoceles in subfertile men. *Cochrane Database Syst Rev* 2009 Jan 21;(1):CD000479.  
<http://www.ncbi.nlm.nih.gov/pubmed/19160180>
- Ficarra V, Cerruto MA, Iguori G et al. Treatment of varicocele in subfertile men: The Cochrane review - a contrary opinion. *Eur Urol* 2006 Feb;49(2):258-63.  
<http://www.ncbi.nlm.nih.gov/pubmed/16426727>
- Grasso M, Lania C, Castelli M, et al. Low-grade left varicocele in patients over 30 years old: the effect of spermatic vein ligation on fertility. *BJU Int* 2000 Feb;85(3):305-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/10671887>
- Yamamoto M, Hibi H, Hirata Y, et al. Effect of varicocelectomy on sperm parameters and pregnancy rates in patients with subclinical varicocele: a randomized prospective controlled study. *J Urol* 1996 May;155(5):1636-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/8627841>
- Unal D, Yeni E, Verit A, et al. Clomiphene citrate versus varicocelectomy in treatment of subclinical varicocele: a prospective randomized study. *Int J Urol* 2001 May;8(5):227-30.  
<http://www.ncbi.nlm.nih.gov/pubmed/11328423>
- Nilsson S, Edvinsson A, Nilsson B. Improvement of semen and pregnancy rate after ligation and division of the internal spermatic vein: fact or fiction? *Br J Urol* 1979 Dec;51(6):591-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/534846>



13. Breznik R, Vlaisavljevic V, Borko E. Treatment of varicocele and male fertility. *Arch Androl* 1993 May-June;30(3):157-60.  
<http://www.ncbi.nlm.nih.gov/pubmed/8498867>
14. Giagulli VA, Carbone MD. Varicocele correction for infertility: which patients to treat? *Int J Androl* 2011 Jun;34(3):236-41.  
<http://www.ncbi.nlm.nih.gov/pubmed/20579135>
15. Baazeem A, Belzile E, Ciampi A, et al. Varicocele and male factor infertility treatment: a new meta-analysis and review of the role of varicocele repair. *Eur Urol* 2011 Oct;60(4):796-808.  
<http://www.ncbi.nlm.nih.gov/pubmed/21733620>
16. Abdel-Meguid, TA, Al-Sayyad A, Tayib A, et al. Does varicocele repair improve male infertility? An evidence-based perspective from a randomized, controlled trial. *Eur Urol* 2011 Mar;59(3):455-61.  
<http://www.ncbi.nlm.nih.gov/pubmed/21196073>
17. Zargooshi J. Sperm count and sperm motility in incidental high-grade varicocele. *Fertil Steril* 2007 Nov;88(5):1470-3.  
<http://www.ncbi.nlm.nih.gov/pubmed/17451695>
18. Tauber R, Johnsen N. Antegrade scrotal sclerotherapy for the treatment of varicocele: technique and late results. *J Urol* 1994 Feb;151(2):386-90.  
<http://www.ncbi.nlm.nih.gov/pubmed/8283530>
19. Sigmund G, Bahren W, Gall H, et al. Idiopathic varicoceles: feasibility of percutaneous sclerotherapy. *Radiology* 1987 Jul;164(1):161-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/3588899>
20. Seyferth W, Jecht E, Zeitler E. Percutaneous sclerotherapy of varicocele. *Radiology* 1981 May;139(2):335-40.  
<http://www.ncbi.nlm.nih.gov/pubmed/7220877>
21. Lenk S, Fahlenkamp D, Glied V, et al. Comparison of different methods of treating varicocele. *J Androl* 1994 Nov-Dec;15(Suppl):34S-37S.  
<http://www.ncbi.nlm.nih.gov/pubmed/7721674>
22. Ivanissevich O. Left varicocele due to reflux; experience with 4,470 operative cases in forty-two years. *J Int Coll Surg* 1960 Dec;34:742-755.  
<http://www.ncbi.nlm.nih.gov/pubmed/13718224>
23. Palomo A. Radical cure of varicocele by a new technique; preliminary report. *J Urol* 1949 Mar;61(3):604-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/18114752>
24. Goldstein M, Gilbert BR, Dicker AP, et al. Microsurgical inguinal varicocelectomy with delivery of the testis: an artery and lymphatic sparing technique. *J Urol* 1992 Dec;148(6):1808-11.  
<http://www.ncbi.nlm.nih.gov/pubmed/1433614>
25. Jungwirth A, Gögüs C, Hauser G, et al. Clinical outcome of microsurgical subinguinal varicocelectomy in infertile men. *Andrologia* 2001 Mar;33(2):71-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/11350369>
26. Miersch WD, Schoeneich G, Winter P, et al. Laparoscopic varicocelectomy: indication, technique and surgical results. *Br J Urol* 1995 Nov;76(5):636-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/8535687>
27. Tan SM, Ng FC, Ravintharan T, et al. Laparoscopic varicocelectomy: technique and results. *Br J Urol* 1995 Apr;75(4):523-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/7788264>

## 7. HYPOGONADISM

### 7.1 Introduction

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. The symptoms and signs of hypoandrogenism presenting before and after completion of puberty are given in Table 10.

**Table 10: Symptoms and signs of hypogonadism debuting before and after completion of puberty\***

Affected organ/function	Before completed puberty	After completed puberty
Larynx	No voice mutation	No voice mutation
Hair	Horizontal pubic hairline Straight frontal hairline Diminished beard growth	Diminished secondary body hair
Skin	Absent sebum production Lack of acne Pallor Skin wrinkling	Decreased sebum production Lack of acne Pallor Skin wrinkling
Bones	Eunuchoid tall stature Osteoporosis	Osteoporosis
Bone marrow	Mild anaemia	Mild anaemia
Muscles	Underdeveloped	Hypotrophy
Prostate	Underdeveloped	Hypotrophy
Penis	Infantile	No change of size
Testes	Possibly maldescended testes Small volume	Decrease of testicular volume
Spermatogenesis	Not initiated	Involuted
Libido and potency	Not developed	Loss

\*Modified from Nieschlag et al. (1998) (1).

The aetiological and pathogenetic mechanisms of male hypogonadism can be divided into three main categories:

1. Primary (hypergonadotrophic) hypogonadism due to testicular failure.
2. Secondary (hypogonadotrophic) hypogonadism caused by insufficient gonadotrophin-releasing hormone (GnRH) and/or gonadotrophin (FSH, LH) secretion.
3. Androgen insensitivity (end-organ resistance).

The most common conditions within these three categories are given in Table 11 (see also Chapter 4: Genetic disorders in infertility).

**Table 11: Disorders with male hypogonadism\***

<b>Primary (hypergonadotropic) hypogonadism (testicular failure)*</b>
Anorchia
Maldescended testes
Klinefelter's syndrome
Y chromosome microdeletions
Numerical and structural chromosomal anomalies
Trauma, testicular torsion, orchitis
Iatrogenic (surgery, medications, irradiation, cytostatic drugs)
Exogenous factors (toxins, heat, occupational hazards)
Systemic diseases (liver cirrhosis, renal failure)
Testicular tumour
Varicocele
Idiopathic
<b>Secondary (hypogonadotropic) hypogonadism (secondary testicular failure)</b>
Congenital
o Idiopathic hypogonadotrophic hypogonadism
o Normosmic
o Iposmic/anosmic (Kallmann syndrome)

Acquired (tumours in the following regions) <ul style="list-style-type: none"> <li>o Dyencephalon (craniopharyngiomas, meningiomas)</li> <li>o Hypothalamus or pituitary</li> </ul>
Empty sella
Granulomatous illnesses
Fractures of the skull base
Ischaemic or haemorrhagic lesions in hypothalamic area
Hyperprolactinaemia
Drugs/anabolic steroids, radiotherapy
<b>Target organ resistance to androgens</b>
Testicular feminisation
Reifenstein's syndrome

## 7.2 Hypogonadotrophic hypogonadism: aetiology, diagnosis and therapeutic management

Idiopathic hypogonadotrophic hypogonadism (IHH) is characterised by low levels of gonadotrophins and sex steroid in the absence of anatomical or functional abnormalities of the hypothalamic-pituitary-gonadal axis (2). Idiopathic HH may be an isolated condition or may be associated with anosmia/hyposmia (Kallmann syndrome). Genetic factors causing a deficit of gonadotrophins may act at the hypothalamic or pituitary level. Mutations in candidate genes (X-linked or autosomal) can be found in about 30% of congenital cases (2) and should be screened prior to assisted reproduction (3).

Acquired hypogonadotrophic hypogonadism can be caused by some drugs, hormones, anabolic steroids, and by tumours. A suspected tumour requires imaging (CT or MR) of the sella region and a complete endocrine work-up.

The failure of hormonal regulation can easily be determined (4). Endocrine deficiency leads to a lack of spermatogenesis and testosterone secretion as a result of decreased secretion of FSH and LH. After having excluded secondary forms (drug, hormones, tumours), the therapy of choice depends on whether the goal is to achieve normal androgen levels or to achieve fertility.

Normal androgen levels and subsequent development of secondary sex characteristics (in cases of onset of hypogonadism before puberty) and eugonadal state can be achieved by androgen replacement alone. However, the stimulation of sperm production requires treatment with human chorionic gonadotrophin (hCG) combined with recombinant FSH or urinary FSH or human menopausal gonadotropins (HMG). In the rare cases of 'fertile eunuchs', who have sufficient production of FSH but not LH, treatment with hCG alone may be sufficient to stimulate sperm production and to achieve normal testosterone levels (5).

If hypogonadotrophic hypogonadism is hypothalamic in origin, an alternative to hCG treatment is therapy with pulsatile GnRH (6). 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. Once pregnancy has been established, patients can return to testosterone substitution.

## 7.3 Hypergonadotrophic hypogonadism: aetiology, diagnosis and therapeutic management

Many conditions are associated in men with hypogonadotrophic hypogonadism (Table 11, see also Chapter 4: Genetic disorders in infertility). Most conditions listed in Table 11 only affect the reproductive function of the testis so that only the FSH level is elevated. However, it has been reported that men with infertility problems are at higher risk for developing impaired Leydig cell function (7), while men with Klinefelter's syndrome often show high LH values and develop hypoandrogenism with ageing (8). 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 (9).

Hypogonadism affecting both reproductive and endocrine functions of the testis occurs after treatment with GnRH analogues or surgical castration for prostatic cancer (10).

The laboratory diagnosis of hypergonadotrophic hypogonadism is based on a high level of FSH, decreased serum testosterone and increased LH levels (3). Testosterone levels should be evaluated in view of the concentration of the serum concentration of sex hormone binding globulin (SHBG). Based on levels of total testosterone and SHBG, free and bioavailable testosterone can be calculated (<http://www.issam.ch/freetesto.htm>).

Due to diurnal variation, blood samples for testosterone assessment should be taken before 10.00 am. The existing guidelines for androgen replacement are based on mainly total testosterone levels. There is general agreement that a total testosterone level > 12 nmol / L (350 ng / dL) does not require substitution. Similarly,

based on the data of younger men, there is consensus that patients with serum total testosterone levels < 8 nmol / L (230 ng / dL) will usually benefit from testosterone treatment. If the serum total testosterone level is between 8 and 12 nmol/L, testosterone supplementation is based on the presence of symptoms.

In obese men, decision-making may be helped by measuring total testosterone with SHBG to calculate free testosterone or measurement of free testosterone by equilibrium dialysis (11). Injectable, oral and transdermal testosterone preparations are available for clinical use (3). The best preparation to use is one that maintains serum testosterone levels as near as possible to physiological concentrations (11-13).

#### 7.4 Conclusion and recommendation for hypogonadism

Conclusion
It is generally agreed that patients with primary or secondary hypogonadism associated with hypoandrogenism should receive testosterone substitution therapy.

Recommendation	GR
Effective drug therapy is available to achieve fertility in men with hypogonadotropic hypogonadism (4).	A

#### 7.5 References

1. Andrology. In: Nieschlag E, Behre HM (eds). *Male Reproductive Health and Dysfunction*. Berlin: Springer Verlag, 1997, Chapter 5, pp. 83-7.
2. Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nat Rev Endocrinol* 2009 Oct;5(10):569-76.  
<http://www.ncbi.nlm.nih.gov/pubmed/19707180>
3. Krausz C. Genetic aspects of male infertility. *European Urological Review* 2009;3(2):93-6.
4. World Health Organization. WHO manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. Cambridge: Cambridge University Press, 2000.
5. Burris AS, Rodbard HW, Winters SJ, et al. Gonadotropin therapy in men with isolated hypogonadotropic hypogonadism: the response to human chorionic gonadotropin is predicted by initial testicular size. *J Clin Endocrinol Metab* 1988 Jun;66(6):1144-51.  
<http://www.ncbi.nlm.nih.gov/pubmed/3372679>
6. Schopohl J, Mehlretter G, von Zumbusch R, et al. Comparison of gonadotropin-releasing hormone and gonadotropin therapy in male patients with idiopathic hypothalamic hypogonadism. *Fertil Steril* 1991 Dec;56(6):1143-50.  
<http://www.ncbi.nlm.nih.gov/pubmed/1743335>
7. Andersson AM, Jørgensen N, Frydelund-Larsen L, et al. Impaired Leydig cell function in infertile men: a study of 357 idiopathic infertile men and 318 proven fertile controls. *J Clin Endocrinol Metab* 2004 Jul;89(7):3161-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/15240588>
8. Lanfranco F, Kamischke A, Zitzmann M, et al. Klinefelter's syndrome. *Lancet* 2004 Jul;364(9430): 273-83.  
<http://www.ncbi.nlm.nih.gov/pubmed/15262106>.
9. Manning M, Junemann KP, Alken P. Decrease in testosterone blood concentrations after testicular sperm extraction for intracytoplasmic sperm injection in azoospermic men. *Lancet* 1998 Jul;352(9121):37.  
<http://www.ncbi.nlm.nih.gov/pubmed/9800753>
10. Daniell HW. Osteoporosis after orchiectomy for prostate cancer. *J Urol* 1997 Feb;157(2):439-44.  
<http://www.ncbi.nlm.nih.gov/pubmed/8996327>
11. Finkelstein JS. Androgens and bone metabolism. In: Nieschlag E, Behre HM (eds). *Testosterone: Action, Deficiency, Substitution*. 2nd edn. Berlin: Springer-Verlag, 1998, pp. 187-207.
12. Nieschlag E, Swerdloff R, Behre HM, et al. Investigation, treatment and monitoring of late-onset hypogonadism in males: ISA, ISSAM, and EAU recommendations. *Int J Androl* 2005 Jun;28(3):125-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/15910536>
13. Nieschlag E, Wang C, Handelsman DJ, et al. Guidelines for the Use of Androgens in Men. Geneva: WHO, 1992.

