Guidelines on Male Infertility

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European Association of Urology

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1. METHODOLOGY

1.1 Introduction

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

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

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 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 2011 (previous update) and October 2012 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*

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 (1).*

It should be noted that when recommendations are graded, the link between the level of evidence (LE) and grade of recommendation (GR) is not directly linear. Availability of randomised controlled trials (RCTs) may not necessarily translate into a grade A recommendation where there are methodological limitations or disparity in published results.

Alternatively, absence of high level of evidence does not necessarily preclude a grade A recommendation, if there is overwhelming clinical experience and consensus. There may be exceptional situations where corroborating studies cannot be performed, perhaps for ethical or other reasons and in this case 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 - although a very important factor - has to be balanced against benefits and burdens, values and preferences, and costs when a grade is assigned (2-4).

The EAU Guidelines Office does not perform structured cost assessments, nor can they address local/national preferences in a systematic fashion. But whenever these data are available, the expert panel will include the information.

Table 2: Grade of recommendation*

Grade	Nature of recommendations
A	Based on clinical studies of good quality and consistency that addressed the specific recommendations, 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 (1).*

1.4 Publication history

The EAU Male Infertility Guidelines were first published in 2001, followed by full-text updates in 2004, 2007, 2010 and 2013. For this 2014 print a scoping search was done covering 2012 and 2013, with a cut off date of September 2013. Embase, Medline and the Cochrane Central Register of Controlled Trails were searched, with a limitation to reviews, meta-analysis or meta-analysis of RCTs. After de-duplication 447 unique records were identified, of which 5 publications were selected for inclusion. A quick reference guide presenting the main findings of the Male Infertility Guidelines is also available (Pocket Guidelines), as well as a number of scientific publications in the EAU journal European Urology (5-7). The Male Infertility panel published a separate scientific paper on Vasectomy in 2012 (7). All texts can be viewed and downloaded for personal use at the society website: http://www.uroweb.org/guidelines/online-guidelines/.

1.5 Potential conflict of interest statement

The expert panel have submitted potential conflict of interest statements which can be viewed on the EAU website: http://www.uroweb.org/guidelines/.

1.6 Definition

“Infertility is the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year”, World Health Organization (WHO) (8).

1.7 Epidemiology and aetiology

About 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility. One in eight couples encounter problems when attempting to conceive a first child and one in six when attempting to conceive a subsequent child. Three percent of women remain involuntarily childless, while 6% of parous women are not able to have as many children as they would wish (9). 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 (8). Male fertility can be reduced as a result of (8):

- congenital or acquired urogenital abnormalities;
- malignancies;
- urogenital tract infections;
- increased scrotal temperature (e.g. as a consequence of varicocele);
- endocrine disturbances;
- genetic abnormalities;
- immunological factors.

In 30-40% of cases, no male-infertility-associated factor is found (idiopathic male infertility). These men present with no previous history of diseases affecting fertility and have normal findings on physical examination and endocrine 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 is assumed to be caused by several factors, including endocrine disruption as a result of environmental pollution, reactive oxygen species, or genetic and epigenetic abnormalities.

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

Diagnosis	Unselected patients (*n* = 12,945)	Azoospermic patients (*n* = 1,446)
All	100%	11.2%
Infertility of known (possible) cause	42.6%	42.6%
Maldescended testes	8.4	17.2
Varicocele	14.8	10.9
Sperm autoantibodies	3.9	-
Testicular tumour	1.2	2.8
Others	5.0	1.2
Idiopathic infertility	30.0	13.3
Hypogonadism	10.1	16.4
Klinefelter syndrome (47, XXY)	2.6	13.7
XX male	0.1	0.6
Primary hypogonadism of unknown cause	2.3	0.8
Secondary (hypogonadotropic) hypogonadism	1.6	1.9
Kallmann syndrome	0.3	0.5
Idiopathic hypogonadotrophic hypogonadism	0.4	0.4
Residual after pituitary surgery	<0.1	0.3
Others	0.8	0.8
Late-onset hypogonadism	2.2	-
Constitutional delay of puberty	1.4	-
General/systemic disease	2.2	0.5
Cryopreservation due to malignant disease	7.8	12.5
Testicular tumour	5.0	4.3
Lymphoma	1.5	4.6
Leukaemia	0.7	2.2
Sarcoma	0.6	0.9
Disturbance of erection/ejaculation	2.4	-
Obstruction	2.2	10.3
Vasectomy	0.9	5.3
Cystic fibrosis (CBAVD)	0.5	3.1
Others	0.8	1.9

1.8 Prognostic factors

Prognostic factors for male infertility are:

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

The cumulative pregnancy rate is 27% in infertile couples with 2 years of follow-up and oligozoospermia as the primary cause of infertility (11). Female age is the most important single variable influencing outcome in assisted reproduction (12). 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.

1.9 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, because this might determine the final outcome (9).	B
The urologist/andrologist should examine any man with fertility problems for urogenital abnormalities. This applies to all men diagnosed with reduced semen quality. A diagnosis is mandatory to start appropriate therapy (drugs, surgery, or assisted reproduction).	C

1.10 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. Updated by Jeremy Howick March 2009. http://www.cebm.net/index.aspx?o=1025 [Access date February 2014]
2. Atkins D, Best D, Briss PA, et al; GRADE Working Group. Grading quality of evidence and strength of recommendations. BMJ 2004 Jun;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 Apr;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;336(7652):1049-51. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2376019/?tool=pubmed
5. Dohle GR, Colpi GM, Hargreave TB, et al; EAU Working Group on Male Infertility. EAU guidelines on male infertility. Eur Urol 2005 Nov;48(5):703-11. http://www.ncbi.nlm.nih.gov/pubmed/16005562
6. Dohle GR, Diemer T, Kopa Z, et al. European Association of Urology Working Group on Male Infertility. European Association of Urology guidelines on vasectomy. Eur Urol 2012 Jan;61(1):159-63. http://www.ncbi.nlm.nih.gov/pubmed/22033172
7. Jungwirth A, Giwercman A, Tournaye H, et al; European Association of Urology Working Group on Male Infertility. European Association of Urology guidelines on Male Infertility: the 2012 update. Eur Urol 2012 Aug;62(2):324-32. http://www.ncbi.nlm.nih.gov/pubmed/22591628
8. World Health Organization. WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple. Cambridge: Cambridge University Press, 2000. http://www.who.int/reproductivehealth/publications/infertility/9780521431361/en/
9. Greenhall E, Vessey M. The prevalence of subfertility: a review of the current confusion and a report of two new studies. Fertil Steril 1990;54:978-83. http://www.ncbi.nlm.nih.gov/pubmed/2245856
10. Andrology. In: Nieschlag E, Behre HM and Nieschlag S (eds). *Male reproductive health and dysfunction*. 3rd edn. Berlin: Springer Verlag, 2010, Chapter 5, pp. 83-7.
11. 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
12. 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). Important treatment decisions are based on the results of semen analysis, therefore, it is essential that the complete laboratory work-up is standardised. Ejaculate analysis has been standardised by the WHO and disseminated by publication of the *WHO Laboratory Manual for the Examination and Processing of Human Semen* (5th edn.) (1). It is the consensus that modern spermatology must follow these guidelines.

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

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

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

*2.1.1 **Frequency of semen analysis***

If the results of semen analysis are normal according to WHO criteria, one test is sufficient. If the results are abnormal in at least two tests, further andrological investigation is indicated. It is important to differentiate between the following:

- oligozoospermia: spermatozoa < 15 million/mL;
- asthenozoospermia: < 32% motile spermatozoa;
- teratozoospermia: < 4% normal forms.

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

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.	A
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 WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edn.) (1).	A*

**Upgraded following panel consensus*

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 Male. Cambridge: Cambridge University Press, 2000. http://www.who.int/reproductivehealth/publications/infertility/0521774748/en/

3. TESTICULAR DEFICIENCY (PRIMARY SPERMATOGENIC FAILURE)

3.1 Definition

Testicular deficiency as a consequence of primary spermatogenic failure is caused by conditions other than hypothalamic-pituitary disease and obstruction 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)
Acquired	Trauma
	Testicular torsion
	Post-inflammatory forms, particularly mumps orchitis
	Exogenous factors (medications, cytotoxic or anabolic drugs, irradiation, heat)
	Systemic diseases (liver cirrhosis, renal failure)
	Testicular tumour
	Varicocele
	Surgery that may compromise vascularisation of the testes and lead to testicular atrophy
Idiopathic	Unknown aetiology
	Unknown pathogenesis

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 toxins;
- gonadotoxic medication including anabolic drugs;
- exposure to radiation or cytotoxic agents ;
- 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 min and a thorough microscopic examination by phase contrast optics at ×200 magnification of the pellet. All samples can be stained and re-examined microscopically (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 maturation arrest exists at the spermatocyte or spermatid level, FSH values are within the normal range.

However, for an individual patient, FSH levels do not accurately predict the spermatogenesis status (3-5).

*3.4.3 **Testicular biopsy***

Testicular biopsy can be part of intracytoplasmic sperm injection (ICSI) treatment in patients with clinical evidence of NOA. Testicular sperm extraction (TESE) is the technique of choice and shows excellent repeatability (6-8). Spermatogenesis may be focal, which means that in about 50% of men with NOA, spermatozoa can be found and used for ICSI. Most authors therefore recommend taking several testicular samples (9,10). 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 (7,11,12). However no threshold value has been found for FSH, inhibin B, or testicular volume and successful sperm harvesting. When there are complete AZFa and AZFb microdeletions, the likelihood of sperm retrieval is almost zero.

