

Guidelines on Male Infertility

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1. INTRODUCTION

1.1 Definition

'Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year' (WHO) (1).

1.2 Epidemiology and aetiology

About 25% of couples do not achieve pregnancy within 1 year, 15% of whom seek medical treatment for infertility and less than 5% remain unwillingly childless. Infertility affects both men and women. Male causes for infertility are found in 50% of involuntarily childless couples. If there is a single factor, the fertile partner may compensate for the less fertile partner. In many couples, however, a male and a female factor coincide. Infertility usually becomes manifest if both partners are subfertile or have reduced fertility (1).

Reduced male fertility can be the result of congenital and acquired urogenital abnormalities, infections of the genital tract, increased scrotal temperature (varicocele), endocrine disturbances, genetic abnormalities and immunological factors (1). No causal factor is found in 60-75% of cases (idiopathic male infertility). These men present with no previous history associated with fertility problems and have normal findings on physical examination and endocrine laboratory testing. Semen analysis reveals a decreased number of spermatozoa (oligozoospermia), decreased motility (asthenozoospermia) and many abnormal forms on morphological examination (teratozoospermia). These abnormalities usually occur together and are described as the oligo-astheno-teratozoospermia (OAT) syndrome. Table 1 summarizes the main aetiological causes of male subfertility.

Unexplained forms of male infertility may be caused by several factors, such as chronic stress, endocrine disruption due to environmental pollution, reactive oxygen species and genetic abnormalities.

Table 1: Aetiology and distribution (%) of male infertility among 7,057 men (1)

Aetiology	Distribution (%)
• Sexual factors	1.7
• Urogenital infection	6.6
• Congenital anomalies	2.1
• Acquired factors	2.6
• Varicocele	12.3
• Endocrine disturbances	0.6
• Immunological factors	3.1
• Other abnormalities	3.0
• Idiopathic abnormal semen (OAT* syndrome) or no demonstrable cause	75.1

* OAT = *Oligo-astheno-teratozoospermia*.

1.3 Prognostic factors

The main factors influencing the prognosis in infertility are:

- Duration of infertility
- Primary or secondary infertility
- Results of semen analysis
- Age and fertility status of the female partner.

When the duration of infertility exceeds 4 years of unprotected sexual intercourse, the conception rate per month is only 1.5%.

At present, in many Western countries, women postpone their first pregnancy until they have finished their education and have started a professional career. However, the fertility of a woman of 35 years of age is only 50% of the fertility potential of a woman aged 25 years. By the age of 38 years, this has reduced to only 25%, and over the age of 40 years it is less than 5%. Female age is the most important single variable influencing outcome in assisted reproduction (2).

1.4 RECOMMENDATIONS (3)

- To categorize infertility, both partners should be investigated simultaneously.
- In the diagnosis and management of male infertility, it is essential to consider the fertility chances of the female partner, since this might determine the final outcome (2) (grade B recommendation).
- As a urogenital expert, the urologist/andrologist should examine any male with fertility problems for urogenital abnormalities. This applies to all males diagnosed with reduced sperm quality. A diagnosis is mandatory to initiate appropriate therapy (drugs, surgery, assisted reproduction) (1) (grade B recommendation).

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2. INVESTIGATIONS

2.1 Semen analysis

Andrological examination is indicated if semen analysis shows abnormalities (Table 2). Because semen analysis still forms the basis of important decisions concerning appropriate treatment, standardization of the complete laboratory work up is highly desirable. Ejaculate analysis has been standardized by the WHO and propagated by continuing work and publications in the *WHO Laboratory Manual for Human Semen and Sperm-Cervical Mucus Interaction* (4th edition) (1). The consensus is that modern spermatology has to follow these guidelines without exception.

Table 2: Overview of standard values for semen analysis according to the 1999 WHO criteria (1)

• Volume	≥ 2.0 mL
• pH	7.0-8.0
• Sperm concentration	≥ 20 million/mL
• Total no. of spermatozoa	≥ 40 million/ejaculate
• Motility	≥ 50% with progressive motility or 25% with rapid motility within 60 min after ejaculation
• Morphology	≥ 14% of normal shape and form*
• Viability	> 50% of spermatozoa
• Leukocytes	< 1 million/mL
• Immunobead test (IBT)	< 50% spermatozoa with adherent particles
• MAR test**	< 50% spermatozoa with adherent particles

* Assessment according to Kruger and Menkfeld criteria.

** MAR = Mixed antiglobulin reaction.

2.1.1 Frequency of semen analysis

If values are normal according to WHO criteria, one test should be sufficient. Further andrological investigation is only indicated if the results are abnormal in at least two tests.

It is important to distinguish between oligozoospermia (< 20 million spermatozoa/mL), astenozoospermia (< 50% motile spermatozoa) and teratozoospermia (< 14% normal forms). Quite often, all three pathologies occur simultaneously as OAT syndrome. In extreme cases of OAT syndrome (< 1 million spermatozoa/mL), as in azoospermia, there is an increased incidence of obstruction of the male genital tract and genetic abnormalities.

2.2 RECOMMENDATIONS

- Andrological investigations are indicated if semen analysis is abnormal in at least two tests.
- Assessment of andrological status has to consider the suggestions made by WHO for the standardized investigation, diagnosis and management of the infertile man and by doing so, implement evidence-based medicine in this interdisciplinary field of reproductive medicine (2) (grade B recommendation).
- Semen analysis has to follow the guidelines of the *WHO Laboratory Manual for Human Semen and Sperm-Cervical Mucus Interaction* (4th edition) (1) (grade B recommendation).

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3. PRIMARY SPERMATOGENIC FAILURE

3.1 Definition

Primary spermatogenic failure is defined as any spermatogenic alteration caused by conditions other than hypothalamic-pituitary disease. The severe forms of primary spermatogenic failure have different aetiologies but present clinically as non-obstructive azoospermia (NOA). The prevalence of azoospermia in the general population has been estimated at 2%. The incidence at a male infertility clinic was found to be as high as 10-20% (1).

3.2 Aetiology

The causes of spermatogenic failure are summarized in Table 3.

Table 3: Causes of spermatogenic failure

• Anorchia
• Congenital factors (testicular dysgenesis)
• Acquired factors (trauma, testicular torsion, tumour, surgery)
• Maldescended testes
• Klinefelter's syndrome*
• Other chromosomal alterations*
• Germ cell aplasia
• Complete and focal germ cell aplasia (Sertoli cell-only syndrome), either congenital or acquired: maldescended testes, irradiation, cytostatic drugs
• Spermatogenic arrest
• Post-inflammatory (orchitis)
• Exogenous factors (medications, toxins, irradiation, heat)
• Systemic diseases (liver cirrhosis, renal failure)
• Testicular tumour
• Varicocele
• Surgeries that can damage vascularization of the testes
• Idiopathic

* See section 4 *Genetic disorders in infertility*.