## 8. CRYPTORCHIDISM

### 8.1 Introduction

Cryptorchidism is the most common congenital abnormality of the male genitalia and is found in 2-5% of newborn boys, depending on gestational age (cryptorchidism occurs more often in premature boys) and age after birth. At the age of 3 months, the incidence of cryptorchidism falls spontaneously to 1-2%. Approximately 20% of undescended testes are non-palpable and may be located within the abdominal cavity.

The aetiology of cryptorchidism is multifactorial, involving disrupted endocrine regulation and several gene defects. The normal descent of the testes requires a normal hypothalamo-pituitary-gonadal axis. Endocrine disruption in early pregnancy can potentially affect gonadal development and normal descent of the testes; however, most boys with maldescended testes show no endocrine abnormalities after birth. 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 (1).

### 8.2 Incidence of cryptorchidism

The Caucasian population has a three-fold higher incidence of cryptorchidism compared to African-Americans. Even between Caucasians, there are significant differences in the risk of cryptorchidism, e.g. it is significantly more common among Danish than Finnish newborns (2). Premature babies have a much higher incidence of cryptorchidism than full-term babies. In a British study, the incidence of cryptorchidism was 2.7% in more than 3,000 boys weighing > 2500 g and 21% in premature boys weighing < 2500 g. At the age of 3 months, spontaneous descent occurred in most boys, and the incidence of cryptorchidism fell to 0.9% and 1.7%, in the > 2500 g and < 2500 g group, respectively (3).

### 8.3 Testicular descent and maldescent

The process of testicular descent has two distinct phases: transabdominal and inguinal. During transabdominal descent, development of the gubernaculum and genitoinguinal ligament plays an important role. The anti-Müllerian hormone regulates the transabdominal descent of the testes. Induction of the gubernaculum depends on a functional *Ins3* gene in mice (4). This gene is expressed in Leydig cells and its targeted deletion causes bilateral cryptorchidism with free-moving testes and genital ducts (5). Androgens play an important role in both phases of testicular descent, while other gene families, e.g. the homeobox (HOX) and GREAT/RXFP2 genes (G-protein-coupled receptor affecting testis descent), are important in the development of genital organs and may be associated with testicular maldescent (6,7).

### 8.4 Hormonal control of testicular descent

Maldescent can be caused by two hormonal factors: hypogonadism and androgen insensitivity. The increasing incidence of reproductive abnormalities in male humans can be explained by increased oestrogen exposure during gestation (8). Some pesticides and synthetic chemicals act as hormonal modulators, often possessing oestrogenic activity (xeno-oestrogens) (9). The oestrogenic and anti-androgenic properties of these chemicals may cause hypospadias, cryptorchidism, reduced sperm density, and an increased incidence of testicular tumours in animal models, via receptor-mediated mechanisms or direct toxic effects associated with Leydig cell dysfunction (10).

### 8.5 Pathophysiological effects in maldescended testes

#### 8.5.1 Degeneration of germ cells

The degeneration of germ cells in maldescended testes is apparent after the first year of life. Degenerative changes vary, depending on the position of the testis (11). During the second year, the number of germ cells declines. In 10-45% of affected patients, the complete loss of germ cells can be detected. Early treatment is therefore recommended to conserve spermatogenesis, especially in bilateral cases. Surgical treatment is the most effective and reliable method of bringing testes into the scrotum. Hormone treatment with hCG has been used widely in the past, but it has now been abolished because of increased germ cell apoptosis after treatment (12).

#### 8.5.2 Relationship with fertility

Semen parameters are often impaired in men with a history of cryptorchidism (13). Surgical treatment during the first or second year of life may have a positive effect on subsequent fertility (14). However, there is no definitive proof of the protective effect of early orchidopexy. 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 unilateral cryptorchidism, paternity is independent of age at orchidopexy and pre-

operative testicular location and testicular size (15). However, a history of unilateral cryptorchidism may result in reduced fertility potential and therefore a longer time to achieve pregnancy.

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%. In cases of bilateral cryptorchidism and azoospermia, orchidopexy performed even in adult life might lead to the appearance of spermatozoa in the ejaculate (16).

### 8.5.3 **Germ cell tumours**

Cryptorchidism is a risk factor for testicular cancer and is associated with testicular microcalcification and intratubular germ cell neoplasia of unclassified type [ITGCNU] former "CIS" of the testis. In 5-10% of testicular cancers, there is a history of cryptorchidism (17). The risk of a germ cell tumour 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 (17). Orchidopexy performed before the age of puberty has been reported to decrease the risk of testicular cancer (18). However, this and other similar reports are based on retrospective data and does not exclude the possibility that boys undergoing early and late orchidopexy represent different pathogenetic groups of testicular maldescent.

## 8.6 **Treatment of undescended testes**

### 8.6.1 **Hormonal treatment**

Human chorionic gonadotrophin or GnRH has been used widely in the past to treat cryptorchidism. However, although 15-20% of retained testes descend during hormonal treatment, one-fifth of these re-ascend later. Also, treatment with hCG may be harmful to future spermatogenesis by increasing the apoptosis of germ cells (12), which is why hormonal treatment is no longer recommended.

### 8.6.2 **Surgical treatment**

The success rate of surgical treatment for undescended testes is 70-90% (19). If the spermatic cords or the spermatic vessels are too short to allow proper mobilisation of the testis into the scrotum, a staged orchidopexy (Fowler-Stephenson procedure) can be performed, using open surgery, laparoscopy or microsurgery.

The optimal age for performing orchidopexy is still debated. Some retrospective studies have indicated early treatment (during the first 2 years of life) has a beneficial effect on preserving future fertility (20), while a recent randomised study showed that surgery at 9 months resulted in a partial catch-up of testicular growth until at least age 4 years versus surgery at 3 years. The results clearly indicate that early surgery has a beneficial effect on testicular growth. Because testicular volume is an approximate indirect measure of spermatogenic activity, it is possible that orchidopexy at an early age might improve future spermatogenesis.

A biopsy at the time of orchidopexy (see section 8.5.3) can reveal intratubular germ cell neoplasia of unclassified type [ITGCNU], which can be removed thereby preventing development of a malignant tumour. If not corrected by adulthood, an 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 (16).

Vascular damage is the most severe complication of orchidopexy and can cause testicular atrophy in 1-2% of cases. In males with non-palpable testes, the post-operative atrophy rate was 12% in those cases with long vascular pedicles that enabled scrotal positioning. Post-operative atrophy in staged orchidopexy has been reported in up to 40% of patients (19).

## 8.7 **Conclusions and recommendations for cryptorchidism**

<b>Conclusions</b>
Cryptorchidism is multifactorial in origin and can be caused by genetic factors and endocrine disruption early in pregnancy.
Cryptorchidism is often associated with testicular dysgenesis and is a risk factor for infertility and germ cell tumours.
Whether early surgical intervention can prevent germ cell loss is still debatable, but in a randomised study it improved testicular growth in boys treated at the age of 9 months compared to those aged 3 years at the time of orchidopexy.
Paternity in men with unilateral cryptorchidism is almost equal to that in men without cryptorchidism.
Bilateral cryptorchidism significantly reduces the likelihood of paternity.



Recommendations	GR
Hormonal treatment of cryptorchidism should be abolished because of the risk of germ cell apoptosis and subsequent reduction of sperm production.	B
Early orchidopexy (6-12 months of age) might be beneficial for testicular development in adulthood.	B
If undescended testes are corrected in adulthood, testicular biopsy for detection of intratubular germ cell neoplasia of unclassified type [ITGCNU; former "CIS] is recommended at the time of orchidopexy (17).	B

## 8.8 References

- Skakkebaek NS, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001 May;16(5):972-8. <http://www.ncbi.nlm.nih.gov/pubmed/11331648>
- Boisen KA, Kaleva M, Main KM, et al. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet* 2004 Apr;363(9417):1264-9. <http://www.ncbi.nlm.nih.gov/pubmed/15094270>
- Heyns CF, Hutson JM. Historical review of theories on testicular descent. *J Urol* 1995 Mar;153(3 Pt 1):754-67. <http://www.ncbi.nlm.nih.gov/pubmed/7861531>
- Scorer CG. The descent of the testis. *Arch Dis Child* 1964 Dec;39:605-9. <http://www.ncbi.nlm.nih.gov/pubmed/14230757>
- Nguyen MT, Showalter PR, Timmons CF, et al. Effects of orchiopexy on congenitally cryptorchid insulin-3 knockout mice. *J Urol* 2002 Oct;168(4 Pt 2):1779-83. <http://www.ncbi.nlm.nih.gov/pubmed/12352358>
- Lewis AG, Pecha BR, Smith EP, et al. Early orchidopexy restores fertility in Hoxa 11 gene knockout mouse. *J Urol* 2003 Jul;170(1):302-5. <http://www.ncbi.nlm.nih.gov/pubmed/12796710>
- Gorlov IP, Kamat A, Bogatcheva NV, et al. Mutations of the GREAT gene cause cryptorchidism. *Hum Mol Genet* 2002 Sep;11(19):2309-18. <http://www.ncbi.nlm.nih.gov/pubmed/12217959>
- Hadziselimovic F, Geneto R, Emmons LR. Elevated placental estradiol: a possible etiological factor of human cryptorchidism. *J Urol* 2000 Nov;164(5):1694-5. <http://www.ncbi.nlm.nih.gov/pubmed/11025750>
- Hosi S, Loff S, Witt K, et al. Is there a correlation between organochlorine compounds and undescended testes? *Eur J Pediatr Surg* 2000 Oct;10(5):304-9. <http://www.ncbi.nlm.nih.gov/pubmed/11194541>
- Mahood IK, Scott HM, Brown R, et al. In utero exposure to di(n-butyl) phthalate and testicular dysgenesis: comparison of fetal and adult end points and their dose sensitivity. *Environ Health Perspect* 2007 Dec;115 Suppl 1:55-61. <http://www.ncbi.nlm.nih.gov/pubmed/18174951>
- Garcia J, González N, Gómez ME, et al. Clinical and anatomopathological study of 2000 cryptorchid testes. *Br J Urol* 1995 Jun;75(6):697-701. <http://www.ncbi.nlm.nih.gov/pubmed/7613821>
- Ritzén EM. Undescended testes: a consensus on management. *Eur J Endocrinol* 2008 Dec;159 Suppl 1:S87-90. <http://www.ncbi.nlm.nih.gov/pubmed/18728121>
- Yavetz H, Harash B, Paz G, et al. Cryptorchidism: incidence and sperm quality in infertile men. *Andrologia* 1992 Sep-Oct;24(5):293-7. <http://www.ncbi.nlm.nih.gov/pubmed/1356318>
- Wilkerson ML, Bartone FF, Fox L, et al. Fertility potential: a comparison of intra-abdominal and intracanalicular testes by age groups in children. *Horm Res* 2001;55(1):18-20. <http://www.ncbi.nlm.nih.gov/pubmed/11423737>
- Miller KD, Coughlin MT, Lee PA. Fertility after unilateral cryptorchidism: paternity, time to conception, pretreatment testicular location and size, hormone and sperm parameters. *Horm Res* 2001; 55(5):249-53. <http://www.ncbi.nlm.nih.gov/pubmed/11740148>
- Giwercman A, Hansen LL, Skakkebaek NE. Initiation of sperm production after bilateral orchiopexy: clinical and biological implications. *J Urol* 2000 Apr;163(4):1255-6. <http://www.ncbi.nlm.nih.gov/pubmed/10737515>

17. Giwercman A, Bruun E, Frimodt-Moller C, et al. Prevalence of carcinoma in situ and other histopathological abnormalities in testes of men with a history of cryptorchidism. *J Urol* 1989 Oct; 142(4):998-1001.  
<http://www.ncbi.nlm.nih.gov/pubmed/2571738>
18. Pettersson A, Richiardi L, Nordenskjold A, et al. Age at surgery for undescended testis and risk of testicular cancer. *N Engl J Med* 2007 May 3;356(18):1835-41.  
<http://www.ncbi.nlm.nih.gov/pubmed/17476009>
19. Jones PF. Approaches to orchidopexy. *Br J Urol* 1995 Jun;75(6):693-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/7613820>
20. Hadziselimovic F, Hocht B, Herzog B, et al. Infertility in cryptorchidism is linked to the stage of germ cell development at orchidopexy. *Horm Res* 2007;68(1):46-52.  
<http://www.ncbi.nlm.nih.gov/pubmed/17356291>

## 9. IDIOPATHIC MALE INFERTILITY

### 9.1 Introduction

No demonstrable cause of male infertility, other than idiopathic OAT syndrome, is found in at least 44% of infertile men (1).