Microsurgical TESE increases retrieval rates versus conventional TESE, and should be preferred in severe cases of of non-obstructive azoospermia (13-16). After opening the testis, an enlarged tubule is excised using micro-scissors or forceps. Then, tubules are minced using mechanical or enzymatic digestion to facilitate sperm search (16). Positive retrievals are reported even in conditions such as Sertoli cell only syndrome type II (1). Percutaneous epididymal sperm aspiration (PESA) results in lower retrieval rates than microsurgical TESE and does not allow histological examination to detect carcinoma *in situ* (CIS) and testicular malignancies (17,18). PESA may also result in more tubular and vascular damage than TESE (19).

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) (20-24). Birth rates are lower in NOA versus OA (19% vs 28%) (25).

- ICSI results in significantly lower fertilisation and implantation rates (26).
- Miscarriage rates are higher in NOA versus OA (11.5% vs 2.5%) (27).
- Neonatal health in terms of birth parameters, major anomalies and chromosomal aberrations in a large cohort of children born after use of non-ejaculated sperm are comparable to the outcome of children born after use of ejaculated sperm (28).

In OA, there were no significant differences in ICSI results between testicular and epididymal sperm (23). Also, no significant differences have been reported in ICSI results between the use of fresh and frozen-thawed sperm (23,25,26).

3.5 Conclusions and recommendations for testicular deficiency

Conclusions	**LE**
Impaired spermatogenesis is often associated with elevated FSH concentration.	3
Spermatozoa are found in about 50% of patients with NOA.	2a
Pregnancies and live births are eventually obtained in 30-50% of couples with NOA, when spermatozoa have been found in the testicular biopsy.	3

Recommendations	**GR**
Men who are candidates for sperm retrieval must receive appropriate genetic counselling.	A
Testicular biopsy is the best procedure to define the histological diagnosis and possibility of finding sperm. Spermatozoa should be cryopreserved for use in ICSI.	A
For patients with NOA who have spermatozoa in their testicular biopsy, ICSI with fresh or cryopreserved spermatozoa is the only therapeutic option.	A
Men with NOA can be offered TESE with cryopreservation of the spermatozoa to be used for ICSI (28).	A
To increase the chances of positive sperm retrieval in men with NOA, TESE (microsurgical or multiple) should be used.	A

3.6 References

1. World Health Organization. WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. Cambridge: Cambridge University Press, 2000. http://www.who.int/reproductivehealth/publications/infertility/0521774748/en/

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. Amer M, Haggar 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
7. 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
8. Vernaeve 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
9. 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
10. 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
11. 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
12. 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
13. 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
14. Deruyver Y, Vanderschueren D, Van der Aa F. Outcome of microdissection TESE compared with conventional TESE in non- obstructive azoospermia: a systematic review. Andrology 2014 Jan;2(1): 20-4.
http://www.ncbi.nlm.nih.gov/pubmed/24193894
15. Marconi M, Keudel A, Diemer T, et al. Combined trifocal and microsurgical testicular sperm extraction is the best technique for testicular sperm retrieval in "low-chance" nonobstructive azoospermia. Eur Urol 2012 Oct;62(4):713-9.
http://www.ncbi.nlm.nih.gov/pubmed/22521095
16. Schwarzer JU, Steinfatt H, Schleyer M, et al. No relationship between biopsy sites near the main testicular vessels or rete testis and successful sperm retrieval using conventional or microdissection biopsies in 220 non-obstructive azoospermic men. Asian J Androl 2013 Nov;15(6):795-8.
http://www.ncbi.nlm.nih.gov/pubmed/24013619
17. Monzo 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
18. Vernaeve V, Tournaye H, Osmanagaoglu K, et al. Intracytoplasmic sperm injection with esticular 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
19. Silber S, Munne 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.

20. Schwarzer J, Fiedler K, Hertwig I, et al. Sperm retrieval procedures and intracytoplasmatic spermatozoa injection with epididymal and testicular sperms. Urol Int 2003;70(2):119-23. http://www.ncbi.nlm.nih.gov/pubmed/12592040
21. 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
22. 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
23. Gil Salom 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
24. 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
25. Vernaeve 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
26. Gil-Salom 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
29. Belva F, De Schrijver F, Tournaye H, et al. Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. Hum Reprod. 2011 Jul;26(7):1752-8. http://www.ncbi.nlm.nih.gov/pubmed/21511713

4. GENETIC DISORDERS IN INFERTILITY

4.1 Introduction

All urologists working in andrology must have an understanding of genetic abnormalities associated with infertility, so that they can provide correct advice to couples seeking fertility treatment. Men with very low sperm counts can be offered a reasonable chance of paternity, using *in vitro* fertilisation (IVF), ICSI, and sperm harvesting from the epididymis or the testes in case of azoospermia. However, the spermatozoa of infertile men show an increased rate of aneuploidy, structural chromosomal abnormalities, and DNA damage, carrying the risk of passing genetic abnormalities to the next generation. Current routine clinical practice is based on the screening of genomic DNA from peripheral blood samples, however, screening of chromosomal anomalies in spermatozoa is also feasible and can be performed in selected cases (1,2).

4.2 Chromosomal abnormalities

Chromosome abnormalities can be numerical (e.g. trisomy) or structural (e.g. inversions or translocations) (3). 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%. In comparison, the incidence of abnormalities was 0.38% in pooled data from three series, with a total of 94,465 newborn male infants, of which 131 (0.14%) were sex chromosome abnormalities and 232 (0.25%) autosomal abnormalities (3). The frequency of chromosomal abnormalities increases as testicular deficiency becomes more severe. Patients with a spermatozoa count < 5 million/mL already show

a 10-fold higher incidence (4%) of mainly autosomal structural abnormalities compared with the general population (5). Men with NOA are at highest risk (6).

Based on the frequencies of chromosomal aberrations in patients with different sperm concentration, karyotype analysis is indicated in men with azoospermia or oligozoospermia (spermatozoa < 10 million/mL) (5,7). If there is a family history of recurrent spontaneous abortions, malformations or mental retardation, karyotype analysis should be requested, regardless of the sperm concentration.

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

Klinefelter's syndrome is the most common sex chromosome abnormality (3,8). Adult men with Klinefelter's syndrome have small firm testicles, devoid of germ cells. The phenotype varies from a normally virilised man to one with the stigmata of androgen deficiency, including female hair distribution, scant body hair, and long arms and legs due to late epiphyseal closure. Leydig cell function is commonly impaired in men with Klinefelter's syndrome (9). 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 should be considered (10). Based on sperm fluorescence *in situ* hybridisation (FISH) studies showing an increased frequency of sex chromosomal abnormalities and increased incidence of autosomal aneuploidy (disomy for chromosomes 13, 18 and 21), concerns have been raised about the chromosomal normality of the embryos generated through ICSI (11).

The production of 24,XY sperm has been reported in 0.9% and 7.0% of men with Klinefelter's mosaicism (12,13) and in 1.36-25% of men with somatic karyotype 47,XXY (14-17). In patients with azoospermia, TESE or (micro-TESE) can be proposed as a therapeutic option since spermatozoa can be recovered in about 30% of cases. To date, 49 healthy children have been born using ICSI without preimplantation genetic diagnosis (PGD) and the conception of one 47,XXY foetus has been reported (8). However, a study of ICSI combined with PGD in 113 embryos reported a significant fall in the rate of normal embryos for couples with Klinefelter's syndrome with respect to controls (54% vs 77.2%) (15). Due to the significant increase of sex chromosomal and autosomal abnormalities in the embryos of Klinefelter's patients, PGD or amniocentesis analysis should be considered.

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

*4.2.2 **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 foetus. As with Klinefelter's syndrome, sperm FISH analysis provide a more accurate risk estimation of affected offspring, however, the diffusion of this genetic test is largely limited by the availability of laboratories able to perform this analysis.

When IVF/ICSI is carried out for men with translocations, PGD or amniocentesis should be performed. Embryos with known unbalanced translocation should not be implanted.

*4.2.3 **Sperm chromosomal abnormalities***

Sperm can be examined for their chromosomal constitution using multicolour FISH both in men with normal karyotype and with anomalies. Aneuploidy in sperm, particularly sex chromosome aneuploidy, is associated with severe damage to spermatogenesis (3,18-20) and with translocations (21).

Florescence *in situ* hybridisation analysis of spermatozoa remains a research investigation, although it has been proposed for clinical use to assess spermatozoa from men with defined andrological conditions (18). Techniques are needed to separate populations of genetically abnormal sperm from normal sperm or to safely screen individual spermatozoa before IVF and ICSI.

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 due to mutation in the KALIG-1 gene on Xp22.3 (22). Several newly identified autosomal gene mutations can also cause Kallmann syndrome (23). Patients with Kallmann syndrome have hypogonadotrophic hypogonadism and anosmia, but may also have other clinical features, including facial asymmetry, cleft palate, colour blindness, deafness, maldescended testes, and unilateral renal aplasia.