3.3 History and physical examination

Typical findings from the history and physical examination of a patient with spermatogenic failure are:

• Cryptorchidism
• Testicular torsion
• Genito-urinary infection
• Testicular trauma
• Environmental toxin exposure
• Gonodadotoxic medication

- | |
|---|
| • Radiation or chemical exposure |
| • Testicular cancer |
| • Absence of testes |
| • Abnormal secondary sexual characteristics |
| • Gynaecomastia |
| • Cryptorchidism |
| • Abnormal testicular volume and/or consistency |
| • Varicocele |

3.4 Investigations

Routine investigations include semen analysis and hormonal determinations. Other investigations are described according to the individual situation.

3.4.1 Semen analysis

In NOA, semen analysis shows normal ejaculate volume and azoospermia after several centrifugations have been performed. A recommended method is semen centrifugation at 600 g for 10 min and thorough microscopic examination of the pellet (x600). The upper fluid is then re-centrifuged (8000 g) for an additional 10 min and examined. All samples can be stained and re-examined under the microscope (2).

3.4.2 Hormonal determinations

Generally, the levels of follicle-stimulating hormone (FSH) are mainly correlated with the number of spermatogonia. When these cells are absent or markedly diminished, FSH values are usually elevated. When the number of spermatogonia is normal, but there is complete spermatocyte or spermatid blockage, FSH values are within normal range. However, on an individual patient basis, FSH levels do not provide an accurate prediction of the status of spermatogenesis (3-5). Preliminary data indicate a stronger correlation between low inhibin B level and spermatogenic damage (6).

3.4.3 Testicular biopsy

A diagnostic testicular biopsy is indicated in patients without evident factors (normal FSH and normal testicular volume) to differentiate between obstructive and NOA.

Testicular biopsy can also be performed as part of a therapeutic process in patients with clinical evidence of NOA who decide to undergo intracytoplasmic sperm injection (ICSI). Spermatogenesis may be focal. In these cases, one or more seminiferous tubules are involved in spermatogenesis while others are not (7,8). About 50-60% of men with NOA have some seminiferous tubules with spermatozoa that can be used for ICSI.

Most authors recommend taking several testicular samples given the potential for regional differences (9,10). Many authors find a good correlation between diagnostic biopsy histology and the likelihood of finding mature sperm cells during testicular sperm retrieval and ICSI (11,12).

3.5 Treatment

Testicular sperm extraction (TESE) and ICSI were introduced in 1993 for treatment of obstructive azoospermia (13-15). It was soon discovered that this technique could also be used for azoospermic men who appeared to have disturbed spermatogenesis (16). If spermatozoa are detected in the testicular biopsy, ICSI with either cryopreserved or fresh spermatozoa can be proposed to the couple.

A karyotype (if not performed previously) and Yq deletions screening are indicated to analyze any therapeutic consequences for the newborn child. If genetic anomalies are detected, the couple has to be properly informed and counselled (see section 4 Genetic disorders in infertility).

In case of azoospermia or ejaculatory failure not responding to vibro- or electro-ejaculation, spermatozoa can be harvested surgically for ICSI. The surgical technique depends on the cause of the azoospermia:

- In NOA, TESE is needed to retrieve spermatozoa.
- In OA, microsurgical or percutaneous epididymal sperm aspiration (MESA/PESA) can be applied. If no spermatozoa are found in the epididymal fluid, TESE is recommended.
- In anejaculation unresponsive to vibration or electro-ejaculation, TESE or seminal tract washout can be applied.

In NOA, the only good predictor of successful retrieval is testicular histology (17). No clear relation was found with FSH, Inhibin B or testicular volume. In case of AZFa and AZFb microdeletions no spermatozoa can be retrieved (18,19). TESE is the technique of choice and shows excellent repeatability (20): TESE results in sperm

retrievals in 50-60% of cases (21,22). Microsurgical TESE may increase retrieval rates (21,23,24): after opening of the testis fluid from large calibre tubules is aspirated with the aid of the operating microscope: complications appears to be lower than with classical TESE (25). Positive retrievals are reported even in conditions, such as Sertoli Cell Only Syndrome (21). Testicular fine needle aspiration (TEFNA) results in lower retrieval rates and does not allow histological examination for the detecting carcinoma in situ and testicular malignancies (26,27). TEFNA may also result in more tubular and vascular damage than (micro)TESE (28).

In OA, surgical retrieval can be combined with reconstruction of the seminal tract. TESE is usually successful and allows retrieval of a large number of spermatozoa suitable for cryopreservation. However, when epididymal tubules are enlarged, MESA enables both quantitatively and qualitatively better recovery of motile spermatozoa, with excellent chances for successful cryopreservation.

The results of ICSI are worse when using sperm retrieved in men with non-obstructive azoospermia as compared to obstructive azoospermia (29-31): birth rates of 19% in NOA versus 28% in OA (32) with significantly lower fertilisation and implantation rates (33) and higher miscarriage rates (11.5% vs. 2.5%) (34). In OA, no significant difference in ICSI results was found between testicular or epididymal sperm (35). Also, no significant differences in ICSI results between the use of fresh and frozen-thawed sperm have been reported (32,35,36-39).

3.6 CONCLUSIONS

- Impaired spermatogenesis is often associated with elevated FSH concentration.
- Testicular biopsy is the best procedure to define the histological diagnosis and the possibility of finding sperm. When spermatozoa are detected, these can be cryopreserved for use in future ICSI cycles.
- Spermatozoa are found in about 60% of patients with NOA. It is crucial that these men who are candidates for sperm retrieval are given appropriate genetic advice.
- For patients with NOA who have spermatozoa in their testicular biopsy, ICSI with fresh or cryopreserved spermatozoa is the only therapeutic option.
- Fertilization and pregnancy are achieved in 30-50% of couples with NOA, when spermatozoa are found in the testicular biopsy.

3.7 RECOMMENDATIONS

- A diagnostic testicular biopsy is indicated only in men with azoospermia, a normal testicular volume and normal FSH (40) (grade B recommendation).
- In couples with a NOA, a TESE with cryopreservation of the spermatozoa to be used for ICSI can be offered (41-43) (grade B recommendation).
- In order to increase the chances of positive sperm retrievals in men with non-obstructive azoospermia testicular sperm extraction (TESE, either single, multiple or microsurgical) should be used rather than testicular fine needle aspiration (TEFNA).