### 9.2 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 (2). Androgens, hCG/human menopausal gonadotrophin, bromocriptine, alpha-blockers, systemic corticosteroids and magnesium supplementation are not effective in the treatment of OAT syndrome. Follicle-stimulating hormone (3) and anti-oestrogens in combination with testosterone (4) might be beneficial in a selection of patients (3,4). A Cochrane analysis showed that men taking oral antioxidants had an associated statistically significant increase in live birth rate (pooled odds ratio (OR) = 4.85; 95% CI: 1.92-12.24; p = 0.0008; I(2) = 0%) when compared with men taking the control. No studies reported harmful side effects from the antioxidant therapy used. The evidence suggests that antioxidant supplementation in subfertile males may improve the outcomes of live birth and pregnancy rate for subfertile couples undergoing ART cycles. Further head-to-head comparisons are necessary to identify the superiority of one antioxidant over another (5).

Recommendation	GR
Medical treatment of male infertility is recommended only for cases of hypogonadotrophic hypogonadism (1).	A

### 9.3 References

1. Pierik FH, Van Ginneken AM, Dohle GR, et al. The advantages of standardized evaluation of male infertility. *Int J Androl* 2000 Dec;23(6):340-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/11114979>
2. Foresta C, Bettella A, Spolaore D, et al. Suppression of the high endogenous levels of plasma FSH in infertile men are associated with improved Sertoli cell function as reflected by elevated levels of plasma inhibin B. *Hum Reprod* 2004 Jun;19(6):1431-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/15117900>
3. Paradisi R, Busacchi P, Seracchioli R, et al. Effects of high doses of recombinant human follicle stimulating hormone in the treatment of male factor infertility: results of a pilot study. *Fertil Steril* 2006 Sep;86(3):728-31.  
<http://www.ncbi.nlm.nih.gov/pubmed/16782097>
4. Adamopoulos DA, Pappa A, Billa E, et al. Effectiveness of combined tamoxifen citrate and testosterone undecanoate treatment in men with idiopathic oligozoospermia. *Fertil Steril* 2003 Oct;80(4):914-20.  
<http://www.ncbi.nlm.nih.gov/pubmed/14556812>
5. Showell MG, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. *Cochrane Database Syst Rev* 2011 Jan 19;(1):CD007411.  
<http://www.ncbi.nlm.nih.gov/pubmed/21249690>



# 10. MALE CONTRACEPTION

## 10.1 Introduction

'Male contribution to contraception' is a more accurate phrase than 'male contraception', as men do not conceive. 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 (1).

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 (2). For men to take more responsibility for family planning, male contraceptive methods must be acceptable, cheap, reversible, and effective.

Research is attempting to (3):

- Prevent sperm production by using exogenic androgens, progestogen and GnRH formulations in various combinations).
- Interfere with the ability of sperm to mature and fertilise, by using an epididymal approach to create a hostile environment for sperm.
- Produce better barrier methods, e.g. polyurethane condoms can be used by those with latex allergy, although they have higher breakage rates (4).
- Produce an antisperm contraceptive vaccine (5).
- Inhibit sperm-egg interactions.

These approaches remain experimental. The method nearest to being generally available clinically is hormonal male contraception, which is based on the suppression of gonadotrophins and testosterone substitution to maintain male sexual function and bone mineralisation and to prevent muscle wasting (6). 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. There are racial differences in the response to androgens alone. However, a combination of testosterone with progestin has resulted in complete suppression of spermatogenesis in all races, and provides contraceptive efficacy equivalent to female hormonal methods (7). Phase III clinical trials of depot preparations of androgen/progestin combinations are in progress.

## 10.2 Vasectomy

Vasectomy is an effective method of permanent male surgical sterilisation (8). 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 (9).

### 10.2.1 Surgical techniques

Various techniques are available for vasectomy. The least invasive approach is the no-scalpel vasectomy (10), which is also associated with a low rate of complications (11). The most effective occlusion technique is cauterisation of the lumen of the vas deferens and fascial interposition (12-14). Most techniques can be carried out safely under local anaesthesia in an outpatient clinic.

### 10.2.2 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 (17).

Acute local complications associated with vasectomy include haematoma, wound infection, and epididymitis in up to 5% of cases (15). The potential long-term complications (e.g. chronic testicular pain) (16) must be discussed with the patient before the procedure. Epididymal tubal damage is common, and is associated with consequent development of sperm granuloma and time-dependent secondary epididymal obstruction, which limits vasectomy reversal.

### 10.2.3 Vasectomy failure

If an effective occlusion technique is used, the risk of recanalisation after vasectomy should be < 1% (12). However, patients should be informed pre-operatively that, although rare, long-term re-canalisation might occur (19). 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' with non-motile spermatozoa < 10,000/mL is still under discussion (18).

#### 10.2.4 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 (15).
- Vasectomy involving cauterisation and fascial interposition appears to be the most effective technique (12-14).

### 10.3 Vasectomy reversal

A wide range of surgical success rates has 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 (vaso-vasostomy or vaso-epididymostomy), and whether reversal was unilateral or bilateral. However, there have been no randomised controlled trials comparing macrosurgery (loops) and microsurgery. Microsurgical techniques with the help of magnification and smaller suture materials should be used (20).

#### 10.3.1 Length of time since vasectomy

Vaso-vasostomy 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%, respectively, for 3-8 years, 79% and 44%, respectively, for 9-14 years, and 71% and 30%, respectively, for > 15 years (21).

#### 10.3.2 Epididymo-vasostomy

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, epididymo-vasostomy is needed to reverse the vasectomy (see above Chapter 5: Obstructive azoospermia) (22).

#### 10.3.3 Microsurgical vasectomy reversal versus epididymal or testicular sperm retrieval and ICSI

According to the calculations of cost per delivery for vasectomy reversal versus 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 (23,24). Sperm retrieval and ICSI must yield an 81% pregnancy rate per cycle to achieve equal costs to vasectomy reversal.

### 10.4 Conclusions and recommendations for male contraception

Conclusions
The most cost-effective approach to treatment of post-vasectomy infertility is microsurgical reversal. This procedure is also associated with the highest chance of pregnancy.
Pregnancy is still achievable after successful vasectomy reversal.
MESA/TESE/PESA (25) and ICSI should be reserved for failed vasectomy reversal surgery.
All available data indicate vasectomy is not associated with any serious, long-term, side effects (15).
Fascial interposition and cauterisation appears to be the most effective vasectomy technique (12-14).

Recommendations	GR
Patients seeking consultation about vasectomy must be informed about the surgical method, risk of failure, irreversibility, the need for post-procedure contraception until clearance, and the risk of complications.	C
Methods of male contraception other than vasectomy are associated with high failure rates or are still experimental (e.g. hormonal approach).	B
Microsurgical vasectomy reversal is a low-risk and (cost-) effective method of restoring fertility.	B

For couples wanting to achieve pregnancy, sperm aspiration together with ICSI is a second-line option for selected cases and in those with failed vaso-vasostomy.

B

## 10.5 References

1. Reproductive Health Strategy. Reproductive Health Research World Health Organisation, Geneva. Adopted at the 57th World Health Assembly, 2004.  
<http://www.who.int/reproductive-health/publications/strategy.pdf>
2. Handelsman D, Waites G. Traditional methods. In: Schill W, Comhaire F, Hargreave T (eds). *Andrology for the Clinician*. Berlin: Springer Verlag, 2006, pp. 122-4.
3. Griffin D, Ringheim K. Male hormonal contraception. What prospects exist and how acceptable are they? *Plan Parent Chall* 1996;(2):20-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/12291936>
4. Gallo MF, Grimes DA, Lopez LM, et al. Non-latex versus latex male condoms for contraception. *Cochrane Database Syst Rev* 2006 Jan 25;(1):CD003550.  
<http://www.ncbi.nlm.nih.gov/pubmed/16437459>
5. Naz RK. Antisperm immunity for contraception. *J Androl* 2006 Mar-Apr;27(2):153-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/16474022>
6. Matthiesson KL, McLachlan RI. Male hormonal contraception: concept proven, product in sight? *Hum Reprod Update* 2006 Jul-Aug;12(4):463-82.  
<http://www.ncbi.nlm.nih.gov/pubmed/16597629>
7. Handelsman DJ, Waites GMH. Hormonal male contraception. In: Schill W, Comhaire F, Hargreave T (eds). *Andrology for the Clinician*. Berlin: Springer Verlag, 2006, pp. 520-4.
8. Schwingl PJ, Guess HA. Safety and effectiveness of vasectomy. *Fertil Steril* 2000 May;73(5):923-36.  
<http://www.ncbi.nlm.nih.gov/pubmed/10785217>
9. Holden CA, McLachlan RI, Cumming R, et al. Sexual activity, fertility and contraceptive use in middle-aged and older men: Men in Australia, Telephone Survey (MATeS). *Hum Reprod* 2005 Dec; 20(12):3429-34.  
<http://www.ncbi.nlm.nih.gov/pubmed/16172145>
10. Li SQ, Goldstein M, Zhu J, et al. The no-scalpel vasectomy. *J Urol* 1991 Feb;145(2):341-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/1988727>
11. Nirapathpongporn A, Huber D, Krieger N. No-scalpel vasectomy at the King's birthday vasectomy festival. *Lancet* 1990 Apr;335(8694):894-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/1969992>
12. Sokal, D, Irsula, B, Hays M, et al; Investigator Study Group. Vasectomy by ligation and excision, with or without fascial interposition: a randomized controlled trial. *BMC Med* 2004 Mar;2:6.  
<http://www.ncbi.nlm.nih.gov/pubmed/15056388>
13. Barone MA, Irsula B, Chen-Mok M, et al; Investigator Study Group. Effectiveness of vasectomy using cautery. *BMC Urol* 2004 Jul;19;4:10.  
<http://www.ncbi.nlm.nih.gov/pubmed/15260885>
14. Sokal DC, Irsula B, Chen-Mok M, et al. A comparison of vas occlusion techniques: cautery more effective than ligation and excision with fascial interposition. *BMC Urol* 2004 Oct;4(1):12.  
<http://www.ncbi.nlm.nih.gov/pubmed/15509302>
15. Schwingl PJ, Guess HA. Safety and effectiveness of vasectomy. *Fertil Steril* 2000 May;73(5):923-36.  
<http://www.ncbi.nlm.nih.gov/pubmed/10785217>
16. Christiansen CG, Sandlow JI. Testicular pain following vasectomy: a review of postvasectomy pain syndrome. *J Androl* 2003 May-Jun;24(3):293-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/12721203>
17. Bernal-Delgado E, Latour-Pérez J, Pradas-Arnal F, et al. The association between vasectomy and prostate cancer: a systematic review of the literature. *Fertil Steril* 1998 Aug;70(2):191-200.  
<http://www.ncbi.nlm.nih.gov/pubmed/9696205>
18. Davies AH, Sharp RJ, Cranston D, et al. The long-term outcome following 'special clearance' after vasectomy. *Br J Urol* 1990 Aug;66(2):211-2.  
<http://www.ncbi.nlm.nih.gov/pubmed/2390708>
19. Verhulst APM, Hoekstra JW. Paternity after bilateral vasectomy. *BJU Int* 1999 Feb;83(3):280-2.  
<http://www.ncbi.nlm.nih.gov/pubmed/10233494>
20. Schroeder-Printzen I, Diemer T, Weidner W. Vasovasostomy. *Urol Int* 2003;70(2):101-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/12592037>
21. Belker AM, Thomas AJ Jr, Fuchs EF, et al. Results of 1,469 microsurgical vasectomy reversals by the Vasovasostomy Study Group. *J Urol* 1991 Mar;145(3):505-11.  
<http://www.ncbi.nlm.nih.gov/pubmed/1997700>

22. Chan PT, Brandell RA, Goldstein M. Prospective analysis of outcomes after microsurgical intussusception vasoepididymostomy. *BJU Int* 2005 Sep;96(4):598-601. <http://www.ncbi.nlm.nih.gov/pubmed/16104917>.
23. Pavlovich CP, Schlegel PN. Fertility options after vasectomy: a cost-effectiveness analysis. *Fertil Steril* 1997 Jan;67(1):133-41. <http://www.ncbi.nlm.nih.gov/pubmed/8986698>
24. Heidenreich A, Altmann P, Engelmann UH. Microsurgical vasovasostomy versus microsurgical epididymal sperm aspiration/testicular extraction of sperm combined with intracytoplasmic sperm injection. A cost-benefit analysis. *Eur Urol* 2000 May;37(5):609-14. <http://www.ncbi.nlm.nih.gov/pubmed/10765102>
25. Esteves SC, Miyaoka R, Agarwal A. Sperm retrieval techniques for assisted reproduction. *Int Braz J Urol* 2011 Sep-Oct;37(5): 570-83 <http://www.ncbi.nlm.nih.gov/pubmed/22099268>

## 11. MALE ACCESSORY GLAND INFECTIONS

### 11.1 Introduction

Infections of the male urogenital tract are potentially curable causes of male infertility (1-3). The WHO considers urethritis, prostatitis, orchitis and epididymitis to be male accessory gland infections (MAGIs) (2). However, specific data are not available to confirm that these diseases have a negative influence on sperm quality and male fertility in general.