Spermatogenesis can be relatively easily induced by hormonal treatment (24), therefore, genetic screening prior to therapy is advisable although it is limited by the rarity of specialised genetic laboratories that can offer this genetic test. Treatment with gonadotropins allows natural conception in most cases, even for men with a relatively low sperm count. Thus, identification of the involved gene (X-linked, autosomal dominant or recessive) can help to provide more accurate genetic counselling, that is, risk estimation for transmission to the offspring.

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 (25). The phenotypic features of complete androgen insensitivity syndrome 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 latter phenotype is also termed Reifenstein syndrome. In the above-mentioned severe forms of androgen resistance, there is no risk of transmission because affected men cannot generate their own biological children using the current technologies. Patients with mild androgen insensitivity syndrome have male infertility as their primary or even sole symptom. Disorders of the androgen receptor causing infertility in the absence of any genital abnormality are rare, and only a few mutations have been reported in infertile (26-29) or fertile (30) men.

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 in particular, premeiotic genes are over-represented on the X-chromosome compared with autosomal chromosomes (31,32). Nevertheless, to date only a few genes have been screened in relatively small populations and none of them appear relevant for male infertility (33,34). Two recent independent studies showed a significantly higher deletion load on the X-chromosome in men with spermatogenic failure with respect to normozoospermic controls (35,36).

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 in 1976 (37). With the advent of molecular genetic tools, microdeletions have been defined in three non-overlapping regions termed AZFa, AZFb and AZFc (38). With knowledge of the precise structure of the Y-chromosome in Yq11, it subsequently became clear that the AZFb and AZFc regions overlap and that there is no AZFd region (39). 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 (40). In each AFZ region, there are several spermatogenesis candidate genes (41). Deletions occur *en bloc* (i.e. removing more than one gene), thus, it is not possible to determine the role of a single AZF gene from the AZF deletion phenotype and it is unclear if they all participate in spermatogenesis. Gene-specific deletions, which remove a single gene, have been reported only in the AZFa region and concern the USP9Y gene. These studies have suggested that USP9Y is most likely to be a “fine tuner” of sperm production, and its specific screening is not advised (42).

4.4.2 *Clinical implications of Y microdeletions*

The clinical significance of Yq microdeletions can be summarised as follows:

- They are not found in normozoospermic men, proving there is a clear cut cause-and-effect relationship between Y-deletions and spermatogenic failure (43).
- The highest frequency of Y-deletions is found in azoospermic men (8-12%), followed by oligozoospermic (3-7%) men.
- Deletions are extremely rare with a sperm concentration > 5 million/mL (~0.7%).
- AZFc deletions are most common (65-70%), followed bY-deletions of the AZFb and AZFb+c or AZFa+b+c regions (25-30%). AZFa region deletions are rare (5%).
- Complete removal of the AZFa region is associated with severe testicular phenotype (Sertoli cell only syndrome), while complete removal of the AZFb region is associated with spermatogenic rest.

Complete removal of the AZFc region causes a variable phenotype ranging from azoospermia to oligozoospermia.

- Classical (complete) AZF deletions do not confer a risk for cryptorchidism or testicular cancer (40).

The specificity and genotype/phenotype correlation reported above means that Y deletion analysis has both a diagnostic and prognostic value for testicular sperm retrieval (40).

4.4.2.1 Testing for Y microdeletions

Indications for AZF deletion screening are based on sperm count and include azoospermia and severe oligozoospermia (spermatozoa count < 5 million/mL). Thanks to the European Academy of Andrology (EAA) guidelines (44) 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 (44). The primers consist of two markers for each region and control markers from the Yp and X-chromosomes. 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 Genetic counselling for AZF deletions

After conception, any Y-deletions are transmitted obligatorily to the male offspring, and genetic counselling is therefore mandatory. In most cases, father and son have the same microdeletion (45-48), but occasionally the son has a larger one (49). The extent of spermatogenic failure (still in the range of azoo-/oligozoospermia) cannot be predicted entirely in the son, due to the different genetic background and the presence or absence of environmental factors with potential toxicity for reproductive function. A significant proportion of spermatozoa from men with complete AZFc deletion are nullisomic for sex chromosomes (50,51), 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%) (52). There are data to support the association of Yq microdeletions with an overall Y-chromosomal instability, which leads to the formation of 45,X0 cell lines (53,54). Despite this theoretical risk, babies born from fathers affected by Yq microdeletions are phenotypically normal (40,44). This could be due to the reduced implantation rate and a likely higher risk of spontaneous abortion of embryos bearing a 45,X0 karyotype.

When ICSI is used in the presence of a Y microdeletion, long-term follow up of any male children is needed with respect to their fertility status and cryopreservation of spermatozoa at a young age can be considered.

4.4.2.3 Y-chromosome: 'gr/gr' deletion

A new type of Yq deletion, known as the gr/gr deletion, has been described in the AZFc region (55). This deletion removes half of the gene content of the AZFc region, affecting the dosage of multicopy genes mapping inside this region. There was an almost eightfold higher risk of developing oligozoospermia [odds ratio (OR) = 7.9, 95% confidence interval (CI): 1.8-33.8; $P < 0.001$] in gr/gr deletion carriers in the largest Caucasian study population published to date (56). The frequency of gr/gr deletion in oligozoospermic patients is ~4%. According to four meta-analyses, gr/gr deletion is a significant risk factor for impaired sperm production (57,58).

However, it is worth noticing that both the frequency of gr/gr deletion and its phenotypic expression vary between different ethnic groups, depending on the Y-chromosome background. For example, in some Y haplogroups, the deletion is fixed and appears to have no negative effect on spermatogenesis. Consequently, the routine screening for gr/gr deletion is a still a debated issue, especially in those laboratories serving diverse ethnic and geographic populations. A large multicentre study has shown that gr/gr deletion is a potential risk factor for testicular germ cell tumours (59). However, these data need further confirmation in an ethnically and geographically matched case-control study setting. For genetic counselling it is worth noticing that partial AZFc deletions (gr/gr and b2/b3) may predispose to complete AZFc deletion in the next generation (60).

4.4.2.4 Conclusions and recommendations on clinical implications of Y microdeletions

Conclusions	LE
gr/gr deletion has been confirmed as a significant risk factor for impaired sperm production, whereas further evidence of the prognostic significance of gr/gr and development of a testicular germ cell tumour is needed.	2b
A son who inherits a complete AZF deletion will have abnormal spermatogenesis because these deletions have not been reported in normozoospermic men.	2a

Recommendations	GR
Testing for microdeletions is not necessary in men with OA (with normal FSH) when ICSI is used because spermatogenesis should be normal.	A
Men with severely damaged spermatogenesis (spermatozoa < 5 million/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).	A
If complete AZFa or AZFb microdeletions are detected, micro-TESE is not necessary because it is extremely unlikely that any sperm will be found.	A
If a man with Yq 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

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-α reductase deficiency	Perineal or scrotal hypospadias, vaginal pouch, immature female phenotype	Autosomal recessive

4.5 Cystic fibrosis mutations and male infertility

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

Congenital bilateral absence of the vas deferens (CBAVD) is associated with *CFTR* gene mutations and was found in ~2% of men with OA attending a clinic in Edinburgh, UK (61). The incidence in men with OA varies between different countries. The clinical diagnosis of absent vasa is easy to miss and all men with azoospermia should be very carefully examined to exclude CBAVD; particularly those with a semen volume < 1.5 mL and pH < 7.0.Approximately 1,500 mutations are listed on the CFTR database (http://www.genet.sickkids.on.ca/cftr/). Many studies have been published of men with CBAVD tested for varying numbers of

mutations. The most frequently found mutations are the ΔF508, R117H and W1282X but their frequency and the presence of other mutations largely depend on the ethnicity of the patient (62,63). Given the functional relevance of a DNA variant (the 5T allele) in a non-coding region of *CFTR* (63), it is now considered a mild *CFTR* mutation rather than a polymorphism and it should be analysed in each CAVD patient.

As more mutations are defined and tested for, almost all men with CBAVD will probably be found to have mutations. It is not practical to test for all known mutations, because many have a very low prevalence in a particular population. Routine testing is usually restricted to the most common mutations in a particular community.

Given that this is a recessive disease, mutations should be found on both alleles of the *CFTR* gene; however, with the routine panel, in most men with CBAVD, mutation is found in only one copy. In these cases a second step analysis is advised which comprises the direct sequencing of the entire gene. Men with CBAVD often have mild clinical stigmata of CF (e.g., history of chest infections).

When a man has CBAVD, it is important to test him and his partner for CF mutations. If the female partner is found to be a carrier of CFTR mutations, the couple must consider very carefully whether to proceed with ICSI using the husband's sperm, as the risk of a having a child with CF or CBAVD will be 50%, depending on the type of mutations carried by the parents. If the female partner is negative for known mutations, the risk of being a carrier of unknown mutations is ~0.4%.

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 and probably has a different genetic causation (64). Consequently, in these subjects *CFTR* mutation screening is not indicated. Men with unilateral absence of the vas deferens are usually fertile, and the condition is most commonly encountered as an incidental finding in the vasectomy clinic. *CFTR* gene mutation screening is indicated in men with unilateral absence of the vas deferens with normal kidneys.

An abdominal ultrasound should be undertaken both in unilateral and bilateral absence of vas deferens. Findings may range from unilateral absence of the vas with ipsilateral absence of the kidney, to bilateral vessel abnormalities and renal abnormalities, such as pelvic kidney (65).