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4. GENETIC DISORDERS IN INFERTILITY

4.1 Introduction

A knowledge of genetic abnormalities in infertility is mandatory for every urologist working in andrology so that correct advice can be given to couples seeking fertility treatment because of male infertility. By means of in-vitro fertilization (IVF), ICSI and TESE men with very low sperm counts can be given a reasonable chance of paternity. However, this also increases the possibility of passing genetic abnormalities on to the next generation because the sperm of infertile men shows an increase in aneuploidy, other genetic abnormalities and DNA damage. Although there are prospects for screening of sperm (1), current routine clinical practice is based on the screening of peripheral blood samples.

4.2 Chromosomal abnormalities

Chromosome abnormalities can be numerical, such as trisomy, or structural, such as inversions or translocations. In a survey of pooled data from 11 publications including 9,766 infertile men, the incidence of chromosomal abnormalities was 5.8% (2). Of these, sex chromosome abnormalities accounted for 4.2% and autosomal abnormalities for 1.5%. For comparison, the incidence of abnormalities in pooled data from three series totalling 94,465 newborn male infants was 0.38%, of which 131 (0.14%) were sex chromosome abnormalities and 232 (0.25%) autosomal abnormalities (3).

Standard karyotype analysis should be offered to all men with damaged spermatogenesis who are seeking fertility treatment by IVF/ICSI.

4.2.1 Sperm chromosomal abnormalities

Multicolour fluorescent *in situ* hybridisation (FISH) analysis makes it possible to examine sperm populations for chromosomal normality. There is an increase in aneuploidy in sperm, and in particular sex chromosome aneuploidy in association with severe damage to spermatogenesis (2,4,5,7-11) and also in men with translocations (6).

FISH analysis of spermatozoa remains a research investigation but should be encouraged, particularly in assessing populations of spermatozoa from men with defined andrological conditions. There is a need to develop techniques to separate populations of genetically abnormal sperm from normal sperm or to identify safe techniques of screening individual spermatozoa prior to IVF and ICSI.

4.2.2 Sex chromosome abnormalities (*Klinefelter's syndrome and variants (47,XXY; 46,XY; 47,XXY mosaicism)*)

Klinefelter's syndrome is the most frequent sex chromosome abnormality. Pooled data from cytogenetic analysis of 9,766 newborn infants showed its occurrence in 66 (0.07%) phenotypical males (3). Adult men with Klinefelter's syndrome have small firm testicles devoid of germ cells. The phenotype can vary from a normally virilised man to one with stigmata of androgen deficiency, including female hair distribution, scanty body hair and long arms and legs because of late epiphyseal closure.

Leydig cell function is commonly impaired in men with Klinefelter's syndrome (12). Testosterone levels may be normal or low, oestradiol levels normal or elevated and FSH levels increased. Surprisingly, libido is often normal despite low testosterone levels, but androgen replacement may be needed with ageing. Germ cell presence and sperm production are variable in men with Klinefelter's mosaicism, 46,XY, 47,XXY. Pre-implantation genetic diagnosis using FISH analysis of cells from embryos can be used to confirm normality (13). The production of 24,XY sperm has been reported in 0.9% and 7.0% of men with Klinefelter's mosaicism (14-16) and in 1.36-25% of men with somatic karyotype 47,XXY (17-21). There is one case report of declining spermatogenesis in a man with Klinefelter's syndrome, with the recommendation that early sperm retrieval sperm should be considered (22). Haploid sperm in men with Klinefelter's syndrome may be the result of a clone of normal cells in a mosaic population, and in certain circumstances some 47,XXY male germ cells may be viable and capable of producing haploid sperm (23). Klinefelter's syndrome patients have an increased chance of producing 47,XXY spermatozoa. When IVF/ICSI is performed, pre-implantation diagnosis should be used or, if not available, amniocentesis and karyotype analysis. Embryos with known Klinefelter's karyotype should probably not be implanted.

Men with Klinefelter's syndrome are at risk for androgen deficiency as they get older and replacement therapy may be needed. Long-term follow up from an endocrine point of view will be needed for all men with Klinefelter's syndrome who have undergone testicular biopsy procedures for sperm retrieval.

4.2.3 Autosomal abnormalities

From time to time, men may ask for fertility treatments, including IVF/ICSI, when there is already a known autosomal defect. In these cases, genetic counselling is also required.

Genetic counselling should be offered to all couples where the male partner is known or found to have

autosomal karyotype abnormality.

4.2.4 *Translocations*

Reciprocal balanced translocations occur in 1 in 500 people. A person with a balanced translocation has a complete set of genetic information and is normal. However, when he or she has children, the child receives unbalanced genetic information, getting either too much or too little genetic material. A parental balanced translocation involving chromosome 21 is one of the causes of Down's syndrome.

When IVF/ICSI is performed for men with translocations, pre-implantation genetic diagnosis should be used or, if not available, amniocentesis and karyotype analysis. Embryos with known unbalanced translocation should probably not be implanted.

4.3 Genetic defects

4.3.1 *X-linked genetic disorders and male fertility*

Each man has only one X-chromosome. An X-linked recessive disorder manifests in males, and the defect will be transmitted to daughters but not to sons.

4.3.2 *Kallmann's syndrome*

The commonest X-linked disorder in infertility practice is Kallmann's syndrome. The predominant form is an X-linked recessive disorder caused by a mutation in the KAL1 gene on Xp22.3 (24). Rarer forms of Kallmann's syndrome include an autosomal-dominant form (25). Patients with Kallmann's syndrome have hypogonadotropic hypogonadism and may have other clinical features, including anosmia, facial asymmetry, cleft palate, colour blindness, deafness, maldescended testes and renal abnormalities. It is important to note that some men with Kallmann's syndrome have an isolated gonadotrophin deficiency without any other phenotypic abnormalities and may present de novo with infertility. It is often possible to stimulate spermatogenesis with replacement therapy (26).

4.3.3 *Androgen insensitivity: Reifstein's syndrome*

The rare disorder of androgen insensitivity may first present with infertility. The condition has X-linked recessive inheritance due to a defect in the androgen receptor gene located on Xq 11-12. The phenotype varies widely, from complete testicular feminization to an apparently normal man with infertility, although the latter is rare. Disorders of the androgen receptor causing infertility in the absence of any genital abnormality seem to be rare (27), although some cases have been identified (28). Longer CAG (cytosine-adenine-guanosine) repeats in exon one have been implicated and there appear to be racial differences.

4.3.4 *Other X-disorders*

A case report exists of an azoospermic man with biopsy-proven spermatogenetic arrest, who was found to have a submicroscopic interstitial deletion on the Xp pseudoautosomal region in peripheral blood and skin fibroblast samples. Other genetic and chromosome examinations were entirely normal, including probing of the Yq region (29). There is also a report about two men with azoospermia and X pseudoautosomal deletions (30).