### 11.2 Urethritis

Infectious, sexually acquired urethritis is caused by various pathogens, most often *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Neisseria gonorrhoea* (4). Non-infectious causes of urethritis include irritations as a result of allergic reactions, trauma and manipulations. Urethral discharge and bladder voiding problems are the predominant symptoms of acute urethritis.

#### 11.2.1 Diagnosis and treatment

Diagnosis is based on the analysis of urethral smear and first-voided urine (VB1). Pathognomonic evidence is > 4 granulocytes per microscopic high-power field (×1000) in an urethral smear, or 15 granulocytes per microscopic field (×400) in the smear of the sediment of 3 mL VB1, is pathognomonic (4). In urethritis, defined by inflammatory discharge, semen analysis for disorders of male fertility is not possible because the anterior urethra is full of infectious and inflammatory material that hampers any useful analysis (5).

The impact of urethritis on semen quality and fertility has not been proven because the ejaculate is contaminated with inflammatory material from the urethra.

It is still debated whether infection with sexually transmitted micro-organisms has a negative effect on sperm function (1,6,7). Male fertility can be impaired by urethral strictures, ejaculatory disturbances (2), or the development of obstruction (8). Obstruction can develop as either a normal urethral stricture or a lesion in the posterior urethra in the area of the verumontanum, both of which can lead to ejaculatory disturbances and central obstruction of the seminal pathway (2).

The Centers for Disease Control and Prevention in Atlanta, GA, USA have published guidelines to standardise the treatment of sexually transmitted diseases (9). Because the aetiology of acute urethritis is usually unknown at the time of diagnosis, empirical therapy is used against potential pathogens. A single dose of a fluoroquinolone is given, followed by a 2-week regimen of doxycycline. Treatment is effective both for gonococcal and (co-existing) chlamydial/ureaplasma infections.

### 11.3 Prostatitis

Prostatitis is the most common urological diagnosis in men < 50 years of age (10). Traditionally, prostatitis has been classified into four clinical entities:

- acute bacterial prostatitis (abp) and prostatic abscess as a sequela/complication of abp;
- chronic bacterial prostatitis (cbp);
- non- or abacterial prostatitis (nbp);
- prostatodynia.

To improve the definition and understanding of prostatitis, a classification system has been proposed by the National Institutes of Health (NIH) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (10) (Table 12).

**Table 12: NIH/NIDDK classification of prostatitis syndrome\***

New NIH category	Clinical entity	Description
I	ABP	Acute infection of the prostate gland
II	CBP	Recurrent infection of the prostate
III	Chronic abacterial prostatitis/CPPS	No demonstrable infection
IIIA	Inflammatory CPPS	White cells in semen, expressed prostatic secretions or post-prostatic massage urine
IIIB	Non-inflammatory CPPS	No white cells in semen, expressed prostatic secretions or post-prostatic massage urine
IV	Asymptomatic inflammatory prostatitis	No subjective symptoms. Inflammation detected either by prostate biopsy or by the presence of white cells in expressed prostatic secretions or semen during evaluation for other disorders

\* Adapted from Wagenlehner et al. (10).

ABP = acute bacterial prostatitis; CBP = chronic bacterial prostatitis; CPPS = chronic pelvic pain syndrome.

### 11.3.1 Microbiology

ABP (NIH I), CBP (NIH II) and, more significantly, prostatic abscesses are clinically relevant but uncommon diseases. The most common causes of bacterial prostatitis are Gram-negative bacteria, mainly strains of *Escherichia coli* (11). The role of Gram-positive bacteria in bacterial prostatitis is controversial. Although enterococci can cause bacterial prostatitis and associated recurrent urinary tract infection (UTI), the importance of other Gram-positive bacteria in chronic prostatitis is doubtful (11), as is that of *C. trachomatis* and *Mycoplasma*, particularly *U. urealyticum* (11-15). Hidden bacteria may be aetiologically involved in patients with chronic idiopathic prostatitis after exclusion of typical bacterial infection (16). Detection of bacteria by molecular techniques has not been evaluated definitively.

### 11.3.2 Diagnosis

Symptoms must be evaluated using standardised scores, especially the NIH symptom score (17). Other investigative procedures include laboratory diagnosis of CBP using the four-specimen test for bacterial localisation (10,11), which measures sequential quantitative bacteriological cultures of the urethra, bladder urine and prostatic secretions, both in expressed prostatic excretion (EPS) and urine after prostatic massage (12).

Simplified techniques compare bacterial and leukocyte counts in the urine before and after prostatic massage (18). Screening of bladder voiding and imaging analysis of the prostate gland must be integrated.

The key to diagnosis is the demonstration of leukocytes in EPS, urine after prostatic massage and/or ejaculate to differentiate between inflammatory and non-inflammatory CPPS.

### 11.3.3 Ejaculate analysis

An ejaculate analysis (see Chapter 2: Investigations) 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 CPPS (NIH IIa vs NIH IIIB).

### 11.3.4 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 made for common urinary tract pathogens, particularly Gram-negative bacteria.

A concentration of  $> 10^3$  cfu/mL urinary tract pathogens in the ejaculate is indicative of significant bacteriospermia. Various micro-organisms are found in the genital tract of men seen in infertility clinics, usually with more than one strain of bacteria present (1). The sampling time can influence the positive rate of micro-organisms in semen and the frequency of isolation of different strains (19). The ideal diagnostic test for *C. trachomatis* in semen has not yet been established (14). 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 (14).

*Ureaplasma urealyticum* is pathogenic only in high concentrations ( $> 10^3$  cfu/mL ejaculate). No more than about 10% of samples analysed for ureaplasma exceed this concentration (20). Normal colonisation of the urethra hampers the clarification of mycoplasma-associated urogenital infections, using samples such as the ejaculate (15).

### 11.3.5 **White blood cells**

The clinical significance of an increased concentration of leukocytes in the ejaculate is controversial (21). Infection is indicated only by an increased level of leukocytes (particularly polymorphonuclear leukocytes) and their products (e.g. leukocyte elastase) secreted into the seminal fluid. Most leukocytes are neutrophilic granulocytes, as suggested by the specific staining of the peroxidase reaction (2). Although leukocytospermia is a sign of inflammation, it is not necessarily associated with bacterial or viral infections (7). Earlier findings have shown that elevated leukocyte numbers are not a natural cause of male infertility (22).

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 (23,24). Both studies found more leukocytes in men with prostatitis compared to those without inflammation (CPPS, type NIH IIIb).

### 11.3.6 **Sperm quality**

The deleterious effects of chronic prostatitis on sperm density, motility and morphology are under debate (1). All investigations have given contradictory results, and have not confirmed that chronic prostatitis has a decisive role in altering conventional semen parameters (25-27).

### 11.3.7 **Seminal plasma alterations**

Seminal plasma elastase is a biochemical indicator of polymorphonuclear lymphocyte activity in the ejaculate (1,28,29), with a suggested cut-off level of approximately 600 ng/mL (1). 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 (30-32), but no correlations have been found. The prostate is the main site of origin of IL-6 in the seminal plasma. Cytokines, especially IL-6, play an important role in the male accessory gland inflammatory process (33). However, elevated cytokine levels do not depend on the number of leukocytes in EPS (34).

### 11.3.8 **Glandular secretory dysfunction**

Infections of the sex glands can impair their excretory function. Decreased quantities of citric acid, phosphatase, fructose, zinc, and  $\alpha$ -glutamyl-transferase activity are indicators of disturbed prostatic secretory parameters (1). Reduced fructose concentration indicates impaired vesicular function (20,35).

### 11.3.9 **Sperm antibodies**

Serum antibodies to sperm antigens are not useful in the diagnosis of immune infertility. Early studies found an association between increased levels of sperm antibodies in serum and NBP (36,37). However, except for suspected chlamydial infections (38), only a history of vasectomy is predictive of sperm antibody formation (39).

### 11.3.10 **Reactive oxygen species**

Reactive oxygen species might be increased in chronic urogenital infections associated with increased leukocyte numbers (40). However, their biological significance in prostatitis remains unclear (1).

### 11.3.11 **Therapy**

Treatment of chronic prostatitis is usually targeted at relieving symptoms (10,41). Andrologically, the aims of therapy for altered semen composition in male adnexitis (acute and chronic infections of the male urogenital tract) are:

- reduction or eradication of micro-organisms in prostatic secretions and semen;
- normalisation of inflammatory (e.g. leukocytes) and secretory parameters;
- improvement of sperm parameters to counteract fertility impairment (42).

Treatment includes antibiotics, anti-inflammatory drugs, surgical procedures, normalisation of urine flow, physical therapy and alterations in general and sexual behaviour.

Only antibiotic therapy of CBP (NIH II) has provided symptomatic relief, eradication of microorganisms and a decrease in cellular and humoral inflammatory parameters in urogenital secretions. The use of alpha-blockers for symptom relief is controversial. Although antibiotics might improve sperm quality (42), there is no evidence that treatment of chronic prostatitis increases the probability of conception (1,43).

## 11.4 **Orchitis and epididymo-orchitis**

### 11.4.1 **Introduction**

Orchitis is an inflammatory lesion of the testis associated with a predominantly WBC exudate inside and outside the seminiferous tubules, which potentially results in tubular sclerosis. The inflammation causes pain and swelling. Chronic inflammatory alterations in the seminiferous tubules disrupt the normal process



of spermatogenesis and alter sperm number and quality (44). Orchitis might also be an important cause of spermatogenic arrest (45), which might be reversible in most cases. Testicular atrophy can develop as a result of tubular sclerosis (45).

#### 11.4.2 **Diagnosis**

Epididymo-orchitis usually presents with unilateral scrotal pain (46). Diagnosis is based on past medical history and palpation. Ultrasonography usually indicates a swollen, enlarged testis. The sonographic features of the tissue do not allow any differential diagnosis (47).

#### 11.4.3 **Ejaculate analysis**

Ejaculate analysis, including leukocyte analysis, indicates persistent inflammatory activity. In many cases, especially in acute epididymo-orchitis, transiently decreased sperm counts and reduced forward motility occur (44,46). Obstructive azoospermia caused by complete obstruction is a rare complication. Mumps orchitis can result in bilateral testicular atrophy (45) and non-obstructive azoospermia. When granulomatous orchitis is suspected, sperm-bound autoantibodies occur.

#### 11.4.4 **Therapy**

Only therapy of acute bacterial epididymo-orchitis and of specific granulomatous orchitis is standardised (45) (Table 13). Several regimens improve the inflammatory lesion. Unfortunately, corticosteroids and non-steroidal anti-inflammatory agents (e.g. diclofenac, indomethacin, acetylsalicylic acid) have not been evaluated for their andrological outcome (47). In mumps orchitis, systemic therapy with interferon  $\alpha$ -2b prevents testicular atrophy and azoospermia (50). In idiopathic granulomatous orchitis, surgical removal of the testis is the therapy of choice.

**Table 13: Treatment of epididymo-orchitis**

Condition and pathogen	Treatment
Acute bacterial epididymo-orchitis	
<i>N. gonorrhoeae</i>	Tetracyclines
<i>C. trachomatis</i>	Tetracyclines
<i>E. coli</i> , <i>Enterobacteriaceae</i>	Fluoroquinolones
Mumps orchitis	Interferon $\alpha$ -2b
Non-specific chronic epididymo-orchitis	Steroidal and non-steroidal inflammatory agents
Granulomatous (idiopathic) orchitis	Semi-castration
Specific orchitis	According to therapy of underlying diseases

### 11.5 **Epididymitis**

#### 11.5.1 **Introduction**

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 *N. gonorrhoea* (51,52). Sexually transmitted epididymitis is usually accompanied by urethritis. Non-sexually transmitted epididymitis is associated with UTI and occurs more often in men aged > 35 years, those who have recently undergone urinary tract instrumentation or surgery, and those who have anatomical abnormalities (52).

#### 11.5.2 **Diagnosis**

In acute epididymitis, inflammation and swelling usually start in the tail of the epididymis and can spread to involve the rest of the epididymis and testicular tissue (46). Although men with epididymitis caused by sexually transmitted micro-organisms always have a history of sexual activity, exposure could have occurred several months before onset. The microbial aetiology of epididymitis is usually easy to determine by Gram-stained examination of both a urethral smear for urethritis and of a mid-stream urine specimen for Gram-negative bacteriuria (51,52). Intracellular Gram-negative diplococci on the smear indicate the presence of *N. gonorrhoea*. Only WBCs on urethral smear indicate non-gonorrhoeal urethritis; *C. trachomatis* will be isolated in about two-thirds of these patients (53).

#### 11.5.3 **Ejaculate analysis**

Ejaculate analysis according to WHO criteria, including leukocyte analysis, might indicate persistent inflammatory activity. In many cases, transiently decreased sperm counts and forward motility are observed



(46,48,51). Ipsilateral low-grade orchitis (54,55) might be the cause of this slight impairment in sperm quality (Table 14) (56).

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: Obstructive azoospermia). The extent of azoospermia after epididymitis is unclear.

**Table 14: Acute epididymitis and impact on sperm parameters.**

Authors	Negative influence			
	Density	Motility	Morphology	Comment
Ludwig & Haselberger (57)	+	+	+	Pyospermia in 19 of 22 cases
Berger <i>et al.</i> (51)		+		
Weidner <i>et al.</i> (47)	+	+	+	Azoospermia in 3 of 70 men
Haidl (58)		+		Chronic infections; macrophages elevated
Cooper <i>et al.</i> (59)				Decrease in epididymal markers: $\alpha$ -glucosidase, L-carnitine

#### 11.5.4 Treatment

Antibiotic therapy is indicated before culture results are available (Table 13). 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. gonorrhoea* or *C. trachomatis* must be told to refer their sexual partners for evaluation and treatment (60).