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, 66, 67, and references therein). The introduction of new analytical approaches is likely to provide major advances in this field (68,69).

Intracytoplasmic sperm injection is used to enable men with severely damaged spermatogenesis to father children in situations formerly considered hopeless and where very few spermatozoa can be obtained. This has led to concern that children may be born with a foetal abnormality, because ICSI may enable defective sperm to bypass the selective processes of the female genital tract and egg covering. Alternatively, eggs may be fertilised that would otherwise not be.

Intracytoplasmic sperm injection babies have a higher risk of *de novo* sex chromosomal aberrations (about a threefold increase compared with natural conceptions) and paternally inherited structural abnormalities. Treatment with assisted reproductive technology was associated with increased risks of cardiovascular, musculoskeletal, urogenital, and gastrointestinal defects and cerebral palsy (70-72).

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 an increase of early pregnancy loss (73,74). DNA damage may improve after varicocele ligation (75,76).

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., *CFTR* mutations), there is up to a 50% chance of the child developing a clinical condition. Many clinicians and infertility clinic personnel may consider it 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	LE
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.	3
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 in the future.	2a

Recommendations	GR
Standard karyotype analysis should be offered to all men with damaged spermatogenesis (spermatozoa < 10 million/mL) who are seeking fertility treatment by IVF.	B
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.	A
All men with Klinefelter's syndrome need long-term endocrine follow-up and may require androgen replacement therapy.	A
For men with severely damaged spermatogenesis (spermatozoa < 5 million/mL), testing for Yq microdeletions is strongly advised.	A
When a man has structural abnormalities of the vas deferens (unilateral or bilateral absence), he and his partner should be tested for CF gene mutations.	A

4.11 References

1. Griffin DK, Finch KA. The genetic and cytogenetic basis of male infertility. Human Fertil Mar 2005 Mar;8(1);19-26. http://www.ncbi.nlm.nih.gov/pubmed/15823847
2. 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
3. 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
4. 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
5. 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
6. Foresta C, Ferlin A, Gianaroli L, et al. Guidelines for the appropriate use of genetic tests in infertile couples. Eur J Hum Genet 2002 May;10(5):303-12. http://www.ncbi.nlm.nih.gov/pubmed/12082505
7. Dul EC, Groen H, van Ravenswaaij-Arts CM, et al. The prevalence of chromosomal abnormalities in subgroups of infertile men. Hum Reprod 2012 Jan;27(1):36-43. http://www.ncbi.nlm.nih.gov/pubmed/22740498
8. 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
9. 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
10. 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
11. 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
12. 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

13. 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
14. 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
15. 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
16. Estop AM, Munne S, Cieply 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
17. 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
18. 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
19. 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
20. 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
21. 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
22. 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
23. 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
24. 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
25. 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
26. 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
27. 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
28. 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
29. 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
30 Giwercman A, Kledal T, Schwartz M, et al. Preserved male fertility despite decreased androgen sensitivity caused by a mutation in the ligand-binding domain of the androgen receptor gene. J Clin Endocrinol Metab 2000 Jun;85(6):2253-9. http://www.ncbi.nlm.nih.gov/pubmed/10852459
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 Tüttelmann F, Simoni M, Kliesch S, et al. Copy number variants in patients with severe oligozoospermia and Sertoli-cell-only syndrome. PLoS One 2011 Apr 29;6(4):e19426.
http://www.ncbi.nlm.nih.gov/pubmed/21559371
36 Krausz C, Giachini C, Lo Giacco D, et al. High Resolution X-chromosome-Specific Array-CGH Detects New CNVs in Infertile Males. PLoS One. 2012;7(10):e44887.
http://www.ncbi.nlm.nih.gov/pubmed/23056185
37. 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
38. 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
39. 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
40. 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
41. 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
42. 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
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. 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
45. 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
46. 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
47. Kamischke A, Gromoll J, Simoni M, et al. Transmisson of a Y chromosomal deletion involving the deleted in azoospermia (DAZ) and chromodomain (CDYI) 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
48. 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

49. 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
50. 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
51. 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
52. 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
53. 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
54. 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
55 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
56. 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
57. Stouffs K, Lissens W, Tournaye H, et al. 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
58. Navarro-Costa P, Goncalves 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
59. 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
60. 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
61. 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
62. De Braekeleer M, Ferec 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
63. Chillon 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
64. 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
65. Drake MJ, Quinn FM. Absent vas deferens and ipsilateral multicystic dysplastic kidney in a child. Br J Urol 1996 May;77(5):756-7. [No abstract available]
http://www.ncbi.nlm.nih.gov/pubmed/8689131
66. 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
67. Tuttelmann 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

68. 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
69. 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
70. 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
71. Davies MJ, Moore VM, Willson KJ et al. Reproductive Technologies and the Risk of Birth Defects. N Engl J Med 2012; 366:1803-1813 http://www.ncbi.nlm.nih.gov/pubmed/
72. 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
73. 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
74. 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
75. 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
76. 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 absence of spermatozoa and spermatogenetic 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 or non-obstructive cause.

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 Epididymis detached from the testis (e.g., in some maldescended testes)	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 (Mullerian cysts)	Post-surgical (bladder neck surgery) Post-infective

5.2 Classification

*5.2.1 **Intratesticular obstruction***

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

*5.2.2 **Epididymal obstruction***

Epididymal obstruction or disjunction is the most common cause of OA, affecting 30-67% of azoospermic men with serum FSH level 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 Chapter 4). Other congenital forms of obstruction are rare, for example, 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 Chapter 11). Acute or chronic traumas can result in epididymal damage (9).

Azoospermia caused by surgery may occur after epididymal surgery, for example, 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 vasovasostomy, 5-10% have epididymal blockage as a result of tubule rupture, making epididymovasostomy mandatory (see Chapter 10). Vasal obstruction may also occur after herniotomy (13). Polypropylene mesh herniorrhaphy appears to be able to induce a fibroblastic response that can 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 Chapter 4). 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 1-3% of cases of OA (1) and is classified as either cystic or post-inflammatory.

Cystic obstructions are usually congenital (i.e., Mullerian duct cyst or urogenital sinus/ejaculatory duct cysts) and are 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 Mullerian duct anomalies, the 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 urethroprostatitis (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 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).

Patients should be asked about:

- haematospermia;
- 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 trauma;
- chronic sinopulmonary infection.

*5.3.2 **Clinical examination***

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

- at least one testis with a volume > 15 mL, although a smaller volume may be found in some patients with OA and concomitant partial testicular failure;
- enlarged and hardened epididymis;
- nodules in the epididymis or vas deferens;
- absence or partial atresia of the vas;
- 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 Chapter 2). Azoospermia means the inability to detect spermatozoa after centrifugation at ×400 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 3,000 g for 15 min. The pellet must be examined for spermatozoa.

Ejaculatory duct obstruction or CBAVD is suggested by a semen volume < 1.5 mL, acid pH, and low fructose level. When semen volume is low, a search must be made for spermatozoa in urine after ejaculation, because 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). FSH level 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, or absent vas deferens) and may demonstrate signs of testicular dysgenesis (e.g., non-homogeneous testicular architecture and microcalcifications) and associated CIS 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 round, 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. Mullerian duct or urogenital sinus/ejaculatory duct cysts (20) and ejaculatory duct calcifications (25) are other known anomalies in OA. TRUS 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 simultaneously.

*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 provided (e.g., Johnsen Score) (27).

5.4 Treatment

*5.4.1 **Intratesticular obstruction***

Intratesticularly, seminal duct recanalisation is impossible. TESE allows sperm retrieval in nearly all OA patients

and is therefore recommended. The spermatozoa retrieved may be used immediately for ICSI or should be cryopreserved.

*5.4.2 **Epididymal obstruction***

Microsurgical epididymal sperm aspiration (MESA) (28) is indicated in men with CBAVD. TESE 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 epididymovasostomy is recommended, with the preferred technique being microsurgical intussusception epididymovasostomy (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 preoperative and intra-operative findings (e.g., concomitant abnormal testicular histology, absence of sperm in the spermatic fluid on sectioning the small epididymal tubules, or extensive fibrosis of the epididymis).

*5.4.3 **Proximal vas obstruction***

Proximal vas obstruction after vasectomy requires microsurgical vasectomy reversal (see Chapter 10). Vasovasostomy is also required in rare cases of proximal vasal obstructions (e.g., iatrogenic, post-traumatic, or post-inflammatory). The absence of spermatozoa in the intraoperative vas deferens fluid suggests the presence of a secondary epididymal obstruction; especially if the seminal fluid of the proximal vas has a thick “toothpaste” appearance. Microsurgical tubulovasostomy is then indicated.

*5.4.4 **Distal vas deferens obstruction***

It is usually impossible to correct large bilateral vas deferens defects, resulting from involuntary excision of the vasa deferentia during hernia surgery in early childhood or previous orchidopexy (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 crossover vasovasostomy or tubulovasostomy.

*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). Intraoperative TRUS makes this procedure safer. If distal seminal tract evaluation is carried out at the time of the procedure, installation of methylene blue dye into the vas deferens can help to document opening of the ducts. The limited success rate of surgical treatment of ejaculatory duct obstruction in terms of spontaneous pregnancies should be weighed against sperm aspiration and ICSI.