4.3.5 *X-linked disorders not associated with male infertility*

A number of rare X-linked disorders are not associated with infertility. When recessive, these appear in male babies but skip several generations and therefore family history is important. Examples of such disorders include:

- Retinitis pigmentosa, a condition that affects 1 in 3,000 people, which may be recessive or dominant and causes visual impairment (31).
- Chronic granulomatous disease, a condition that predisposes to severe bacterial and fungal infections (32).
- Menkes disease, an X-linked recessive disturbance of copper metabolism associated with progressive neurological symptoms (33).

The couple should be given choices after appropriate genetic counselling, which should include the severity of any disorder that may result. It may be appropriate to offer pre-implantation sex determination and replacement of female embryos or amniocentesis and abortion.

4.4 Y genes and male infertility

4.4.1 *Introduction*

The first cases of Y microdeletions and male infertility were reported in 1992 (34) and since that time many case series have been published. Although microdeletions may occur in fertile men, they are more prevalent in infertile men (35). Microdeletions have been found in three non-overlapping regions of the Y chromosome, AZF a-b-c (36). A fourth region AZFd overlaps with AZFc and is considered by some to be a separate area (37,38).

The most commonly found microdeletion is in the AZFc region, encompassing the DAZ gene. However, there is a poor correlation between AZFc microdeletions or DAZ gene deletion and spermatogenesis; different men with apparently similar microdeletions can have different degrees of damage to their spermatogenesis (35,39). TESE can be used for men with AZFc Y microdeletions (40). AZFa and AZFb microdeletions are much rarer. If the AZFa microdeletion is large enough to remove both the USP9Y (DFFRY) and the DDX3Y (DBY) genes, azoospermia occurs (41-43). There is no recorded case of sperm recovery by micro-TESE (40). Azoospermia also occurs in men with larger AZFb Y microdeletions (40). Microdeletions are a subset of rearrangements of the long arm of the Y chromosome, others include duplications and inversions. The biological significance of these haplotypes has yet to be determined (44), but it is possible that some of them are associated with reduced fertility (45).

4.4.2 Clinical implications of Y microdeletions

Generally, genes on the Y chromosome do not affect vital processes but code for male characteristics (half of humanity does not have a Y chromosome!). Thus, men with Y microdeletions are unlikely to have phenotypic abnormalities other than abnormalities of the male reproductive system. Y microdeletions are associated with varying degrees of derangement of spermatogenesis (36,38,39,42,46). The AZFc microdeletion 51gr/51gr is associated with male infertility (47). It is a rare low penetrance allele that confers susceptibility to testicular germ cell tumour (TGCT) (48). This finding has implications when ICSI is used to achieve fertility for a man with a gr/gr microdeletion, and there is a need for more information about Y genes and Y microdeletions and diseases of the male reproductive organs. There is also one report of a higher frequency of AZFc microdeletions in the husbands of women with recurrent pregnancy loss (49).

Y microdeletions can be transmitted to male offspring. However, this is rare in the normal population because, without ICSI treatment, men with very low sperm counts are less likely to father children (35,36,50-56). In most cases, the microdeletion in the son is the same as in the father, but there have been reports that the size of the microdeletion can increase (52,56). More information is needed from father/son pairs about the fertility status of the son and whether the microdeletions remain the same size. When ICSI is used in the presence of a Y microdeletion, consideration needs to be given to long-term follow up of any male children with respect to their fertility status, and in the case of gr/gr microdeletions their risk of developing germ cell tumours.

4.4.2.1 Testing for Y microdeletions

Testing for microdeletions is now widespread, and methodology is becoming standardised (36,57,58). The following genes are located in the AZF regions and specifically expressed in the testis, including RBMY1A1, DAZ, VCY, XKRY, CDY1, DY2, HSFY, PRY, and BPY2. Other genes are expressed in more than one tissue, including RPS4Y2, USP9Y, DDX3Y, UTY, JARID1D, and EIF1AY, and there are probably others.

There is a need for studies to correlate testis histopathology with different combinations of loss of one or more of these genes. (For complete listings of Y genes, see the NCBI website <http://www.ncbi.nlm.nih.gov>). In the future, gene array analysis may replace testing for Y microdeletions. At present, the best test is to use selected STS (sequence tagged sites) probes chosen to define the most likely microdeletions. Direct testing for genes remains a research procedure.

4.4.2.2 CONCLUSIONS

- Testing for microdeletions is not necessary in men with obstructive azoospermia where ICSI is used, because spermatogenesis should be normal.
- For men with severely damaged spermatogenesis, testing for microdeletions before ICSI is desirable. However, as other risks (e.g. the risk of developing TGCT) are remote, it is reasonable to take into account the cost and availability of testing and to discuss this with the couple.
- If AZFa or AZFb Y microdeletions are detected, then it is doubtful whether micro-TESE is worthwhile because the chance of finding sperm is extremely low.
- There is a need for further evidence of the prognostic significance of gr/gr AZFc microdeletions and the development of TGCTs.
- If a man with microdeletion and his partner wish to proceed with ICSI, they can be advised that microdeletions will be passed to sons, but not to daughters.
- It is likely that a son who inherits a microdeletion will in turn have a fertility problem.
- There is some evidence that the gr/gr AZFc microdeletion may increase the risk of testicular cancer, but the actual increase in risk although unknown is probably very small.

4.4.3 Autosomal defects with severe phenotypic abnormalities as well as infertility

There are a number of inherited disorders with severe or considerable generalized abnormalities as well as infertility (Table 4). Such patients will be well known to doctors often from childhood and any fertility problem

has to be managed in the context of the care of the man as a whole and with consideration of his and his partner's ability to care for a child should treatment be successful.

Table 4: Less common inherited disorders associated with infertility and other alterations to phenotype

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

4.5 Cystic fibrosis mutations and male infertility

Cystic fibrosis (CF), a fatal autosomal-recessive disorder, is the most common genetic disease of Caucasians; 4% are carriers of gene mutations involving the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This gene is located on the short arm of chromosome 7. It encodes a membrane protein that functions as an ion channel and also 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 mutations and was found in approximately 2% of men with obstructive azoospermia attending a clinic in Edinburgh (59). However, the incidence in men with obstructive azoospermia varies between different countries.

The clinical diagnosis of absent vasa is easy to miss and all men with azoospermia should be very carefully examined to exclude CBAVD, particularly those with a semen volume of < 1.5 mL and pH less than 7.0.