## 11.6 Conclusions and recommendations for male accessory gland infections

Conclusions
Urethritis and prostatitis are not associated clearly with male infertility.
Antibiotic treatment often only eradicates micro-organisms; it has no positive effect on inflammatory alterations, and cannot reverse functional deficits and anatomical dysfunction.

Recommendations	GR
In most cases, the aetiology of acute urethritis is unknown at the time of diagnosis; empirical therapy is therefore suggested using a single dose of a fluoroquinolone, followed by a 2-week regimen of doxycycline. Treatment is effective both for gonococcal and (co-existing) chlamydial/ureaplasma infections (9).	B
Antibiotic therapy of (chronic) bacterial prostatitis has been shown to provide symptomatic relief, eradication of micro-organisms, and a decrease in cellular and humoral inflammatory parameters in urogenital secretions (61-64).	B
Although antibiotic procedures for MAGI might provide improvement in sperm quality, therapy does not necessarily enhance the probability of conception (1,43).	B
Patients with epididymitis that is known or suspected to be caused by <i>N. gonorrhoea</i> or <i>C. trachomatis</i> must be instructed to refer their sexual partners for evaluation and treatment (60).	B

## 11.7 References

1. Weidner W, Krause W, Ludwig M. Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis. *Hum Reprod Update* 1999 Sep-Oct;5(5):421-32.  
<http://www.ncbi.nlm.nih.gov/pubmed/10582781>

2. World Health Organization. *WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male*. Cambridge: Cambridge University Press, 2000.
3. Purvis K, Christiansen E. Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. *Int J Androl* 1993 Feb;16(1):1-13.  
<http://www.ncbi.nlm.nih.gov/pubmed/8468091>
4. Schiefer HG. Microbiology of male urethroadnexitis: diagnostic procedures and criteria for aetiologic classification. *Andrologia* 1998;30(Suppl 1):7-13.  
<http://www.ncbi.nlm.nih.gov/pubmed/9629437>
5. Chambers RM. The mechanism of infection in the urethra, prostate and epididymis. In: Keith LG, Berger GS, Edelman DA (eds). *Infections in Reproductive Health: Common Infections*. Lancaster: MTP Press, pp. 283-96.
6. Ness RB, Markovic N, Carlson CL, et al. Do men become infertile after having sexually transmitted urethritis? An epidemiologic examination. *Fertil Steril* 1997 Aug;68(2):205-13.  
<http://www.ncbi.nlm.nih.gov/pubmed/9240243>
7. Trum JW, Mol BW, Pannekoek Y, et al. Value of detecting leukocytospermia in the diagnosis of genital tract infection in subfertile men. *Fertil Steril* 1998 Aug;70(2):315-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/9696227>
8. Purvis K, Christiansen E. The impact of infection on sperm quality. *J Br Fertil Soc* 1995;1:31-41.
9. Krieger JN. New sexually transmitted diseases treatment guidelines. *J Urol* 1995 Jul;154(1):209-13.  
<http://www.ncbi.nlm.nih.gov/pubmed/7776428>
10. Wagenlehner FM, Diemer T, Naber KG, et al. Chronic bacterial prostatitis (NIH type II): diagnosis, therapy and influence on the fertility status. *Andrologia* 2008 Apr;40(2):100-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/18336459>
11. Naber KG, Weidner W. Chronic prostatitis-an infectious disease? *J Antimicrob Chemother* 2000 Aug;46(2):157-61.  
<http://www.ncbi.nlm.nih.gov/pubmed/10933636>
12. Weidner W, Schiefer HG, Krauss H, et al. Chronic prostatitis: a thorough search for etiologically involved microorganisms in 1,461 patients. *Infection* 1991;19 Suppl 3:S119-25.  
<http://www.ncbi.nlm.nih.gov/pubmed/2055646>
13. Bruce AW, Reid G. Prostatitis associated with *Chlamydia trachomatis* in 6 patients. *J Urol* 1989 Oct;142(4):1006-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/2677408>
14. Taylor-Robinson D. Evaluation and comparison of tests to diagnose *Chlamydia trachomatis* genital infections. *Hum Reprod* 1997 Nov;12(11 Suppl):113-20.  
<http://www.ncbi.nlm.nih.gov/pubmed/9433967>
15. Taylor-Robinson D. Infections due to species of *Mycoplasma* and *Ureaplasma*: an update. *Clin Infect Dis* 1996 Oct;23(4):671-684; quiz 683-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/8909826>
16. Krieger JN, Riley DE, Roberts MC, et al. Prokaryotic DNA sequences in patients with chronic idiopathic prostatitis. *J Clin Microbiol* 1996 Dec;34(12):3120-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/8940458>
17. Litwin MS, McNaughton-Collins M, Fowler FJ Jr, et al. The National Institutes of Health chronic prostatitis symptom index: development and validation of a new outcome measure. Chronic Prostatitis Collaboration Research Network. *J Urol* 1999 Aug;162(2):369-75.  
<http://www.ncbi.nlm.nih.gov/pubmed/10411041>
18. Ludwig M, Schroeder-Printzen I, Ludecke G, et al. Comparison of expressed prostatic secretions with urine after prostatic massage-a means to diagnose chronic prostatitis/inflammatory chronic pelvic pain syndrome. *Urology* 2000 Feb;55(2):175-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/10688073>
19. Liversedge NH, Jenkins JM, Keay SD, et al. Antibiotic treatment based on seminal cultures from asymptomatic male partners in in-vitro fertilization is unnecessary and may be detrimental. *Hum Reprod* 1996 Jun;11(6):1227-31.  
<http://www.ncbi.nlm.nih.gov/pubmed/8671429>
20. Weidner W, Krause W, Schiefer HG, et al. Ureaplasma infections of the male urogenital tract, in particular prostatitis, and semen quality. *Urol Int* 1985;40(1):5-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/3883615>
21. Aitken RJ, Baker HW. Seminal leukocytes: passengers, terrorists or good samaritans? *Hum Reprod* 1995 Jul;10(7):1736-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/8582971>

22. Tomlinson MJ, Barratt CLR, Cooke ID. Prospective study of leukocytes and leukocyte subpopulations in semen suggests they are not a cause of male infertility. *Fertil Steril* 1993 Dec;60(6):1069-75.  
<http://www.ncbi.nlm.nih.gov/pubmed/8243688>
23. Krieger JN, Berger RE, Ross SO, et al. Seminal fluid findings in men with nonbacterial prostatitis and prostatodynia. *J Androl* 1996 Dec;17(3):310-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/8792222>
24. Weidner W, Jantos C, Schiefer HG, et al. Semen parameters in men with and without proven chronic prostatitis. *Arch Androl* 1991 May-Jun;26(3):173-83.  
<http://www.ncbi.nlm.nih.gov/pubmed/1872650>
25. Giamarellou H, Tympanidis K, Bitos NA, et al. Infertility and chronic prostatitis. *Andrologia* 1984 Sep-Oct;16(5):417-22.  
<http://www.ncbi.nlm.nih.gov/pubmed/6496959>
26. Christiansen E, Tollefsrud A, Purvis K. Sperm quality in men with chronic abacterial prostatovesiculitis verified by rectal ultrasonography. *Urology* 1991 Dec;38(6):545-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/1746084>
27. Leib Z, Bartoov B, Eltes F, et al. Reduced semen quality caused by chronic abacterial prostatitis: an enigma or reality? *Fertil Steril* 1994 Jun;61(6):1109-16.  
<http://www.ncbi.nlm.nih.gov/pubmed/8194626>
28. Wolff H, Bezold G, Zebhauser M, et al. Impact of clinically silent inflammation on male genital tract organs as reflected by biochemical markers in semen. *J Androl* 1991 Sep-Oct;12(5):331-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/1765569>
29. Wolff H. The biologic significance of white blood cells in semen. *Fertil Steril* 1995 Jun;63(6):1143-57.  
<http://www.ncbi.nlm.nih.gov/pubmed/7750580>
30. Dousset B, Hussenet F, Daudin M, et al. Seminal cytokine concentrations (IL-1beta, IL-2, IL-6, sR IL-2, sR IL-6), semen parameters and blood hormonal status in male infertility. *Hum Reprod* 1997 Jul;12(7):1476-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/9262280>
31. Huleihel M, Lunenfeld E, Levy A, et al. Distinct expression levels of cytokines and soluble cytokine receptors in seminal plasma of fertile and infertile men. *Fertil Steril* 1996 Jul;66(1):135-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/8752625>
32. Shimonovitz S, Barak V, Zacut D, et al. High concentration of soluble interleukin-2 receptors in ejaculate with low sperm motility. *Hum Reprod* 1994 Apr;9(4):653-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/8046017>
33. Zalata A, Hafez T, van Hoecke MJ, et al. Evaluation of beta-endorphin and interleukin-6 in seminal plasma of patients with certain andrological diseases. *Hum Reprod* 1995 Dec;10(12):3161-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/8822435>
34. Alexander RB, Ponniah S, Hasday J, et al. Elevated levels of proinflammatory cytokines in the semen of patients with chronic prostatitis/chronic pelvic pain syndrome. *Urology* 1998 Nov;52(5):744-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/9801092>
35. Comhaire F, Verschraegen G, Vermeulen L. Diagnosis of accessory gland infection and its possible role in male infertility. *Int J Androl* 1980 Feb;3(1):32-45.  
<http://www.ncbi.nlm.nih.gov/pubmed/7409893>
36. Jarow JP, Kirkland JA Jr, Assimos DG. Association of antisperm antibodies with chronic nonbacterial prostatitis. *Urology* 1990 Aug;36(2):154-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/2385884>
37. Witkin SS, Zelikovsky G. Immunosuppression and sperm antibody formation in men with prostatitis. *J Clin Lab Immunol* 1986 Sep;21(1):7-10.  
<http://www.ncbi.nlm.nih.gov/pubmed/3543373>
38. Munoz MG, Witkin SS. Autoimmunity to spermatozoa, asymptomatic Chlamydia trachomatis genital tract infection and gamma delta T lymphocytes in seminal fluid from the male partners of couples with unexplained infertility. *Hum Reprod* 1995 May;10(5):1070-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/7657743>
39. Jarow JP, Sanzone JJ. Risk factors for male partner antisperm antibodies. *J Urol* 1992 Dec;148(6):1805-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/1433613>
40. Depuydt CE, Bosmans E, Zalata A, et al. The relation between reactive oxygen species and cytokines in andrological patients with or without male accessory gland infection. *J Androl* 1996 Nov-Dec;17(6):699-707.  
<http://www.ncbi.nlm.nih.gov/pubmed/9016401>

41. Schaeffer AJ. Clinical practice. Chronic prostatitis and chronic pelvic pain syndrome. *N Engl J Med* 2006 Oct 19;355(16):1690-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/17050893>
42. Weidner W, Ludwig M, Miller J. Therapy in male accessory gland infection-what is fact, what is fiction? *Andrologia* 1998;30(Suppl 1):87-90.  
<http://www.ncbi.nlm.nih.gov/pubmed/9629448>
43. Comhaire FH, Rowe PJ, Farley TM. The effect of doxycycline in infertile couples with male accessory gland infection: a double blind prospective study. *Int J Androl* 1986 Apr;9(2):91-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/3539821>
44. Purvis K, Christiansen E. Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. *Int J Androl* 1993 Feb;16(1):1-13.  
<http://www.ncbi.nlm.nih.gov/pubmed/8468091>
45. Diemer T, Desjardins C. Disorders of Spermatogenesis. In: Knobil E, Neill JD (eds). *Encyclopedia of Reproduction*. Vol 4. San Diego: Academic Press, 1999, pp. 546-56.
46. [No authors listed.] Association of Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases: National guideline for the management of epididymo-orchitis. *Sex Transm Infect* 1999 Aug;75(Suppl 1):S51-3.  
<http://www.ncbi.nlm.nih.gov/pubmed/10616385>
47. Weidner W, Garbe C, Weissbach L, et al. [Initial therapy of acute unilateral epididymitis using ofloxacin. II. Andrological findings.] *Urologe A* 1990 Sep;29(5):277-280. [Article in German]  
<http://www.ncbi.nlm.nih.gov/pubmed/2120839>
48. Weidner W, Krause W. Orchitis. In: Knobil E, Neill JD (eds). *Encyclopedia of Reproduction*. Vol. 3. San Diego: Academic Press, 1999, pp. 92-5.
49. Vicari E, Mongioi A. Effectiveness of long-acting gonadotrophin-releasing hormone agonist treatment in combination with conventional therapy on testicular outcome in human orchitis/epididymo-orchitis. *Hum Reprod* 1995 Aug;10(8):2072-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/8567844>
50. Ruther U, Stilz S, Rohl E, et al. Successful interferon-alpha 2, a therapy for a patient with acute mumps orchitis. *Eur Urol* 1995;27(2):174-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/7744163>
51. Berger RE, Alexander RE, Harnisch JP, et al. Etiology, manifestations and therapy of acute epididymitis: prospective study of 50 cases. *J Urol* 1979 Jun;121(6):750-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/379366>
52. Berger RE. Epididymitis. In: Holmes KK, Mardh PA, Sparling PF et al. (eds). *Sexually Transmitted Diseases*. New York: McGraw-Hill, 1984, pp. 650-62.
53. Weidner W, Schiefer HG, Garbe C. Acute nongonococcal epididymitis. Aetiological and therapeutic aspects. *Drugs* 1987;34(Suppl 1):111-17.  
<http://www.ncbi.nlm.nih.gov/pubmed/3481311>
54. Nilsson S, Obrant KO, Persson PS. Changes in the testis parenchyma caused by acute non-specific epididymitis. *Fertil Steril* 1968 Sep-Oct;19(5):748-57.  
<http://www.ncbi.nlm.nih.gov/pubmed/5676481>
55. Osegbe DN. Testicular function after unilateral bacterial epididymo-orchitis. *Eur Urol* 1991;19(3):204-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/1855525>
56. Weidner W, Krause W, Ludwig M. Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis. *Hum Reprod Update* 1999 Sep-Oct;5(5):421-32.  
<http://www.ncbi.nlm.nih.gov/pubmed/10582781>
57. Ludwig G, Haselberger J. [Epididymitis and fertility. Treatment results in acute unspecific epididymitis.] *Fortschr Med* 1977 Feb;95(7):397-9. [Article in German]  
<http://www.ncbi.nlm.nih.gov/pubmed/849851>
58. Haidl G. Macrophages in semen are indicative of chronic epididymal infection. *Arch Androl* 1990;25(1):5-11.  
<http://www.ncbi.nlm.nih.gov/pubmed/2389992>
59. Cooper TG, Weidner W, Nieschlag E. The influence of inflammation of the human genital tract on secretion of the seminal markers alpha-glucosidase, glycerophosphocholine, carnitine, fructose and citric acid. *Int J Androl* 1990 Oct;13(5):329-36.  
<http://www.ncbi.nlm.nih.gov/pubmed/2283178>
60. Robinson AJ, Grant JB, Spencer RC, et al. Acute epididymitis: why patient and consort must be investigated. *Br J Urol* 1990 Dec;66(6):642-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/2265337>