Complications following TURED include retrograde ejaculation due to bladder neck injury and urine reflux into the ejaculatory ducts, seminal vesicles, and vasa (causing poor sperm motility, semen acid 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	LE
Obstructive lesions of the seminal tract should be suspected in azoospermic or severely oligozoospermic patients with normal-sized testes and normal endocrine parameters.	3

Recommendation	GR
In azoospermia caused by epididymal obstruction, standard procedures include vasovasostomy and tubulovastomy.	B
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	B
In azoospermia caused by epididymal obstruction, scrotal exploration with microsurgical epididymal sperm aspiration and cryopreservation of spermatozoa should be performed. Microsurgical reconstruction should be performed, if applicable. Results of reconstructive microsurgery depend on the cause and location of the obstruction, and the surgeon's expertise.	B

5.6 References

1. Hendry WF. Azoospermia and surgery for testicular obstruction. In: Hargreave TB (ed). *Male Infertility*. Berlin: Springer-Verlag, 1997, pp. 319-36.
2. 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
3. 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
4. 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
5. 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
6. 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
7. 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
8. 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
9. 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. [No abstract available]
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. [No abstract available]
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. [No abstract available]
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, Zumbe 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/clsysrev/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. [No abstract available] 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) with the following andrological implications:

- failure of ipsilateral testicular growth and development;
- symptoms of pain and discomfort;
- male 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 Valsava manoeuvre, but can be shown by special tests (Doppler ultrasound studies) (3);
- grade 1: palpable during Valsava 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 should 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 due to germ cell dysfunction, testicular hypoxia, retrograde flow of metabolites, increased temperature and/or decreased secretion of gonadotropins and androgens, and consequent reduction in fertility. However, the pathophysiology of varicocele still remains unsolved. 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 several 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 varicocele improves a couples' chance of conception (7). This meta-analysis was criticised for including several heterogeneous studies, men with normal semen analysis, and men with a subclinical varicocele (8). In three RCTs repair of a subclinical varicocele was found to be ineffective (9-11). Also, studies of men with a varicocele and normal semen analysis have shown no clear benefit of treatment over observation (12,13).

The duration of infertility also seems to be important. In a recent study it was shown that couples with infertility of > 2 years duration had a significantly higher pregnancy rate after varicocelectomy 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 four RCTs of varicocelectomy in men with a clinical varicocele, oligospermia and otherwise unexplained infertility, there was a trend in favour of surgical correction (15). The combined OR was 2.23 (95% CI, 0.86-5.78; *P* = 0.091), indicating that varicocelectomy was 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). Although 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. Current evidence indicates that microsurgical varicocelectomy is the most effective and least morbid method among the varicocelectomy techniques (17).

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
Open operation			
Scrotal operation		-	Testicular atrophy, arterial damage with risk of devascularisation and testicular gangrene, scrotal haematoma, postoperative 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	Postoperative 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; postoperative pain in right shoulder (due to diaphragmatic stretching during pneumoperitoneum); pneumoscrotum: wound infection

6.6 Conclusions and recommendations for varicocele

Conclusions	LE
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.	2a
Although the treatment of varicocele in adolescents may be effective, there is a significant risk of overtreatment.	3
Varicocele repair may be effective in men with subnormal semen analysis, a clinical varicocele and otherwise unexplained infertility.	1a

Recommendations	GR
Varicocele treatment is recommended for adolescents with progressive failure of testicular development documented by serial clinical examination.	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, infertility duration of ≥ 2 years and otherwise unexplained infertility in the couple.	A

6.7 References

1. 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
2. World Health Organization. WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. Cambridge: Cambridge University Press, 2000. http://www.who.int/reproductivehealth/publications/infertility/0521774748/en/
3. 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
4. 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. http://www.ncbi.nlm.nih.gov/pubmed/1601152
5. Argawal A, Deepinder F, Cocuzza M, et al. Efficacy of varicocelectomy in improving semen parqameters: new meta-analytical approach. Urology 2007 Sep;70(3):532-8. http://www.ncbi.nlm.nih.gov/pubmed/17905111
6. 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
7. 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
8. 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
9. Grasso M, Lania C, Castelli M, et al. Low-grade left varicocoele 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
10. Yamamoto M, Hibi H, Hirata Y, et al. Effect of varicocoelectomy 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
11. Unal D, Yeni E, Verit A, et al. Clomiphene citrate versus varicocoelectomy in treatment of subclinical varicocoele: a prospective randomized study. Int J Urol 2001 May;8(5):227-30. http://www.ncbi.nlm.nih.gov/pubmed/11328423
12. 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 varicocoele 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 metaanalysis 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. Ding H, Tian J, Du W, et al. Open non-microsurgical, laparoscopic or open microsurgical varicocelectomy for male infertility: a meta-analysis of randomized controlled trials. BJU Int 2012 Nov;110(10):1536-42.
http://www.ncbi.nlm.nih.gov/pubmed/22642226
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, Gliech 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. [No abstract available]
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. [No abstract available]
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, Gogus 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 provided in Table 10.

Table 10: Symptoms and signs of hypogonadism appearing 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. (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 gonadotropin-releasing hormone (GnRH) and/or gonadotropin (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).

Table 11: Disorders associated with male hypogonadism*

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

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

7.2 Hypogonadotrophic hypogonadism: aetiology, diagnosis and therapeutic management

Idiopathic hypogonadotrophic hypogonadism (IHH) is characterised by low levels of gonadotropins and sex steroid in the absence of anatomical or functional abnormalities of the hypothalamic-pituitary-gonadal axis (2). IHH may be an isolated condition or may be associated with anosmia/hyposmia (Kallmann syndrome). Genetic factors causing a deficit of gonadotropins may act at the hypothalamic or pituitary level.

Mutations in candidate genes (X-linked or autosomal) can be found in ~30% of congenital cases (2)

and should be screened prior to assisted reproduction (3).

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

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 (drugs, hormones, or tumours), the therapy of choice depends on whether the goal is to achieve normal androgen levels or fertility.

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

If hypogonadotrophic hypogonadism is hypothalamic in origin, an alternative to hCG treatment is 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 in men are associated with hypergonadotrophic hypogonadism (Table 11, see also Chapter 4). Most conditions listed in Table 11 only affect the reproductive function of the testes so that only FSH level is elevated. However, it has been reported that men with infertility are at higher risk for developing impaired Leydig cell function (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 testes occurs after treatment with GnRH analogues or surgical castration for prostatic cancer (10).

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 serum concentration of sex hormone binding globulin (SHBG). Based on levels of total testosterone, albumin 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 h. The existing guidelines for androgen replacement are based on presence of symptoms of hypogonadism and 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. For the group with serum total testosterone level between 8 and 12 nmol/L (in repeated samples), a 3-6-month trial period with testosterone supplementation can be considered. Generally, androgen replacement should not be given to men not presenting with symptoms of hypogonadism.

Testosterone suppresses pituitary production of LH and FSH, therefore, replacement therapy should not be given for infertility.

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 within the physiological concentration (11-13). *See also EAU Guidelines on Hypogonadism (14).*

7.4 Conclusion and recommendation for hypogonadism

Conclusion	LE
It is generally agreed that patients with primary or secondary hypogonadism associated with hypoandrogenism should receive testosterone substitution therapy.	1b

Recommendation	GR
Effective drug therapy is available to achieve fertility in men with hypogonadotrophic hypogonadism (4).	A*
Testosterone replacement is strictly contraindicated for the treatment of male infertility (13).	A*

**Upgraded following panel consensus*

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. http://www.who.int/reproductivehealth/publications/infertility/0521774748/en/
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, Mehltretter 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, Jorgensen 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. [No abstract available] 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. http://apps.who.int/iris/handle/10665/60776
14. Dohle G, Arver S, Bettocchi C, et al. Members of the EAU Guidelines Office. Guidelines on Male Hypogonadism. In: EAU Guidelines, edn. presented on the 27th EAU Annual Congress, Paris 2012. ISBN: 978-90-79754-83-0.

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 hypothalamic-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 threefold higher incidence of cryptorchidism compared to African-Americans. Even between Caucasians, there are significant differences in the risk of cryptorchidism, for example, 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 > 3,000 boys weighing > 2.5 kg and 21% in premature boys weighing < 2.5 kg. 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 > 2.5 kg and < 2.5 kg 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-Mullerian hormone regulates transabdominal descent of the testes. Induction of the gubernaculum depends on a functional Insl3 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, whereas other gene families, for example, 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 antiandrogenic 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 preoperative testicular location and 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); formerly CIS of the testes. In 5-10% of testicular cancers, there is a history of cryptorchidism (17). The risk of a germ cell tumour (GCT) is 3.6-7.4 times higher than in the general population and 2-6% of men with a history of cryptorchidism will develop a testicular tumour (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 do 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 gonadotropin or GnRH has been used widely in the past to treat cryptorchidism. Although 15-20% of retained testes descend during hormonal treatment, one-fifth of these reascend 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 that early treatment (during the first 2 years of life) has a beneficial effect on preserving future fertility (20), whereas 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 (21). The results clearly indicate that early surgery has a beneficial effect on testicular growth. Testicular volume is an approximate indirect measure of spermatogenic activity, therefore, 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 (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 men with non-palpable testes, the postoperative atrophy rate was 12% in those cases with long vascular pedicles that enabled scrotal positioning. Postoperative atrophy in staged orchidopexy has been reported in up to 40% of patients (19).