There are approximately 1,500 mutations listed on the CFTR database (<http://www.genet.sickkids.on.ca/cftr/>). There are many published series of men with CBAVD, who were tested for varying numbers of mutations. In general, the more mutations tested for, the higher the percentage of men found to have them. In a review of published series of 449 men with CBAVD, the Delta F508 mutation was detected in 244 men, the R117H mutation in 54 men and the W1282X mutation in 37; 63 other mutations were found in between one and nine men, but not all mutations were tested for in all case series (60). It seems likely that as more mutations are defined and tested for, almost all men with CBAVD will be found to have mutations. At present, it is not practical to test for all known mutations as many have a very low prevalence in a particular population. Testing is usually restricted to the mutations that occur most commonly in a particular community.

Mutations may be found in both copies of the CFTR gene. However, in most men with CBAVD, mutation is found in only one copy. In some of these supposedly heterozygous cases, there may be an unknown second mutation, but there is also another mechanism. In two-thirds of these, a DNA variant – the 5t allele – can be detected in a non-coding region of CFTR (61). Men with CBAVD often have mild clinical stigmata of CF, e.g. history of chest infections. It is therefore important to follow-up children born after ICSI where the father has CBAVD and is either hetero- or homozygous.

When a man has CBAVD, it is important to test him and his partner for CF mutations. If she is also found to be a carrier, the couple must very carefully consider whether to proceed with ICSI using the husband's sperm, as the chance of a having a baby with CF will be 25% if the man is heterozygous and 50% if the man is homozygous. If the female partner is negative for known mutations, her chance of being a carrier of unknown mutations is about 0.4%. In these circumstances, the possibility of her heterozygous partner fathering a child with CF is approximately 1:410.

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 (62) and probably has a different genetic causation. Men with unilateral absence of the vas deferens are usually fertile,

and the condition is most commonly encountered as an incidental finding in the vasectomy clinic. Nevertheless, men with unilateral absence and CF mutations may have the same underlying genetic diseases as men with true CBAVD. It has also been found that men with bilateral absence of vas deferens and renal abnormalities do not have CFTR abnormalities (63).

Tests for CF mutations should be undertaken in men who are found to have unilateral absence of the vas and normal kidneys or bilateral absence or bilateral abnormality. If the results are negative and renal anatomy has not been defined, it is worthwhile obtaining an abdominal ultrasound. Findings may range from unilateral absence of the vas with ipsilateral absence of the kidney to bilateral vessel abnormalities and renal abnormalities, such as pelvic kidney.

4.7 Other single gene disorders

There is intense research into genes that control spermatogenesis, and in particular unique Y genes as their products may be targets for non-hormonal contraception. There are now many research publications of candidate genes for spermatogenesis, for example:

- Ubiquitin protease 26 gene (64,65)
- Polymorphisms in the oestrogen receptor gene (66,67)
- Polymorphisms of the gonadotrophin-regulated testicular helicase gene (68)
- UTP14c (69)
- SPAG16L (70)
- BGR-like gene (71)
- SPO11, EIF5A2, ACT (72)
- N372H variant of the BRCA2 gene (73)
- Heat shock transcription factor in AZFb (74).

At present, the clinical application of these findings is limited. It is likely that single gene mutations of these various genes account for only a small proportion of male infertility and therefore the practicality of testing will be constrained by expense and availability of the testing techniques. However, this may change with the advent of cheap gene array testing techniques, which will have the capacity to screen all or most of the known single gene defects with one test.

4.8 Unknown genetic disorders

ICSI is now 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 worries that children may be born with a fetal abnormality, because ICSI may enable defective sperm to bypass the selective processes of the female genital tract and egg covering. Alternatively, eggs may be fertilized that would otherwise not be fertilized. It is therefore very reassuring that the collected fetal abnormality statistics from ICSI centres do not indicate any increase in congenital malformations compared with the general population. However, the indications for ICSI are constantly being extended to include fertilization with immature sperm forms and it is particularly important to continue to monitor fetal abnormality rates, using detailed subgroup analysis according to the clinical and molecular diagnosis of the father.

4.9 Genetic and DNA abnormalities in sperm

There is an increase in the amount of DNA damage in spermatozoa from men with oligozoospermia. This is associated with reduced chances of natural conception and to a lesser extent after IVF/ICSI (75) and an increase in early pregnancy loss (76). There is one report that DNA damage may improve after varicocele ligation (77).

4.10 Genetic counselling and ICSI

The best initial management is to give the couple full information about the risks to the child to help them decide whether to proceed or not 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. The best management is to agree treatment with the couple, providing them with full information about the genetic risk.

However, in the situation where both partners are known to carry defects (e.g. CF mutations), there can be up to a 50% chance of child developing clinical CF and dying early after a number of years of morbidity. Many clinicians and infertility clinic personnel may feel it is unethical to proceed on the basis that 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 pre-implantation diagnosis and replacement only of normal embryos.

4.11 CONCLUSIONS

New insights into the genetic basis of infertility and the advent of ICSI necessitate a good understanding of genetics by clinicians and the public at large.

Advances in diagnostic modalities will allow identification of the genetic basis of more disorders, as well as providing diagnosis of known disorders at a lower cost, for some of which gene therapy may become practical.

4.12 RECOMMENDATIONS

- Standard karyotype analysis should be offered to all men with damaged spermatogenesis who are seeking fertility treatment by IVF/ICSI (2) (grade A recommendation).
- For men with severely damaged spermatogenesis, testing for Yq microdeletions before ICSI is desirable. However, as these men and their male children are unlikely to have any phenotypic abnormality other than impaired spermatogenesis, it is reasonable to take into account the cost and limitations of current testing methods and to discuss this with the couple (55-58) (grade B recommendation).
- When a man has structural abnormalities of the vas deferens (CBAVD, unilateral absence of the vas), it is important to test him and his partner for CF gene mutations (60) (grade A recommendation).
- Genetic counselling is mandatory in couples with a genetic abnormality found in clinical or genetic investigation and in patients who carry a (potential) inheritable disease (1) (grade A recommendation).

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5. OBSTRUCTIVE AZOOSPERMIA

5.1 Definition

Obstructive azoospermia means the absence of both spermatozoa and spermatogenetic cells in semen and post-ejaculate urine due to bilateral obstruction of the seminal ducts. Obstructive azoospermia is less common than NOA and occurs in 15-20% of men with azoospermia. Common causes of obstructive azoospermia are summarized in Table 5.

Table 5: A classification of obstructive azoospermia on the basis of ductal obstruction due to congenital and acquired causes

Classification	Conditions
<i>Epididymal obstruction</i>	
• Congenital forms	Idiopathic epididymal obstruction
• Acquired forms	Post-infective (epididymitis) Post-surgical (epididymal cysts)
<i>Vas deferens obstruction</i>	
• Congenital forms	Congenital absence of the vas deferens
• Acquired forms	Post-vasectomy Post-surgical (hernia, scrotal surgery)
<i>Ejaculatory duct obstruction</i>	
• Congenital forms	Prostatic cysts (Müllerian cysts)
• Acquired forms	Post-surgical (bladder neck surgery) Post-infective

Men with obstructive azoospermia present with normal size testes and normal FSH. On examination, enlargement of the epididymis can be found. Sometimes, the vas deferens appears absent due to congenital factors or previous inguinal or scrotal surgery. Although obstructions in primary infertile men are 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.