61. Schneede P, Tenke P, Hofstetter AG. Urinary Tract Infection Working Group of the Health Care Office of the European Association of Urology. Sexually transmitted diseases (STDs)-a synoptic overview for urologists. *Eur Urol* 2003 Jul;44(1):1-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/12814668>
62. Schaeffer AJ, Weidner W, Barbalias GA, et al. Summary consensus statement: diagnosis and management of chronic prostatitis/chronic pelvic pain syndrome. *Eur Urol* 2003;(Suppl 2):1-4.
63. Alexander RB, Probert KJ, Schaeffer AJ, et al; Chronic Prostatitis Collaborative Research Network. Ciprofloxacin or tamsulosin in men with chronic prostatitis/chronic pelvic pain syndrome: a randomized, double-blind trial. *Ann Intern Med* 2004 Oct;141(8):581-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/15492337>
64. Nickel JC, Narayan P, McKay J, et al. Treatment of chronic prostatitis/chronic pelvic pain syndrome with tamsulosin: a randomized double blind trial. *J Urol* 2004 Apr;171(4):1594-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/15017228>

## 12. GERM CELL MALIGNANCY AND TESTICULAR MICROCALCIFICATION

### 12.1 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 always preceded by CIS, and untreated germ cell neoplasia of unclassified type ([ITGCNU] former CIS) will eventually progress to invasive cancer (1,2).

The most convincing evidence for a general decline in male reproductive health is the increase in testicular cancer seen in Western countries (3). In almost all countries with reliable cancer registers, the incidence of testicular cancer has increased (4). 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 dysgenetic testes have an increased risk of developing testicular cancer in adulthood. These cancers arise from premalignant gonocytes or CIS cells (5). Testicular microlithiasis, seen on ultrasound, can be associated with germ cell tumours and CIS of the testis.

### 12.2 Testicular germ cell cancer and reproductive function

Men with TGCT have decreased semen quality, even before cancer is diagnosed (6). 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 14: Semen cryopreservation). Treatment of TGCT can result in additional impairment of semen quality (7).

In addition to spermatogenic failure, patients with TGCT have Leydig cell dysfunction, even in the contralateral testis (8). The risk of hypogonadism may therefore be increased in men treated for TGCT. The measurement of pretreatment 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 (9).

The risk of hypogonadism is most pronounced in TGCT patients treated with  $\geq 3$  cycles of chemotherapy and in patients who have received 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 (10). Even the risk of low libido and erectile dysfunction is increased in TGCT patients (11).

### 12.3 Testicular microlithiasis

Microcalcification inside the testicular parenchyma can be found in 0.6-9% of men referred for testicular ultrasound (12-14). Although the true incidence of microcalcification in the general population is unknown, it is probably rare. However, ultrasound findings of testicular microlithiasis (TM) are common in men with TGCT, cryptorchidism, testicular dysgenesis, infertility, testicular torsion and atrophy, Klinefelter's syndrome,



hypogonadism, male pseudohermaphroditism, varicocele, epididymal cysts, pulmonary microlithiasis and non-Hodgkin's lymphoma. The incidence reported seems to be higher with high-frequency ultrasound machines (16).

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% (17-19), and 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 (20). 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 a 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 and those with a history of TGCT, and contralateral TM (21).

## 12.4 Recommendations for germ cell malignancy and testicular microcalcification

Recommendations	GR
It is important to encourage and educate patients with TM about self-examination, as this might result in early detection of TGCT.	B
Testicular biopsy should be offered to men with TM, who belong to one of the following high-risk groups: infertility and bilateral TM, atrophic testes, undescended testes, and men with a history of TGCT and contralateral TM (21).	B
If there are suspicious findings on physical examination or ultrasound in patients with TM and associated lesions, surgical exploration with testicular biopsy or orchidectomy should be considered.	B
Testicular biopsy, follow-up scrotal ultrasound, routine use of biochemical tumour markers, or abdominal or pelvic computed tomography is not justified for men with isolated TM without associated risk factors (e.g. infertility, cryptorchidism, testicular cancer, atrophic testis) (15).	B
Men with TGCT are at increased risk of developing hypogonadism and sexual dysfunction and should therefore be followed up (10,11).	B

TGCT = testicular germ cell tumour; TM = testicular microlithiasis

## 12.5 References

- Skakkebaek NE. Carcinoma in situ of the testis: frequency and relationship to invasive germ cell tumours in infertile men. *Histopathology* 1978 May;2(3):157-70.  
<http://www.ncbi.nlm.nih.gov/pubmed/27442>
- von der Maase H, Rørth M, Walbom-Jørgensen S, et al. Carcinoma in situ of contralateral testis in patients with testicular germ cell cancer: study of 27 cases in 500 patients. *Br Med J* 1986 Nov;293(6559):1398-401.  
<http://www.ncbi.nlm.nih.gov/pubmed/3026550>
- Jacobsen R, Bostofte E, Engholm G, et al. Risk of testicular cancer in men with abnormal semen characteristics: cohort study. *BMJ* 2000 Sep;321(7264):789-92.  
<http://www.ncbi.nlm.nih.gov/pubmed/11009515>
- Huyghe E, Matsuda T, Thonneau P. Increasing incidence of testicular cancer worldwide: a review. *J Urol* 2003 Jul;170(1):5-11.  
<http://www.ncbi.nlm.nih.gov/pubmed/12796635>
- Giwercman A, Muller J, Skakkebaek NE. Carcinoma in situ of the undescended testis. *Semin Urol* 1988 May;6(2):110-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/2903524>
- Petersen PM, Skakkebaek NE, Vistisen K, et al. Semen quality and reproductive hormones before orchiectomy in men with testicular cancer. *J Clin Oncol* 1999 Mar;17(3):941-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/10071288>
- Eberhard J, Stahl O, Giwercman Y, et al. Impact of therapy and androgen receptor polymorphism on sperm concentration in men treated for testicular germ cell cancer: a longitudinal study. *Hum Reprod* 2004 Jun;19(6):1418-25.  
<http://www.ncbi.nlm.nih.gov/pubmed/15105386>

8. Willemse PH, Sleijfer DT, Sluiter WJ, et al. Altered Leydig cell function in patients with testicular cancer: evidence for bilateral testicular defect. *Acta Endocrinol (Copenh)* 1983 Apr;102(4):616-24. <http://www.ncbi.nlm.nih.gov/pubmed/6133401>
9. Nord C, Bjoro T, Ellingsen D, et al. Gonadal hormones in long-term survivors 10 years after treatment for unilateral testicular cancer. *Eur Urol* 2003 Sep;44(3):322-8. <http://www.ncbi.nlm.nih.gov/pubmed/12932930>
10. Eberhard J, Ståhl O, Cwikiel M, et al. Risk factors for post-treatment hypogonadism in testicular cancer patients. *Eur J Endocrinol* 2008 Apr;158(4):561-70. <http://www.ncbi.nlm.nih.gov/pubmed/18362304>
11. Eberhard J, Ståhl O, Cohn-Cedermark G, et al. Sexual function in men treated for testicular cancer. *J Sex Med* 2009 Jul;6(7):1979-89. <http://www.ncbi.nlm.nih.gov/pubmed/19453896>
12. Parra BL, Venable DD, Gonzalez E, et al. Testicular microlithiasis as a predictor of intratubular germ cell neoplasia. *Urology* 1996 Nov;48(5):797-9. <http://www.ncbi.nlm.nih.gov/pubmed/8911532>
13. Peterson AC, Bauman JM, Light DE, et al. The prevalence of testicular microlithiasis in an asymptomatic population of men 18 to 35 years old. *J Urol* 2001 Dec;166(6):2061-4. <http://www.ncbi.nlm.nih.gov/pubmed/11696707>
14. von Eckardstein S, Tsakmakidis G, Kamischke A, et al. Sonographic testicular microlithiasis as an indicator of premalignant conditions in normal and infertile men. *J Androl* 2001 Sep-Oct;22(5):818-24. <http://www.ncbi.nlm.nih.gov/pubmed/11545295>
15. Thomas K, Wood SJ, Thompson AJ, et al. The incidence and significance of testicular microlithiasis in a subfertile population. *Br J Radiol* 2000 May;73(869):494-7. <http://www.ncbi.nlm.nih.gov/pubmed/10884745>
16. Pierik FH, Dohle GR, van Muiswinkel JM, et al. Is routine scrotal ultrasound advantageous in infertile men? *J Urol* 1999 Nov;162(5):1618-20. <http://www.ncbi.nlm.nih.gov/pubmed/10524881>
17. Derogee M, Bevers RF, Prins HJ, et al. Testicular microlithiasis, a premalignant condition: prevalence, histopathologic findings, and relation to testicular tumor. *Urology* 2001 Jun;57(6):1133-7. <http://www.ncbi.nlm.nih.gov/pubmed/11377326>
18. Miller FN, Sidhu PS. Does testicular microlithiasis matter? A review. *Clin Radiol* 2002 Oct;57(10):883-90. <http://www.ncbi.nlm.nih.gov/pubmed/12413911>
19. Giwercman A, Muller J, Skakkebaek NE. Prevalence of carcinoma in situ and other histopathological abnormalities in testes from 399 men who died suddenly and unexpectedly. *J Urol* 1991 Jan;145(1):77-80. <http://www.ncbi.nlm.nih.gov/pubmed/1984105>
20. de Gouveia Brazao CA, Pierik FH, Oosterhuis JW, et al. Bilateral testicular microlithiasis predicts the presence of the precursor of testicular germ cell tumors in subfertile men. *J Urol* 2004 Jan;171(1):158-60. <http://www.ncbi.nlm.nih.gov/pubmed/14665866>
21. van Casteren NJ, Looijenga LH, Dohle GR. Testicular microlithiasis and carcinoma in situ overview and proposed clinical guideline. *Int J Androl* 2009 Aug;32(4):279-87. <http://www.ncbi.nlm.nih.gov/pubmed/19207616>

## 13. DISORDERS OF EJACULATION

### 13.1 Definition

Disorders of ejaculation are uncommon, but important, causes of male infertility. This group includes several heterogeneous dysfunctions, which can be either organic or functional.

### 13.2 Classification and aetiology

#### 13.2.1 Anejaculation

Anejaculation involves complete absence of antegrade or retrograde ejaculation and is caused by failure of emission of semen from the seminal vesicles, the prostate and the ejaculatory ducts into the urethra (1). True anejaculation is usually associated with a normal orgasmic sensation. Occasionally (e.g. in incomplete spinal cord injuries), this sensation is altered or decreased. True anejaculation is always associated with central or



peripheral nervous system dysfunction or with drugs (2) (Table 15).

**Table 15: Aetiology of anejaculation**

<b>Neurogenic</b>	<b>Drug-related</b>
Spinal cord injury	Antihypertensives
Cauda equina lesion	Antipsychotics
Retroperitoneal lymphadenectomy	Antidepressants
Aortoiliac or horseshoe-kidney surgery	Alcohol
Colorectal surgery	
Multiple sclerosis	
Parkinson's disease	
Autonomic neuropathy (diabetes mellitus)	

**13.2.2 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. Some patients report sporadic events of nocturnal emission or of ejaculation occurring during great emotional excitement unrelated to sexual activity (3).

**13.2.3 Delayed ejaculation**

In delayed ejaculation, abnormal stimulation of the erect penis is needed to achieve orgasm with ejaculation (1). Delayed ejaculation can be considered a mild form of anorgasmia, and both conditions can be found alternately in the same patient. The causes of delayed ejaculation can be psychological or organic, e.g. incomplete spinal cord lesion (3), iatrogenic penile nerve damage (4), or pharmacological, e.g. antidepressants, antihypertensives, antipsychotics (5).

**13.2.4 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, except in paraplegia. Partial antegrade ejaculation must not be confused with the secretion of bulbo-urethral glands. The causes of retrograde ejaculation can be divided into neurogenic, pharmacological, urethral, or bladder neck incompetence (Table 16).