8.7 Conclusions and recommendations for cryptorchidism

Conclusions	LE
Cryptorchidism is multifactorial in origin and can be caused by genetic factors and endocrine disruption early in pregnancy.	3
Cryptorchidism is often associated with testicular dysgenesis and is a risk factor for infertility and GCT.	2b
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.	3
Bilateral cryptorchidism significantly reduces the likelihood of paternity.	3

Recommendations	GR
Hormonal treatment of cryptorchidism in adults is not recommended.	A
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 ITGCNU (formerly CIS) is recommended at the time of orchidopexy.	B

8.8 References

1. Skakkebaek NS, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an ncreasingly common developmental disorder with environmental aspects. Hum Reprod 2001 May;16(5):972-8. http://www.ncbi.nlm.nih.gov/pubmed/11331648
2. 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
3. Heyns CF, Hutson JM. Historical review of theories on testicular descent. J Urol 1995 Mar;153 (3 Pt 1):754-67. [No abstract available] http://www.ncbi.nlm.nih.gov/pubmed/7861531
4. Scorer CG. The descent of the testis. Arch Dis Child 1964 Dec;39:605-9. http://www.ncbi.nlm.nih.gov/pubmed/14230757

5. 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
6. 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
7. 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
8. 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
9. 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
10. 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
11. Garcia J, Gonzalez N, Gomez 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
12. 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
13. 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
14. 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
15. 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
16. 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. [No abstract available]
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. [No abstract available]
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
21. Kollin C, Stukenborg JB, Nurmio M, et al. Boys with Undescended Testes: Endocrine, Volumetric and Morphometric Studies on Testicular Function before and after Orchidopexy at Nine Months or Three Years of Age. J Clin Endocrinol Metab 2012 Dec;97(12):4588-95
http://www.ncbi.nlm.nih.gov/pubmed/23015652

9. IDIOPATHIC MALE INFERTILITY

9.1 Introduction

No demonstrable cause of infertility 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). Clomiphen citrate and tamoxifen have been widely used in idiopathic OAT but there is no proven evidence for their benefit. A recent meta-analysis reported some improvement in sperm quality and spontaneous pregnancy rate (3). Androgens, hCG/HMG, bromocriptine, alpha-blockers, systemic corticosteroids and magnesium supplementation are not effective in the treatment of OAT syndrome. Follicle-stimulating hormone (3) might be beneficial in a selection of patients (3). A Cochrane analysis showed that men taking oral antioxidants had an associated significant increase in live birth rate (pooled OR = 4.85; 95% CI: 1.92-12.24; P = 0.0008; I(2) = 0%) when compared with men taking the control treatment. No studies have reported harmful side effects from antioxidant therapy. The evidence suggests that antioxidant supplementation in subfertile men may improve the outcomes of live birth and pregnancy rate for subfertile couples undergoing assisted reproduction technique (ART) cycles. Further head-to-head comparisons are necessary to identify the superiority of one antioxidant over another (4).

Recommendation	GR
Medical treatment of male infertility is recommended only for cases of hypogonadotrophic hypogonadism.	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. Chua ME, Escusa KG, Luna S, et al. Revisiting oestrogen antagonists (clomiphene or tamoxifen) as medical empiric therapy for idiopathic male infertility: a meta-analysis. Andrology 2013 Sep;1(5): 749-57. http://www.ncbi.nlm.nih.gov/pubmed/23970453
4. 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
5. Showell MG, Brown J, Yazdani A, et al. Antioxidants for male subfertility. Cochrane Database Syst Rev 2011 Jan;(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”, because 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 gonadotropins and testosterone substitution to maintain male sexual function and bone mineralization, 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 results 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). Extensive guidelines on vasectomy were published by the EAU in 2012 (9). 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 (10).

10.2.1 ***Surgical techniques***

Various techniques are available for vasectomy. The least invasive approach is no-scalpel vasectomy (11), which is also associated with a low rate of complications (12). The most effective occlusion technique is cauterisation of the lumen of the vas deferens and fascial interposition (13-15). 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 (16,17). Acute local complications associated with vasectomy include haematoma, wound infection, and epididymitis in up to 5% of cases (17). The potential long-term complications (e.g., chronic testicular pain) (18) 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 preoperatively that, although rare, long-term recanalisation 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 (20).

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 (vasovasostomy or vasoepididymostomy), and whether reversal was unilateral or bilateral. However, there have been no RCTs comparing macrosurgery (loops) and microsurgery. Microsurgical techniques with the help of magnification and smaller suture materials should be used (21).

*10.3.1 **Length of time since vasectomy***

Vasovasostomy results have shown patency rates up to 90%. The longer the interval is from vasectomy to reversal, the lower is the pregnancy rate. In a study of 1,469 men who had undergone microsurgical vasectomy reversal, patency and pregnancy rates were 97% and 76%, respectively, for an interval up to 3 years after vasectomy; 88% and 53% for 3-8 years, 79% and 44% for 9-14 years, and 71% and 30% for > 15 years (22).

*10.3.2 **Tubulovasostomy***

The chance of secondary epididymal obstruction after vasectomy increases with time. After an interval of 10 years, 25% of men appear to have epididymal blockage. If secondary epididymal obstruction occurs, tubulovasostomy is needed to reverse the vasectomy (see Chapter 5) (23).

*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 (24,-27). 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	LE
Vasectomy is considered the gold standard for the male contribution to contraception.	1
All available data indicate that vasectomy is not associated with any serious, long term side effects.	1b
Pregnancy is still achievable after successful vasectomy reversal.	2a
Methods of male contraception other than vasectomy are associated with high failure rates or are still experimental (e.g., hormonal approach).	3

Recommendations	GR
Vasectomy meets best the criteria for the male contribution to contraception, with regard to efficacy, safety and side effects. Cauterisation and fascial interposition are the most effective techniques.	A
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.	A*
Microsurgical vasectomy reversal is a low-risk and (cost-) effective method of restoring fertility.	B
MESA/TESE/PESA and ICSI should be reserved for failed vasectomy reversal surgery.	A
For couples wanting to achieve pregnancy, sperm aspiration together with ICSI is a second-line option for selected cases and in those with failed vasovasostomy.	B

**Upgraded following panel consensus*

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. Tradional 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;(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. Dohle G, Diemer Th, Kopa Z, et al. European Association of Urology Guidelines on Vasectomy. Eur Urol 2012 Jan;61(1):159-63.
http://www.ncbi.nlm.nih.gov/pubmed/22033172
10. 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
11. 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
12. 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
13. 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
14. 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
15. 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
16. Bernal-Delgado E, Latour-Perez 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
17. 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
18. 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
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. Korthorst RA, Consten D, van Roijen JH. Clearance after vasectomy with a single semen sample containing < than 100 000 immotile sperm/mL: analysis of 1073 patients. BJU Int 2010 Jun;105(11):1572-5.
http://www.ncbi.nlm.nih.gov/pubmed/20002679
21 Schroeder-Printzen I, Diemer T, Weidner W. Vasovasostomy. Urol Int 2003;70(2):101-7.
http://www.ncbi.nlm.nih.gov/pubmed/12592037
22. 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
23. 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
24. 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
25. 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
26. 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

27. Cook LA, Van Vliet HA, Pun A, et al. Vasectomy techniques for male sterilization: systematic Cochrane review of randomized controlled trials and controlled clinical trials. Hum Reprod 2004 Nov;19(11):2431-8.
http://www.ncbi.nlm.nih.gov/pubmed/15496598

11. MALE ACCESSORY GLAND INFECTIONS AND INFERTILITY

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.

In order to keep the guidelines as short as possible, the MAGI section is discussed in detail in the Guidelines for Urological Infections and Chronic Pelvic Pain (4,5).

11.2. Ejaculate analysis

11.2.1 ***Introduction***

Ejaculate analysis (see Chapter 2) clarifies whether the prostate is involved as part of a generalised MAGI and provides information about sperm quality. In addition, leukocyte analysis allows differentiation between inflammatory and non-inflammatory chronic pelvic pain syndrome (CPPS) (NIH IIa vs NIH IIIb).

11.2.2 ***Microbiological findings***

After exclusion of urethritis and bladder infection, > 10^6 peroxidase-positive white blood cells (WBCs) per millilitre of ejaculate indicate an inflammatory process. In this case, a culture should be performed for common urinary tract pathogens, particularly Gram-negative bacteria.

A concentration of > 10^3 cfu/mL urinary tract pathogens in the ejaculate is indicative of significant bacteriospermia. Various microorganisms 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 microorganisms in semen and the frequency of isolation of different strains (6). The ideal diagnostic test for *Chlamydia trachomatis* in semen has not yet been established (7). 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 (7).

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 (8). Normal colonisation of the urethra hampers the clarification of mycoplasma-associated urogenital infections, using samples such as the ejaculate (9).

11.2.3 ***White blood cells***

The clinical significance of an increased concentration of leukocytes in the ejaculate is controversial (10). 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 (11). Earlier findings have shown that elevated leukocyte numbers are not a natural cause of male infertility (12). 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 (13,14). Both studies found more leukocytes in men with prostatitis compared to those without inflammation (CPPS, type NIH IIIb).