5.2 Classification

5.2.1 Intratesticular obstruction

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

5.2.2 Epididymal obstruction

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

Congenital epididymal obstruction usually manifests as congenital bilateral absence of the vas deferens (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 section 4 Genetic disorders in infertility). Other congenital forms of obstruction (dysjunction between efferent ductules and corpus epididymis, agenesis/atresia of a short part of the epididymis) are rare.

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

Among the acquired forms, those secondary to acute (gonococcal) and subclinical (e.g. chlamydial) epididymitis are considered to be most frequent (7,8) (see section 11 Male accessory gland infections). Acute or chronic traumas may result in epididymal damage (9).

Azoospermia caused by surgery may occur after epididymal cyst removal. Epididymal obstruction secondary to long-lasting distal obstruction must be taken into account 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 sterilization, with reports of subsequent germ cell impairment and fibrosis (11,12). About 2-6% of these men request vasectomy reversal. Of those undergoing vasovasostomy, 5-10% appear to have epididymal blockage due to tubule rupture, making epididymovasostomy mandatory (see section 10 Male contraception). Vasal obstruction may also occur after herniotomy (13). Polypropylene mesh herniorrhaphy seems to induce a fibroblastic response able to entrap, or obliterate, the vas deferens (14).

The most common congenital vasal obstruction is CBAVD, often accompanied by CF. Unilateral agenesis or a partial defect is associated with contralateral seminal duct anomalies or renal agenesis in 80% and 26% of cases, respectively (15) (see section 4 Genetic disorders in infertility). Distal vas deferens obstruction includes CBAVD and accidental injury to the vas deferens during hernia surgery (16).

5.2.4 Ejaculatory duct obstruction

Ejaculatory duct obstruction is found in about 1-3% of obstructive azoospermia (1). These obstructions can be classified as cystic or post-inflammatory.

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

Paramedian or lateral intraprostatic cysts are Wolffian in origin and rarely found 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

This might be attributed to local neuropathy (22). Because of the vasographic patterns of ampullovesicular atony or of ejaculatory duct hypertony, this abnormality seems to be very often associated with urodynamic dysfunctions. Although it has been observed in patients suffering from juvenile diabetes and in polycystic kidney disease (23), no relevant pathology has been found in most cases described. Results of semen analysis vary between azoospermia, cryptozoospermia and severe OAT syndrome.

5.3 Diagnostic management

5.3.1 Clinical history

Clinical history taking should follow the suggestions for investigation of infertile men (see section 2 Investigations), including asking about the presence of:

- 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 traumas
- Chronic sinopulmonary infections.

5.3.2 Clinical examination

This follows the suggestions for investigation of the infertile man. The following findings are indicative for obstructive azoospermia:

- At least one testis > 15 mL volume (although a smaller testicular volume may be found in some patients with obstructive azoospermia 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 performed at an interval of 2-3 months, according to the WHO (see section 2 Investigations). Azoospermia means absence of spermatozoa after centrifugation at x400 magnification. Careful repeat observation of several smears after semen liquefaction is necessary. Finding no spermatozoa in wet preparation should result in the centrifugation of aliquots or of the whole semen sample (600 rpm for 15 min). The pellet must be examined for spermatozoa.

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

5.3.4 Hormone levels

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

5.3.5 Ultrasonography

Scrotal ultrasound can be helpful in finding signs of obstruction (e.g. dilatation of rete testis, enlarged epididymis with cystic lesions and absence of vas deferens) and to exclude signs of testicular dysgenesis, such as non-homogenous testicular architecture and microcalcifications.

Transurethral ultrasound (TRUS) must be performed on patients with a low seminal volume and in whom distal obstruction is suspected. If possible, TRUS should be performed at high resolution and with high-frequency (7 MHz) biplane transducers. Seminal vesicle enlargement (anterior-posterior diameter 15 mm) (21) and roundish, anechoic areas in the seminal vesicle (24) are TRUS anomalies more often associated with ejaculatory duct obstruction, especially when semen volume is 1.5 mL. Other known anomalies in cases of obstructive azoospermia are Müllerian duct cysts or urogenital sinus/ejaculatory duct cysts (20) and ejaculatory duct calcifications (25). TRUS may also be applied to aspirate seminal vesicle fluid (26).

Invasive diagnosis, including testicular biopsy, scrotal exploration and distal seminal duct evaluation, are indicated in patients with obstructive azoospermia in whom an acquired obstruction of the seminal ducts is suspected. It is advisable to perform explorative and recanalization surgery at the same time.

5.3.6 Testicular biopsy

This may be indicated to exclude spermatogenic failure in selective cases. The same surgical procedure may also be used to extract testicular spermatozoa (i.e. TESE) for cryopreservation and subsequent ICSI, when surgical recanalization cannot be performed or has failed. A scoring system for testicular biopsies is given in Table 6 (27).

Table 6: Scoring system for testicular biopsies (Johnsen score) (27)

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

5.4 Treatment

5.4.1 Intratesticular obstruction

Since seminal duct recanalization at this level is impossible, TESE or fine-needle aspiration are recommended. The spermatozoa retrieved may be immediately used for ICSI or may be cryopreserved. Both TESE and fine-needle aspiration allow sperm retrieval in nearly all obstructive azoospermic patients.

5.4.2 Epididymal obstruction

Microsurgical epididymal sperm aspiration (MESA) (28) is indicated in men with CBAVD. Retrieved spermatozoa are usually used for ICSI. In general, one MESA procedure provides sufficient material for several ICSI cycles (29). For sperm recovery, there is limited evidence that a micropuncture with nerve stimulation may be preferable to a simple MESA technique as it produces higher pregnancy and fertilisation rates (30). In patients with azoospermia due to acquired epididymal obstruction, end-to-end or end-to-side microsurgical epididymovasostomy is recommended: microsurgical intussusception vasoepididymostomy seems to be the most favourable technique (31).

Reconstruction may be done unilaterally or bilaterally; patency and pregnancy rates are usually higher with bilateral reconstruction. Before performing microsurgery, it is important to check that there is full patency downstream of the epididymis. Anatomical recanalization following surgery may require 3-18 months. Before performing microsurgery (and also in all cases where recanalization is impossible), epididymal spermatozoa should be aspirated and cryopreserved to be used for ICSI in case of surgical failure (29).