**Table 16: Aetiology of retrograde ejaculation**

<b>Neurogenic</b>	<b>Pharmacological</b>
Spinal cord injury	Antihypertensives
Cauda equina lesions	$\alpha$ 1-adrenoceptor antagonists
Multiple sclerosis	Antipsychotics
Autonomic neuropathy (juvenile diabetes)	Antidepressants
Retroperitoneal lymphadenectomy	<b>Bladder neck incompetence</b>
Sympathectomy	Congenital defects/dysfunction of hemitrigone
Colorectal and anal surgery	Bladder extrophy
<b>Urethral</b>	Bladder neck resection
Ectopic ureterocele	Prostatectomy
Urethral stricture	
Urethral valves or verumontanum hyperplasia	
Congenital dopamine $\beta$ -hydroxylase deficiency	

**13.2.5 Asthenic ejaculation**

Asthenic ejaculation, also defined as partial ejaculatory incompetence or 'ejaculation baveuse' (5), is characterised by an altered propulsive phase, with a normal emission phase. The orgasmic sensation is reduced and the typically rhythmical contractions associated with ejaculation are missing, whereas in asthenic

ejaculation caused by urethral obstruction, these contractions are present. Asthenic ejaculation generally is caused by the neurogenic or urethral pathologies already listed in Table 16. Asthenic ejaculation does not usually affect semen quality.

#### **13.2.6 Premature ejaculation**

Premature ejaculation is the inability to control ejaculation for a sufficient length of time during vaginal penetration. Although a universally accepted definition of sufficient length of time does not exist, some patients are unable to delay ejaculation beyond a few coital thrusts, or even after vaginal penetration. Premature ejaculation may be strictly organic (e.g. prostatitis-related) or psychogenic, primary or acquired, partner-related or non-selective, and can be associated with erectile dysfunction. Premature ejaculation does not impair fertility, provided intravaginal ejaculation occurs. For more extensive discussion on this topic, the EAU Male Sexual Dysfunction guidelines should be consulted.

#### **13.2.7 Painful ejaculation**

Painful ejaculation is usually an acquired condition that is often related to lower urinary tract symptoms (6). It sometimes causes moderate sexual dysfunction. The painful sensation might be felt in the perineum, or urethra and urethral meatus (7). It can be caused by ejaculatory duct obstruction, all types of chronic prostatitis/CPPS, urethritis, urethrocele, antidepressant drugs, and psychological problems.

### **13.3 Diagnosis**

Diagnostic management includes the following recommended procedures.

#### **13.3.1 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, primary or acquired disorder), as well as to psychosexual aspects (education, features of affective relationship, pre-existent psychological trauma, previous psychological therapy).

#### **13.3.2 Physical examination**

Genital and rectal examinations are conducted, including evaluation of the prostate, bulbo-cavernosus reflex and anal sphincter tone. Minimal neurological tests include:

- sensitivity of scrotum, testes, and perineum
- cremasteric and abdominal cutaneous reflex
- leg osteotendinous and plantar reflexes.

#### **13.3.3 Post-ejaculatory urinalysis**

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

#### **13.3.4 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 is also suggested (8).

#### **13.3.5 Optional diagnostic work-up**

This diagnostic workup can include:

- neurophysiological tests (bulbocavernosus evoked response and dorsal nerve somatosensory evoked potentials);
- tests for autonomic neuropathy;
- psychosexual evaluation;
- video-cystometry;
- cystoscopy;
- transrectal ultrasonography;
- uroflowmetry;
- vibratory stimulation of the penis.

### **13.4 Treatment**

Infertility caused by disorders of ejaculation is seldom treated on the basis of aetiology. Treatment usually involves retrieving 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.

### 13.5 Aetiological treatment

If possible, any pharmacological treatment that is interfering with ejaculation should be stopped. In painful ejaculations, tamsulosin can be administered during antidepressant treatment (9). Treatment should be given for urogenital infections (i.e. in cases of painful ejaculation) (8). Dapoxetine, a selective serotonin re-uptake inhibitor (SSRI) has been introduced for the therapy of premature ejaculation (PE) (10), since it appears that PE is related to serotonin levels. If possible, any underlying urethral pathology or metabolic disorder (e.g. diabetes) should be corrected. Psychotherapy is usually not very effective.

### 13.6 Symptomatic treatment

#### 13.6.1 Premature ejaculation (PE)

Premature ejaculation can be treated with the selective SSRI dapoxetine, topical anaesthetic agents to increase intravaginal ejaculation latency time, behavioural therapy and/or psychotherapy. Off-label use of SSRIs (e.g. paroxetine, fluoxetine) should be applied with caution.

#### 13.6.2 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 17). Alternatively, the patient can be encouraged to ejaculate when his bladder is full to increase bladder neck closure (11).

**Table 17: Drug therapy for retrograde ejaculation**

Drug	Dosage regimen	Ref.
Ephedrine sulphate	10-15 mg four times daily	12
Midodrin	5 mg three times daily	13
Brompheniramine maleate	8 mg twice daily	14
Imipramine	25-75 mg three times daily	15
Desipramine	50 mg every second day	16

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.;

Sperm retrieval is timed to coincide with the partner's ovulation. Urine must be alkalinised (pH 7.2-7.8) and osmolarity must be 200-300 mOsmol/kg. The patient is asked to have intercourse or to masturbate. Within 10 minutes after ejaculation, urine must be voided and centrifuged, and the pellet resuspended in 0.5 mL Tyrode's or Ham's F-10 medium, and immediately inseminated (17). Alternatively, a catheter can be applied to the bladder and 10-50 mL Tyrode's or Ham's F-10 medium instilled into the bladder. The patient must ejaculate, and a second catheterisation is carried out immediately to retrieve spermatozoa. The latter treatment minimises contact between spermatozoa and urine (18). If the biological sperm preparation is not of sufficient quality for intrauterine insemination, the couple must undergo *in vitro* reproductive procedures (i.e. ICSI) with fresh or cryopreserved spermatozoa.

#### 13.6.3 Anejaculation

Drug treatment for anejaculation caused by lymphadenectomy and neuropathy or psychosexual therapy in anorgasmic men is not very effective. In all these cases, and in men who have a spinal cord injury, vibrostimulation (i.e. the application of a vibrator to the penis) is first-line therapy.

In anejaculation, vibrostimulation evokes the ejaculation reflex (19), which requires an intact lumbosacral spinal cord segment. Complete spinal injuries and injuries above T10 show a better response to vibrostimulation. Once the safety and efficacy of this procedure has been assessed, patients can manage the process in their own home. Intravaginal insemination using a 10-mL syringe during ovulation can be carried out. If the quality of semen is poor, or ejaculation is retrograde, the couple may enter an IVF programme.

If vibrostimulation has failed, electro-ejaculation is the therapy of choice (20). Electro-ejaculation

involves electric stimulation of the periprostatic nerves via a probe inserted into the rectum, which seems unaffected by reflex arc integrity. Anaesthesia is required except in cases of complete spinal cord injury. In 90% of patients, electrostimulation induces ejaculation, which is retrograde in one-third of cases. Semen quality is often poor and most couples will need to enter an IVF programme (21).

When electro-ejaculation fails or cannot be carried out, sperm can be retrieved from the seminal ducts by aspiration from the vas deferens (22) (see Chapter 5 Obstructive azoospermia) or seminal tract washout (23).

When sperm cannot be retrieved, epididymal obstruction or testicular failure must be suspected. TESE can then be used (8,24). Anejaculation following either surgery for testicular cancer or total mesorectal excision can be prevented using monolateral lymphadenectomy or autonomic nerve preservation (24), respectively.

### 13.7 Conclusion and recommendations for disorders of ejaculation

Conclusion
Ejaculation disorders can be treated using a wide range of drugs and physical stimulation, with a high level of efficacy.

Recommendations	GR
Aetiological treatments for ejaculatory disorders should be offered before sperm collection and ART is performed.	B
Premature ejaculation can be treated successfully with either topical anaesthetic creams or SSRIs (22).	A
In men with spinal cord injury, vibrostimulation and electro-ejaculation are effective methods of sperm retrieval.	B

### 13.8 References

- Buvat J, Glossaire. [Disruptions in ejaculation] In: Buvat J, Jouannet P (eds). *[Ejaculation and its Disruptions.]* Lyon-Villeurbanne: SIMEP, 1984, p. 9. [Book in French]
- Wang R, Monga M, Hellstrom WJG. Ejaculatory dysfunction. In: Comhaire FH (ed). *Male Infertility: Clinical Investigation. Cause, Evaluation and Treatment.* London: Chapman Hall, 1996, pp. 205-221.
- Pryor JP. Erectile and ejaculatory problems in infertility. In: Hargreave TB (ed). *Male Infertility.* Berlin: Springer-Verlag, 1997, pp. 319-36.
- Yachia D. Our experience with penile deformations: incidence, operative techniques, and results. *J Androl* 1994 Nov-Dec;15(Suppl):63S-68S.  
<http://www.ncbi.nlm.nih.gov/pubmed/7721682>
- Rudkin L, Taylor MJ, Hawton K. Strategies for managing sexual dysfunction induced by antidepressant medication. *Cochrane Database Syst Rev* 2004 Oct18;(4):CD003382.  
<http://www.ncbi.nlm.nih.gov/pubmed/15495050>
- Vallancien G, Emberton M, Harving N, et al; Alf-One Study Group. Sexual dysfunction in 1,274 European men suffering from lower urinary tract symptoms. *J Urol* 2003 Jun;169(6):2257-61.  
<http://www.ncbi.nlm.nih.gov/pubmed/12771764>
- Hermabessiere J, Bouquet de la Joliniere J, Buvat J. [Painful ejaculation. Researching organic causes.] In: Buvat J, Jouannet P (eds). *[Ejaculation and its Disruptions.]* Lyon-Villeurbanne: SIMEP, 1984, pp. 129-134. [Book in French]
- Abdel-Hamid IA, El Naggar EA, El Gilany AH. Assessment of as needed use of pharmacotherapy and the pause-squeeze technique in premature ejaculation. *Int J Impot Res* 2001 Feb;13(1):41-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/11313839>
- Demyttenaere K, Huygens R. Painful ejaculation and urinary hesitancy in association with antidepressant therapy: relief with tamsulosin. *Eur Neuropsychopharmacol* 2002 Aug;12(4):337-41.  
<http://www.ncbi.nlm.nih.gov/pubmed/12126873>
- McMahon CG, Porst H. Oral agents for the treatment of premature ejaculation: review of efficacy and safety in the context of the recent international society for sexual medicine criteria for lifelong premature ejaculation. *Sex Med* 2011 Oct;8(10):2707-25.  
<http://www.ncbi.nlm.nih.gov/pubmed/21771283>
- Crich JP, Jequier AM. Infertility in men with retrograde ejaculation: the action of urine on sperm motility, and a simple method for achieving antegrade ejaculation. *Fertil Steril* 1978 Nov;30(5):572-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/720646>

12. Gilja I, Parazajder J, Radej M, et al. Retrograde ejaculation and loss of emission: possibilities of conservative treatment. *Eur Urol* 1994;25(3):226-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/8200405>
13. Jonas D, Linzbach P, Weber W. The use of midodrin in the treatment of ejaculation disorders following retroperitoneal lymphadenectomy. *Eur Urol* 1979;5(3):184-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/87324>
14. Schill WB. Pregnancy after brompheniramine treatment of a diabetic with incomplete emission failure. *Arch Androl* 1990;25(1):101-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/2389987>
15. Brooks ME, Berezin M, Braf Z. Treatment of retrograde ejaculation with imipramine. *Urology* 1980 Apr;15(4):353-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/7190335>
16. Hendry WF. Disorders of ejaculation: congenital, acquired and functional. *Br J Urol* 1998 Sep;82(3):331-41.  
<http://www.ncbi.nlm.nih.gov/pubmed/9772867>
17. Schill WB. Diagnosis and treatment of ejaculatory sterility. In: Paulson JD, Nigro-Vilar A, Lucena E, Martini L (eds). *Andrology. Male Fertility and Sterility*. Orlando: Academic Press, 1986, pp. 599-617.
18. Hotchkiss RS, Pinto AB, Kleegman S. Artificial insemination with semen recovered from the bladder. *Fertil Steril* 1954 Jan-Feb;6(1):37-42.  
<http://www.ncbi.nlm.nih.gov/pubmed/13220644>
19. Brindley GS. Reflex ejaculation under vibratory stimulation in paraplegic men. *Paraplegia* 1981;19(5):299-302.  
<http://www.ncbi.nlm.nih.gov/pubmed/7279433>
20. Elliott S, Rainsbury PA. Treatment of anejaculation. In: Colpi GM, Balerna M (eds). *Treating Male Infertility: New Possibilities*. Basel: Karger AG, 1994, pp. 240-54.
21. Denil J, Kuczyk MA, Schultheiss D, et al. Use of assisted reproductive techniques for treatment of ejaculatory disorders. *Andrologia* 1996;28(Suppl 1):43-51.  
<http://www.ncbi.nlm.nih.gov/pubmed/9082877>
22. Waldinger MD. The neurobiological approach to premature ejaculation. *J Urol* 2002 Dec;168(6):2359-67.  
<http://www.ncbi.nlm.nih.gov/pubmed/12441918>
23. Jankowicz E, Drozdowski W, Pogumirski J. [Idiopathic autonomic neuropathy (pandysautonomia)]. *Neurol Neurochir Pol* 2001 Mar-Apr;35(3):439-52. [Article in Polish]  
<http://www.ncbi.nlm.nih.gov/pubmed/11732267>
24. Maurer CA, Z'Graggen K, Renzulli P, et al. Total mesorectal excision preserves male genital function compared with conventional rectal cancer surgery. *Br J Surg* 2001 Nov;88(11):1501-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/11683749>

## 14. SEMEN CRYOPRESERVATION

### 14.1 Definition

Cryopreservation is the storage of biological material at subzero temperatures [e.g. -80°C 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.