11.2.4 ***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 (15-17).

11.2.5 ***Seminal plasma alterations***

Seminal plasma elastase is a biochemical indicator of polymorphonuclear lymphocyte activity in the ejaculate (1,18,19), 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 (20-22), but no correlations have been found. The prostate is the main site of origin of IL-6 and IL-8 in the seminal plasma. Cytokines, especially IL-6, play an important role in the male accessory gland inflammatory process (23). However, elevated cytokine levels do not depend on the number of leukocytes in expressed prostatic secretion (EPS) (24).

*11.2.6 **Glandular secretory dysfunction***

Infections of the sex glands can impair their excretory function. Decreased quantities of citric acid, phosphatase, fructose, zinc, and α-glutamyl-transferase activity are indicators of disturbed prostatic secretory parameters (1). Reduced fructose concentration indicates impaired vesicular function (8,25).

*11.2.7 **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 non- or abacterial prostatitis (26,27). However, except for suspected chlamydial infections (28), only a history of vasectomy is predictive of sperm antibody formation (29).

*11.2.8 **Reactive oxygen species***

Reactive oxygen species might be increased in chronic urogenital infections associated with increased leukocyte numbers (30). However, their biological significance in prostatitis remains unclear (1).

*11.2.9 **Therapy***

Treatment of chronic prostatitis is usually targeted at relieving symptoms (31,32). 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 microorganisms in prostatic secretions and semen;
- normalisation of inflammatory (e.g., leukocytes) and secretory parameters;
- improvement of sperm parameters to counteract fertility impairment (33).

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 chronic bacterial prostatitis (NIH II) has provided symptomatic relief, eradication of microorganisms, and a decrease in cellular and humoral inflammatory parameters in urogenital secretions. The use of α-blockers for symptom relief is controversial. Although antibiotics might improve sperm quality (33), there is no evidence that treatment of chronic prostatitis increases the probability of conception (1,34).

11.3 Epididymitis

*11.3.1 **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 *Neisseria gonorrhoea* (35,36). Sexually transmitted epididymitis is usually accompanied by urethritis. Non-sexually transmitted epididymitis is associated with urinary tract infection and occurs more often in men aged > 35 years, those who have recently undergone urinary tract instrumentation or surgery, and those who have anatomical abnormalities (37).

*11.3.2 **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 (36,38,39). Ipsilateral low-grade orchitis (40,41) might be the cause of this slight impairment in sperm quality (Table 14) (42).

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). 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 (43)	+	+	+	Pyospermia in 19 of 22 cases
Berger *et al.* (36)		+		
Weidner *et al.* (44)	+	+	+	Azoospermia in 3 of 70 men
Haidl (45)		+		Chronic infections; macrophages Elevated
Cooper *et al.* (46)				Decrease in epididymal markers: α-glucosidase, L-carnitine

11.3.3 ***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. gonorrhoeae* or *C. trachomatis* must be told to refer their sexual partners for evaluation and treatment (47).

11.4 Conclusions and recommendations for male accessory gland infections

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

Recommendations	GR
Patients with epididymitis that is known or suspected to be caused by *N. gonorrhoeae* or *C. trachomatis* must be instructed to refer their sexual partners for evaluation and treatment.	B

11.5 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. http://www.who.int/reproductivehealth/publications/infertility/0521774748/en/
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. Engeler D, Baranowski AP, Dinis Oliveira P, et al. Members of the EAU Guidelines Office. EAU Guidelines on Chronic Pelvic Pain. In: EAU Guidelines, edition presented at the 27th EAU Annual Congress, Paris 2012. ISBN 978-90-79754-83-0.
5. Grabe M, Bjerklund Johansen TE, Botto H, et al. Members of the EAU Guidelines Office. EAU Guidelines on Urological Infections. In: EAU Guidelines, edition presented at the 27th EAU Annual Congress, Paris 2012. ISBN 978-90-79754-83-0.
6. 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

7. 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
8. Weidner W, Krause W, Schiefer HG, et al. Ureaplasmal 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
9. 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
10. 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
11. Trum JW, Mol BW, Pannekoek Y, et al. Value of detecting leukocytospermia in the diagnosis f genital tract infection in subfertile men. Fertil Steril 1998 Aug;70(2):315-9.
http://www.ncbi.nlm.nih.gov/pubmed/9696227
12. 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
13. 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
14. 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
15. 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
16. 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
17. 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
18. 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
19. 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
20. 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
21. 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
22. 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
23. 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
24. 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
25. 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
26. 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

27. 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
28. 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
29. 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
30. 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
31 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
32. 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
33. 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
34. 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
35. 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
36. Berger RE. Epididymitis. In: Holmes KK, Mardh PA, Sparling PF et al. (eds). *Sexually Transmitted Diseases*. New York: McGraw-Hill, 1984, pp. 650-62.
37. 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
38. [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. [No abstract available] http://www.ncbi.nlm.nih.gov/pubmed/10616385
39. Weidner W, Krause W. Orchitis. In: Knobil E, Neill JD (eds). *Encyclopedia of Reproduction*. Vol. 3. San Diego: Academic Press, 1999, pp. 92-5. [No abstract available] http://www.ncbi.nlm.nih.gov/pubmed/10616385
40. 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
41. Osegbe DN. Testicular function after unilateral bacterial epididymo-orchitis. Eur Urol 1991;19(3):204-8. http://www.ncbi.nlm.nih.gov/pubmed/1855525
42. 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
43. 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
44. 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
45. 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

46. 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
47. 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

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 preceded by CIS, and untreated ITGCNU 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 dysgenic testes have an increased risk of developing testicular cancer in adulthood. These cancers arise from premalignant gonocytes or CIS cells (5). Testicular microlithiasis (TM), seen on ultrasound, can be associated with GCT and CIS of the testes.

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). 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 ≥ 3 cycles of chemotherapy or irradiation of retroperitoneal lymph nodes. However, this risk is greatest at 6-12 months post-treatment. This suggests there may be some improvement in Leydig cell function, and why it is reasonable to expect initiation of androgen replacement, until the patient shows continuous signs of testosterone deficiency, even at 2 years follow-up (10). The risk of low libido and erectile dysfunction is also 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 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 TGCT. However, available data indicate that men in whom TM is found by ultrasound, and who have an increased risk of TGCT, should be offered testicular biopsy for detection of CIS. The list of high-risk patients includes men with infertility and bilateral TM, atrophic testes, undescended testes, a history of TGCT, and contralateral TM (21).

12.4 Recommendations for germ cell malignancy and testicular microcalcification

Recommendations	GR
As for all men, patients with TM and without special risk factors (see below) should be encouraged to perform self-examination because 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, a history of TGCT, or contralateral TM.	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 CT is not justified in men with isolated TM without associated risk factors (e.g., infertility, cryptorchidism, testicular cancer, and atrophic testis).	B
Men with TGCT are at increased risk of developing hypogonadism and sexual dysfunction and should therefore be followed up.	B

12.5 References

1. 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
2. von der Maase H, Rorth M, Walbom-Jorgensen 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
3. 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
4. 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
5. Giwercman A, Muller J, Skakkebaek NE. Carcinoma in situ of the undescended testis. Semin Urol 1988 May;6(2):110-9. [No abstract available] http://www.ncbi.nlm.nih.gov/pubmed/2903524
6. 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
7. 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, Stahl O, Cwikiel M, et al. Risk factors for post-treatment hypogonadism in yesticular cancer patients. Eur J Endocrinol 2008 Apr;158(4):561-70. http://www.ncbi.nlm.nih.gov/pubmed/18362304

11. Eberhard J, Stahl 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. It is caused by failure of semen emission from the seminal vesicles, prostate and 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).

*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 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, organic (e.g., incomplete spinal cord lesion (3) or iatrogenic penile nerve damage (4), or pharmacological [e.g., selective serotonin re-uptake inhibitors (SSRIs), antihypertensives, or 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 bulbourethral glands. The causes of retrograde ejaculation can be divided into neurogenic, pharmacological, or urethral, or bladder neck incompetence (Table 15).

Table 15: Aetiology of anejaculation and retrograde ejaculation

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

The International Society for Sexual Medicine (ISSM) has adopted the first evidence-based definition of lifelong premature ejaculation (PE): “Premature ejaculation is a male sexual dysfunction characterised by ejaculation which always or nearly always occurs prior to or within about one minute of vaginal penetration; and inability to delay ejaculation on all or nearly all vaginal penetrations; and negative personal consequences, such as distress, bother, frustration and/or the avoidance of sexual intimacy”.

Premature ejaculation may be strictly organic (e.g., prostatitis-related) or psychogenic, partner-related or non-selective, and can be associated with erectile dysfunction. It does not impair fertility, provided intravaginal ejaculation occurs.

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

*13.3.2 **Physical examination***

Genital and rectal examinations are conducted, including evaluation of the prostate, bulbocavernosus 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 of centrifuged urine 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 or biochemical infection marker tests are also suggested (8).