Patency rates range between 60% and 87% (32-34) and cumulative pregnancy rates between 10% and 43%. Recanalization success rates may be adversely affected by pre-operative and operative findings, such as concomitant abnormal testicular histology, absence of sperm in the spermatic fluid on sectioning the small epididymal tubules and wide fibrosis of the epididymis.

The finding of motile or immotile spermatozoa at the level of the anastomosis does not appear to be related to the patency rate, but moving from the corpus to the caput epididymidis has a significant adverse effect upon patency and pregnancy outcome. Spermatozoa need to pass through at least part of the epididymis to mature and be able to fertilize oocytes in a natural cycle. Concomitant presence of ultrasonographic abnormalities in the prostate and seminal vesicles is also associated with a less favourable outcome (10).

In terms of delivery rate, vasoepididymostomy in patients with epididymal obstruction secondary to vasectomy has proved more successful and more cost-effective than MESE and ICSI (35) (see section 10 Male contraception).

5.4.3 Proximal vas obstruction

Proximal vas obstruction after vasectomy requires microsurgical vasectomy reversal (see section 10 Male contraception). Vasovasostomy must also be performed in the rare cases of proximal vasal obstructions (iatrogenic, post-traumatic, post-inflammatory). When spermatozoa are absent in the intraoperative vas fluid, a secondary epididymal obstruction may be present, especially if the seminal fluid of the proximal vas has a thick 'toothpaste' appearance. Microsurgical vasoepididymostomy is indicated.

5.4.4 Distal vas deferens obstruction

Large bilateral vas defects resulting from involuntary vas excision during hernia surgery in early childhood or previous orchidopexy are usually incorrectable (16). In these cases, one can resort to proximal vas deferens sperm aspiration (36) or TESE/MESA to be used for ICSI. In large monolateral vas defects associated with contralateral testicular atrophy, the vas of the atrophic testis can be used for a crossover vasovasostomy or vasoepididymostomy.

5.4.5 Ejaculatory duct obstruction

The treatment of ejaculatory duct obstruction depends on the aetiology. In large post-inflammatory obstruction and when one or both ejaculatory ducts empty into an intraprostatic midline cyst, transurethral resection of the ejaculatory ducts (TURED) (20,37) can be performed. 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 and more effective. If distal seminal tract evaluation is carried out at the time of the procedure, installation of methylene blue dye into the vas may be helpful to document opening of the ducts.

Complications following TURED include retrograde ejaculation due to bladder neck injury, reflux of urine into ducts, seminal vesicles and vasa (causing poor sperm motility, acid semen pH and epididymitis). Alternatives to TURED are MESA, TESE, proximal vas deferens sperm aspiration, seminal vesicle aspiration and direct ultrasonically guided cyst aspiration.

In cases of functional obstruction of the distal seminal ducts, TURED often fails to improve the sperm output. Spermatozoa may then be retrieved by antegrade seminal tract washout (37). Spermatozoa retrieved by any of the aforementioned surgical techniques should always be cryopreserved for assisted reproductive procedures.

5.5 CONCLUSIONS

- Obstructive lesions of the seminal tract should be suspected in azoospermic or severely oligozoospermic patients with normal-sized testes and normal endocrine parameters.
- Results of reconstructive microsurgery depend on the cause and location of the obstruction and the expertise of the surgeon. Standardized procedures include vasovasostomy and epididymovasostomy.
- Sperm retrieval techniques such as MESA, TESE and testicular fine-needle aspiration can be applied additionally. The consensus is that these methods should only be performed with the facility for cryostorage of the material obtained.

5.6 RECOMMENDATIONS

- In cases of azoospermia due to epididymal obstruction a scrotal exploration with MESA and cryopreservation of the spermatozoa should be performed together with a microsurgical reconstruction (35) (grade B recommendation).

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6. VARICOCELE

6.1 Introduction

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

- Failure of ipsilateral testicular growth and development
- Symptoms of pain and discomfort
- Infertility.

6.2 Classification

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

- Subclinical: Not palpable or visible at rest or during Valsalva manoeuvre, but demonstrable by special tests (reflux found upon Doppler examination) (3).
- Grade 1: Palpable during Valsalva manoeuvre but not otherwise.
- Grade 2: Palpable at rest, but not visible.
- Grade 3: Visible and palpable at rest.

6.3 Diagnosis

The diagnosis of varicocele has been defined by the WHO (2). The consensus is that diagnostic procedure and classification of a varicocele, including analysis, have to follow these accepted criteria (2).

The diagnosis of varicocele is made by clinical examination and may be confirmed by colour Doppler analysis. In centres where treatment is performed by antegrade or retrograde sclerotherapy or embolization, the diagnosis is additionally confirmed by X-ray.

6.4 Basic considerations

Various studies have been conducted on the epidemiology of varicocele, its association with male infertility and whether treatment is beneficial.

- Varicocele is a physical abnormality present in 20-24% of the adult male population (4,5). It is more common in men of infertile marriages, affecting 25% of those with abnormal semen analysis (6).
- The incidence of pain and discomfort associated with varicocele is 2-10% (7). Treatment to relieve symptoms is often recommended, but there have been few outcome studies; however, most urologists accept discomfort as a valid indication.
- The exact association between reduced male fertility and varicocele is not known, but analysis of the WHO data (8) clearly indicates that varicocele is related to semen abnormalities, decreased testicular volume and decline in Leydig cell function.
- Two prospective randomized studies showed increased ipsi- and contralateral testis growth in adolescents who had received varicocele treatment compared with those who did not (9,10). A cohort follow-up study, which took serial measurements of testicular size in children, showed that varicocele halted testicular development. However, following treatment for varicocele, catch-up growth occurred to the expected growth percentile (11).
- A series of studies suggested that altered endocrine profiles in men with varicocele might predict those who would benefit from treatment (12,13).
- Five prospective randomized studies of varicocele treatment in adults gave conflicting results (6,14-18), with the largest study indicating benefit (16,18). The externally randomized study involved 10 centres and included men of infertile couples who had moderate oligozoospermia ($5-20 \times 10^6/\text{mL}$) and grade II or III varicocele. Immediate therapy was significantly more effective than delaying treatment for 1 year with regard to achieving pregnancy and the pregnancy rate per menstrual cycle (fecundability). However, a meta-analysis of the five trials indicated no benefit (common odds ratio was 0.85% (95% CI: 0.49-1.45) (19).

- The only prospective randomized study of treatment of subclinical varicocele failed to show that therapy benefited fertility (20).
- Analysis of the large WHO infertility study (21) indicated that there was an excess of couples where both partners had factors associated with reduced fertility compared with the expected rate of coincidence in the general population. This implied that a minor cause of impaired fertility, such as varicocele, will only manifest in couples in which the female partner also has reduced fertility.