### 14.2 Introduction

Cryopreservation was first developed in the 1940s by veterinarians and adapted for human sperm in the 1950s. The first pregnancy that used cryopreservation took place in 1954 (1). In fertility practice, clinical indications for cryopreservation include storage of sperm, testicular and ovarian tissue and early embryos.

### 14.3 Indications for storage

Storage of sperm is available in many clinics for the following indications:

- Before potentially sterilising chemotherapy or radiotherapy for cancer (2) or for non-malignant diseases.
- Before surgery that might interfere with fertility (e.g. bladder neck surgery in a younger man or removal of a testical in a man with testicular malignancy, before vasectomy).
- 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, multiple sclerosis).

- For men with paraplegia when sperm have been obtained by electro-ejaculation or obtained by using penile vibratory stimulation.
- For men with psychogenic anejaculation, after sperm have been obtained either by electro-ejaculation or a sperm retrieval procedure.
- After gonadotrophin treatment has induced spermatogenesis in men with hypogonadotropic hypogonadism.
- For men with NOA, the chance of finding sperm using micro-TESE is approximately 60-70%. Cryopreservation can be used to separate sperm collection from ICSI, thus avoiding unnecessary hyperstimulation of the female partner. It can also be used to avoid repeated sperm retrieval procedures.
- In any situation where 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 and a quarantine period of 3-6 months reduces the risk of transmission of infection from sperm donors; in most countries, fresh sperm are no longer used.

## **14.4 Precautions and techniques**

### **14.4.1 Freezing and thawing process**

The cryopreservation techniques currently used are not yet optimal as 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 that 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 (3-6). Further damage can be caused by contamination of samples with micro-organisms and high levels of superoxide radicals (7,8). 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 albumen. After freezing, the tissues are immersed in liquid nitrogen.

Several techniques have been developed to try to reduce damage caused by freezing and thawing, including:

- Rapid method (9,10): sample is held in the vapour phase for 10 minutes before being plunged into liquid nitrogen.
- Slow method (11): sample is gradually cooled in the vapour phase for approximately 40 minutes.
- 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 increased storage time and repeated freezing and thawing. The maximum viable storage time for human sperm is not known. Many laboratory or regulatory authorities apply a storage time limit of up to 10 years (12). However, longer storage times are sometimes needed (e.g. for a 17-year-old man who has had sperm stored before undergoing chemotherapy for testicular cancer).

### **14.4.2 Cryopreservation of very small numbers of sperm**

Standard cryopreservation in straws is an efficient way of storing large number of sperm (e.g. for a donor insemination programme). However, in micro-TESE, very few sperm might be obtained, and the choice is either to freeze testicular tissue and find sperm after thawing the tissue, or to freeze very small numbers of sperm. If sperm are frozen in straws, it can be very difficult to find any sperm after thawing. Instead, the sperm should be frozen in a pellet (13) or in a container (14).

### **14.4.3 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. The most widely used safeguard is to accept samples for storage only from patients whose semen samples have been tested for infection and confirmed as safe. Donor samples should be tested for viral (hepatitis B and C, human immunodeficiency virus [HIV]) and sexually transmitted (*C. trachomatis*, gonorrhoea, syphilis) infections.

Until the test results are known, samples must be stored in an individual quarantine vessel (15)



([http://www.hfea.gov.uk/docs/8th\\_Code\\_of\\_Practice\(2\).pdf](http://www.hfea.gov.uk/docs/8th_Code_of_Practice(2).pdf)) [access date December 2011]. Some laboratories use the additional safeguard of double-wrapping the straws before freezing, although this is more costly and can interfere with the freezing process, thus reducing sample quality upon thawing. Some centres carry out cytomegalovirus (CMV) testing and store CMV-negative and CMV-positive samples separately.

Considerable ethical issues surround the storage of samples before cancer chemotherapy for a man who is hepatitis-virus- or HIV-positive. Very 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, as sperm-washing techniques fail in about 5%.

#### 14.4.4 **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. The level of precaution depends on the cost and resources available to the laboratory, but if possible the following safeguards should be in place:

- All in-use storage vessels should be fitted with an alarm system that is activated by rising temperature or liquid nitrogen leakage.
- The alarm system should alert a laboratory staff member, according to a 24-h, 365-day rota.
- Ideally, there should be a spare storage container, in which samples can be transferred following a vessel failure.

#### 14.4.5 **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.

It is best to obtain instructions from the owner of the sample at the time of, or very shortly after storage, about what to do with the sample in the event of death or untraceability. In some countries, owners are legally required to provide instructions/consent. Choices available for the owner of the sample depend on the laws of the country, might not be appropriate in all situations, and include:

- a request that the sample should be destroyed;
- use of the sample by their wife or partner;
- use of the sample in research;
- donation of the sample to help another infertile couple.

### 14.5 **Biological aspects**

Cryopreservation induces deterioration of the seminal quality. After the sample has been thawed, motility (16) and morphology (17,18) are worsened, including mitochondrial acrosomal and sperm tail damage (19). Sperm freezing decreases motility by 31% and mitochondrial activity by 36%, and causes morphological disruption in 37% of sperm (9). 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 (13).

### 14.6 **Conclusions and recommendations for semen cryopreservation**

<b>Conclusions</b>
The purpose of sperm cryopreservation is to enable future ART procedures.
Cryopreservation techniques are not optimal, and future efforts are needed to improve the outcome of sperm banking.
Cryopreservation should be offered and explained in patients with specific diseases, or before a patient undergoes surgery, chemotherapy or radiotherapy that might damage his reproductive integrity.
If testicular biopsies are indicated, sperm cryopreservation is strongly advised.



Recommendations	GR
Cryopreservation of semen should be offered to all men who are candidates for chemotherapy, radiation or surgical interventions that might interfere with spermatogenesis or cause ejaculatory disorders.	B
If cryopreservation is not available locally, patients should be advised about the possibility of visiting, or transferring to, the nearest cryopreservation unit before therapy starts.	C
Consent for cryopreservation should include a record of the man's wishes for his samples if he dies or is otherwise untraceable.	C
Precautions should be taken 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. Samples from men who are positive for hepatitis virus or HIV should not be stored in the same container as samples from men who have been tested and are free from infection.	C

## 14.7 References

- Bunge RG, Keettel WC, Sherman JK. Clinical use of frozen semen; report of four cases. *Fertil Steril* 1954 Nov-Dec;5(6):520-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/13210484>
- Saito K, Suzuki K, Iwasaki A, et al. Sperm cryopreservation before cancer chemotherapy helps in the emotional battle against cancer. *Cancer* 2005 Aug;104(3):521-4.  
<http://www.ncbi.nlm.nih.gov/pubmed/15968690>
- Desrosiers P, Légaré C, Leclerc P, et al. Membranous and structural damage that occur during cryopreservation of human sperm may be time-related events. *Fertil Steril* 2006 Jun;85(6):1744-52.  
<http://www.ncbi.nlm.nih.gov/pubmed/16643911>
- Donnelly ET, McClure N, Lewis SE. Cryopreservation of human semen and prepared sperm: effects on motility parameters and DNA integrity. *Fertil Steril* 2001 Nov;76(5):892-900.  
<http://www.ncbi.nlm.nih.gov/pubmed/11704107>
- Chohan KR, Griffin JT, Carrell DT. Evaluation of chromatin integrity in human sperm using acridine orange staining with different fixatives and after cryopreservation. *Andrologia* 2004 Oct;36(5):321-6.  
<http://www.ncbi.nlm.nih.gov/pubmed/15458552>
- Askari HA, Check JH, Peymer N, et al. Effect of natural antioxidants tocopherol and ascorbic acids in maintenance of sperm activity during freeze-thaw process. *Arch Androl* 1994 Jul-Aug;33(1):11-5.  
<http://www.ncbi.nlm.nih.gov/pubmed/7979804>
- Smith KD, Steinberger E. Survival of spermatozoa in a human sperm bank. Effects of long-term storage in liquid nitrogen. *J Am Med Assoc* 1973 Feb;223(7):774-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/4739258>
- Agarwal A, Said TM. Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. *BJU Int* 2005 Mar;95(4):503-7.  
<http://www.ncbi.nlm.nih.gov/pubmed/15705068>
- Grischenko VI, Dunaevskaya AV, Babenko VI. Cryopreservation of human sperm using rapid cooling rates. *Cryo Letters* 2003 Mar-Apr;24(2):67-76.  
<http://www.ncbi.nlm.nih.gov/pubmed/12819827>
- Sherman JK, Bunge RG. Observations on preservation of human spermatozoa at low temperatures. *Proc Soc Exp Biol Med* 1953 Apr;82(4):686-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/13055973>
- Sawada Y, Ackerman D, Behrman SJ. Motility and respiration of human spermatozoa after cooling to various low temperatures. *Fertil Steril* 1967 Nov-Dec;18(6):775-81.  
<http://www.ncbi.nlm.nih.gov/pubmed/6073928>
- Henry MA, Noiles EE, Gao D, et al. Cryopreservation of human spermatozoa. IV. The effects of cooling rate and warming rate on the maintenance of motility, plasma membrane integrity, and mitochondrial function. *Fertil Steril* 1993 Nov;60(5):911-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/8224279>
- Bahadur G, Ling KL, Hart R, et al. Semen quality and cryopreservation in adolescent cancer patients. *Hum Reprod* 2002 Dec;17(12):3157-61.  
<http://www.ncbi.nlm.nih.gov/pubmed/12456617>

14. Hallak J, Hendin BN, Thomas AJ Jr, et al. Investigation of fertilizing capacity of cryopreserved spermatozoa from patients with cancer. *J Urol* 1998 Apr;159(4):1217-20.  
<http://www.ncbi.nlm.nih.gov/pubmed/9507838?dopt=AbstractPlus>
15. Clarke GN. Sperm cryopreservation: is there a significant risk of cross-contamination? *Hum Reprod* 1999 Dec;14(12):2941-3.  
<http://humrep.oxfordjournals.org/content/14/12/2941.long#sec-1>
16. O'Connell M, McClure N, Lewis SE. The effects of cryopreservation on sperm morphology, motility and mitochondrial function. *Hum Reprod* 2002 Mar;17(3):704-9.  
<http://www.ncbi.nlm.nih.gov/pubmed/11870124>
17. Woolley DM, Richardson DW. Ultrastructural injury to human spermatozoa after freezing and thawing. *J Reprod Fertil* 1978 Jul;53(2):389-94.  
<http://www.ncbi.nlm.nih.gov/pubmed/567693>
18. Watson PF. Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. *Reprod Fertil Dev* 1995;7(4):871-91.  
<http://www.ncbi.nlm.nih.gov/pubmed/8711221>
19. Donnelly ET, McClure N, Lewis SE. Cryopreservation of human semen and prepared sperm: effects on motility parameters and DNA integrity. *Fertil Steril* 2001 Nov;76(5):892-900.  
<http://www.ncbi.nlm.nih.gov/pubmed/11704107>

## 15. ABBREVIATIONS USED IN THE TEXT

*This list is not comprehensive for the most common abbreviations*

ABP	acute bacterial prostatitis
ART	assisted reproduction techniques
CAIS	complete androgen insensitivity syndrome
CBAVD	congenital bilateral absence of the vas deferens
CBP	chronic bacterial prostatitis
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CIS	carcinoma <i>in situ</i>
CMV	cytomegalovirus
CPPS	chronic pelvic pain syndrome
EAA	European Academy of Andrology
EPS	expressed prostatic excretion
FISH	(multicolour) fluorescent <i>in situ</i> hybridisation
FSH	follicle-stimulating hormone
GnRH	gonadotrophin-releasing hormone
GR	grade of recommendation
GREAT	G-protein-coupled receptor affecting testis descent
hCG	human chorionic gonadotrophin
HIV	human immunodeficiency virus
ICSI	intracytoplasmic sperm injection
IHH	idiopathic hypogonadotrophic hypogonadism
IL-6	interleukin-6
ITGCNU	intratubular germ cell neoplasia of unclassified type
IVF	in vitro fertilisation
LE	level of evidence
LH	luteinising hormone
MAGI	male accessory gland infection
MAR	mixed antiglobulin reaction
MESA	microsurgical epididymal sperm aspiration
NBP	non- or abacterial prostatitis
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
NOA	non-obstructive azoospermia
OA	obstructive azoospermia
OAT	oligo-astheno-teratozoospermia [syndrome]
PE	premature ejaculation
PGD	preimplantation genetic diagnosis
SHBG	sex hormone binding globulin
SSRIs	selective serotonin reuptake inhibitors
TDS	testicular dysgenesis syndrome
TEFNA	testicular fine-needle aspiration
TESE	testicular sperm extraction
TGCT	testicular germ cell tumour
TM	testicular microlithiasis
TRUS	transurethral ultrasound
TURED	transurethral resection of the ejaculatory ducts
UTI	urinary tract infection
WBC	white blood cell
VB1	first-voided urine
WHO	World Health Organization

### Conflict of interest

All members of the Male Infertility guidelines writing panel have provided disclosure statements of all relationships they have that may be perceived as a potential source of conflict of interest. This information is kept on file in the European Association of Urology Central Office database. This guidelines 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.