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

This diagnostic work-up can include:

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

13.4 Treatment

Infertility caused by disorders of ejaculation is seldom treated on the basis of aetiology. Treatment usually involves retrieval of spermatozoa for use in 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 ejaculation, tamsulosin can be administered during antidepressant treatment (9). Treatment should be given for urogenital infections (i.e., in cases of painful ejaculation) (8). Dapoxetin is an SSRI that has been introduced for the therapy of PE (10), because 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***

Primature ejaculation can be treated with the SSRI dapoxetine, topical anaesthetic agents to increase intravaginal ejaculation latency time, behavioural therapy, and/or psychotherapy. Off-label use of SSRIs (e.g., paroxetine and 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 16). Alternatively, the patient can be encouraged

to ejaculate when his bladder is full to increase bladder neck closure (11).

Table 16: Drug therapy for retrograde ejaculation

Drug	Dosage regimen	Ref.
Ephedrine sulphate	10-15 mg four times daily	12
Midodrine	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. Alternatively, a catheter can be inserted into the bladder to allow instillation of 10-50 mL Tyrode's or Ham's F-10 medium. The patient must ejaculate, and a second catheterisation is carried out immediately to retrieve spermatozoa. The latter treatment minimises contact between spermatozoa and urine (17,18).

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

*13.6.3 **Anejaculation***

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

In anejaculation, vibrostimulation evokes the ejaculation reflex (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, electroejaculation is the therapy of choice (20). Electroejaculation involves electrical 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 electroejaculation fails or cannot be carried out, sperm can be retrieved from the seminal ducts by aspiration from the vas deferens (22) (see Chapter 5) or seminal tract washout (23).

When sperm cannot be retrieved, epididymal obstruction or testicular failure must be suspected). If only immotile sperm can be retrieved, DNA damage is very likely and will yield poor IVF results. 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	LE
Ejaculation disorders can be treated using a wide range of drugs and physical stimulation, with a high level of efficacy.	3

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.	A
In men with spinal cord injury, vibrostimulation and electroejaculation are effective methods of sperm retrieval.	B

13.8 References

1. 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]
2. 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.
3. Pryor JP. Erectile and ejaculatory problems in infertility. In: Hargreave TB (ed). *Male Infertility*. Berlin:Springer-Verlag, 1997, pp. 319-36.
4. 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
5. 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
6. 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
7. 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]
8. 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
9. 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
10. 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
11. 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. Mahadevan.M., Leeton,J.F. and Trounson.A.O. Noninvasive method of semen collection for successful artificial insemination. Fertil Steril 1981; 36, 243-7. [No abstract available] http://www.ncbi.nlm.nih.gov/pubmed/7262339
18. Yavetz H, Yogev L, Hauser R. Retrograde Ejaculation. Human Reprod. 1994; 9:381-6. http://www.ncbi.nlm.nih.gov/pubmed/8006123
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 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 and testicular tissue.

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 testicle in a man with testicular malignancy, or before vasectomy or transgender surgery).
- For men with progressive decrease in semen quality as a result of diseases that have an associated risk of subsequent azoospermia (i.e., pituitary macroadenoma, craniopharyngioma, empty sella syndrome, chronic nephropathy, uncontrolled diabetes mellitus, and multiple sclerosis).
- For men with paraplegia when sperm have been obtained by electroejaculation or obtained by penile vibratory stimulation.
- For men with psychogenic anejaculation, after sperm have been obtained either by electroejaculation or a sperm retrieval procedure.
- After gonadotropin treatment has induced spermatogenesis in men with hypogonadotrophic hypogonadism.
- For men with NOA, the chance of finding sperm using micro-TESE is ~50%.

Cryopreservation can be used for sperm collected through TESE, avoiding repeated sperm retrieval procedures and unnecessary hyperstimulation of the female partner.

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

14.4 Precautions and techniques

*14.4.1 **Freezing and thawing process***

The cryopreservation techniques currently used are not yet optimal because damage occurs to cells during cryopreservation and prolonged storage. Most damage occurs during freezing and thawing. Major causes of damage during freezing are ice crystal formation and cell dehydration, which disrupt the cell wall and intracellular organelles. Sperm morphology, motility and vitality decrease significantly after thawing, and cryopreservation increases the damage done to sperm DNA (3-6). Further damage can be caused by contamination of samples with microorganisms 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 albumin. After freezing, the

samples are immersed in liquid nitrogen.
Several techniques have been developed to try to reduce damage caused by freezing and thawing, including:

- One-step freezing method (9,10): sample is held in the vapour phase for 10 min before being plunged into liquid nitrogen.
- Slow or multi-step method (11): sample is gradually cooled in the vapour phase for approximately 40 min. A programmable automatic freezing machine, which is preset to cool at a rate of 1-10°C/min is used.

The method available depends on the resources of the laboratory. Whichever freezing technique is used, it should be tested using donor sperm and post-thaw examination, and should regularly undergo a quality-control programme.

The likelihood of sperm survival decreases with repeated freezing and thawing. The maximum viable storage time for human sperm is not known. Many laboratory or regulatory authorities apply a storage limit of up to 10 years (12). However, longer storage is 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 small numbers of sperm***

Standard cryopreservation in straws is an efficient way of storing large numbers of sperm (e.g., for a donor insemination programme). However, in micro-TESE, few sperm might be obtained, and the choice is either to freeze testicular tissue and find sperm after thawing the tissue, or to freeze small numbers of sperm. If sperm are frozen in straws, it can be difficult to find any sperm after thawing. Instead, the sperm should be frozen in a pellet (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 use so-called high security closed straws. According to the European directives 2004/23 and 2006/17, samples should be tested for hepatitis B and C and human immunodeficiency virus (HIV). In case of non-partner donation, samples are also tested for C. Trachomatis (by NAT) and syphilis, as well as genetics, that is, karyotype and most prevalent genetic disorders in the population to which the non-partner donor belongs.

Until the test results are known, samples must be stored in an individual quarantine vessel (separate storage). If open straws are used (e.g., for vitrification purposes) some laboratories use the additional safeguard of double-wrapping the straws before freezing, although this is more costly. Some centres carry out cytomegalovirus testing and store negative and positive samples separately.

Considerable ethical issues surround the storage of samples before cancer chemotherapy in men who are hepatitis-virus- or HIV-positive. Few clinics have separate storage facilities for HIV-positive samples. However, the success of antiretroviral treatment is increasing the number of HIV-positive men who may wish to store sperm. There is also concern about HIV transmission to children conceived using HIV-positive sperm, because sperm-washing techniques fail in ~5% of cases.

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.

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.

14.5 Biological aspects

Cryopreservation induces deterioration of semen 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 Cryopreservation of testicular stem cells

Spermatogonial stem cell (SSC) preservation and transplantation have been proposed as a promising strategy for fertility preservation in young boys facing SSC loss (20). Since the first publication of SSC transplantation in mice in 1994, remarkable progress has been made towards a clinical application. Cryopreservation protocols for testicular tissue have been developed in animal models, translated to humans, and are already used clinically. Transplantation methods are being used in human testes, and the efficiency and safety of the technique has been evaluated in a mouse model. The application of this technique in humans looks possible, therefore, banking testicular biopsies from prepubertal boys for future stem cell transplantation is being introduced in many centres.

14.7 Conclusions and recommendations for semen cryopreservation

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

Recommendations	GR
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.	A
If testicular biopsies are indicated, sperm cryopreservation is strongly advised.	A
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

14.8 References

1. Bunge RG, Keettel WC, Sherman JK. Clinical use of frozen semen; report of four cases. Fertil Steril 1954 Nov-Dec;5(6):520-9. [No abstract available] http://www.ncbi.nlm.nih.gov/pubmed/13210484
2. 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
3. Desrosiers P, Legare 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
4. 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
5. 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
6. 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
7. 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
8. 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

9. 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
10. Sherman JK, Bunge RG. Observations on preservation of human spermatozoa at low temperatures. Proc Soc Exp Biol Med 1953 Apr;82(4):686-8. [No abstract available]
http://www.ncbi.nlm.nih.gov/pubmed/13055973
11. 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. [No abstract available]
http://www.ncbi.nlm.nih.gov/pubmed/6073928
12. 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
13. 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
15. Clarke GN. Sperm cryopreservation: is there a significant risk of cross-contamination? Hum Reprod 1999 Dec;14(12):2941-3. [No abstract available]
http://www.ncbi.nlm.nih.gov/pubmed/10601075
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
20. Geens M, Goossens E, De Block G, et al. Autologous spermatogonial stem cell transplantation in man: current obstacles for a future clinical application. Hum Reprod Update 2008 Mar-Apr;14(2):121-30.
http://www.ncbi.nlm.nih.gov/pubmed/18187526

15. ABBREVIATIONS USED IN THE TEXT

This list is not comprehensive for the most common abbreviations

ABP	acute bacterial prostatitis
ART	assisted reproduction technique
CBAVD	congenital bilateral absence of the vas deferens
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CIS	carcinoma *in situ*
CPPS	chronic pelvic pain syndrome
EAA	European Academy of Andrology
EPS	expressed prostatic excretion
FISH	fluorescent *in situ* hybridisation
FSH	follicle-stimulating hormone
GCT	germ cell tumour
GnRH	gonadotrophin-releasing hormone
GR	grade of recommendation
GREAT	G-protein-coupled receptor affecting testis descent
hCG	human chorionic gonadotrophin
HIV	human immunodeficiency virus
HMG	human menopausal gonadotropin
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
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
WBC	white blood cell
VB1	first-voided urine
WHO	World Health Organization

Conflict of interest

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