The studies mentioned above were summarized in a recent review (22), criticising the recent Cochrane analyses of randomized controlled trials on varicocele treatment and pregnancies (23). The authors concluded that the Cochrane meta-analysis conclusions should not support guidelines recommendation against varicocele treatment in subfertile patients. Data from ongoing studies should provide more information on this topic.

6.5 Treatment

Several treatment modalities can be chosen (Table 7). The type of intervention is mainly dependent on the therapist's experience. Although laparoscopic varicocelectomy is feasible, it needs to be justified in terms of cost effectiveness.

Table 7: Recurrence and complication rates of different treatment methods for varicocele

Treatment	Recurrence/persistence rates	Complications
• Antegrade sclerotherapy	9%	Complication rate 0.3-2.2%; testicular atrophy; scrotal haematoma; epididymitis; left-flank erythema
• Retrograde sclerotherapy	Recurrence and persistence rate 9.8% (24)	Adverse reaction to the contrast medium; flank pain; persistent thrombophlebitis; vascular perforation (25)
• Retrograde embolization	3.8-10% (26,27)	Pain due to thrombophlebitis (27); bleeding haematoma; infection; venous perforation; hydrocele; radiological complication, e.g. reaction to contrast media; misplacement or migration of the coils (28); retroperitoneal haemorrhage; fibrosis; ureteric obstruction (5)
<i>Open operation</i>		
• Scrotal operation	–	Testicular atrophy (5); arterial damage with risk of devascularization and gangrene of the testicle
• Inguinal approach	13.3% (29)	Possibility of missing out a branch of testicular vein
• High ligation	29% (29)	5-10% incidence of hydrocele (30)
• Microsurgical	0.8-4% (31,32)	Post-operative hydrocele arterial injury; scrotal haematoma
• Laparoscopy	3-7% (33-35)	Injury to testicular artery and lymph vessels; intestinal, vascular and nerve damage; pulmonary embolism; peritonitis (35); bleeding; post-operative pain in right shoulder (due to diaphragmatic stretching during pneumoperitoneum) (34); pneumoscrotum; wound infection (35)

6.6 CONCLUSIONS

- Current information supports the hypothesis that in some men the presence of varicocele is associated with progressive testicular damage from adolescence onwards and consequent reduction in fertility. However, in infertile couples this impaired fertility potential will only be manifest if female fertility is also reduced.
- Although treatment of varicocele in adolescents may be effective, there is a significant risk of overtreatment.

6.7 RECOMMENDATIONS

- Varicocele treatment is recommended for adolescents who have progressive failure of testicular development documented by serial clinical examination (9,10) (grade B recommendation).
- There is no evidence indicating benefit from varicocele treatment in adolescents who have no ipsilateral testicular atrophy and no endocrine abnormalities. In this situation, varicocele treatment cannot be recommended except in the context of clinical trials (9,10) (grade B recommendation).
- Reviews of randomized clinical trials have raised doubts about the benefit of varicocele treatment in subfertile men. Varicocele treatment for infertility should not be done unless there has been full discussion with the infertile couple about the uncertainties of treatment benefit (19,22,23) (grade B recommendation).

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7. HYPOGONADISM

7.1 Introduction

Hypogonadism is defined as deficient androgen secretion. The symptoms of hypogonadism depend on the degree of androgen deficiency and whether the condition develops before or after the pubertal development of the secondary sex characteristics. The symptoms and signs of hypogonadism debuting before and after completion of puberty are given in Table 8.

Table 8: The symptoms and signs of hypogonadism debuting before and after completion of puberty

Affected organ/function	Before completed puberty	After completed puberty
• Larynx	No voice mutation	No voice mutation
• Hair	Horizontal pubic hairline Straight frontal hairline Diminished beard growth	Diminished secondary body hair
• Skin	Absent sebum production Lack of acne Pallor Skin wrinkling	Decreased sebum production Lack of acne Pallor Skin wrinkling
• Bones	Eunuchoid tall stature Osteoporosis	Osteoporosis
• Bone marrow	Low-level anaemia	Low-level anaemia
• Muscles	Underdeveloped	Atrophy
• Prostate	Underdeveloped	Atrophy
• 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

The aetiological and pathogenetic mechanisms behind male hypogonadism can be divided into three main categories.

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

The most common conditions within these three categories are given in Table 9.

Table 9: Disorders with male hypogonadism. Modified from Nieschlag et al. (1)

Primary (hypergonadotropic) hypogonadism (= testicular insufficiency)
• Anorchia
• Congenital factors (testicular dysgenesis)
• Acquired factors (trauma, testicular torsion, tumour, surgery)
• Maldescended testes
• Klinefelter's syndrome*
• Other chromosomic alteration*
• Germ cell aplasia
• Complete and focal germ cell aplasia (Sertoli cell-only syndrome), either congenital or acquired: maldescended testes, irradiation, cytostatic drugs
• Spermatogenic arrest
• Post-inflammatory (orchitis)
• Exogenous factors (medications, toxins, irradiation, heat)
• Systemic diseases (liver cirrhosis, renal failure)
• Testicular tumour
• Varicocele
• Surgeries that can damage vascularization of the testes
• Idiopathic
Secondary (hypothalamic or pituitary origin) (hypogonadotropic state with secondary hypogonadism)
• Idiopathic hypogonadotropic hypogonadism (including Kallmann's syndrome)
• Delay of puberty
• Hyperprolactinaemia
• Drugs/anabolic steroids
Target organ resistance to androgens
• Testicular feminization
• Reifenstein's syndrome

* See section 4 Genetic disorders in infertility.

7.2 Hypogonadotropic hypogonadism: aetiology, diagnosis and therapeutic management

Primary hypogonadotropic hypogonadism is caused either by hypothalamic or pituitary diseases. The failure of hormonal regulation can easily be determined (2). Endocrine deficiency leads to a lack of spermatogenesis and testosterone secretion due to decreased secretion patterns of luteinizing hormone (LH) and FSH. The therapy of choice depends on whether the goal is to achieve normal androgen levels or to achieve fertility.

Normal androgen levels and subsequent development of secondary sex characteristics (in cases of onset of hypogonadism before puberty) and eugonadal state can be achieved by androgen replacement only. However, stimulation of sperm production requires treatment with human chorionic gonadotrophin (hCG) combined with recombinant FSH. In the rare cases of 'fertile eunuchs' having sufficient production of FSH but not LH, treatment with hCG only may be sufficient to stimulate sperm production and to achieve normal testosterone levels (3).

If hypogonadotropic hypogonadism is hypothalamic in origin, an alternative to hCG treatment is therapy with pulsatile GnRH (4). In patients who have developed hypogonadism prior to puberty and not been treated with gonadotropins or GnRH, 1-2 years of therapy may be necessary to achieve sperm production. Once pregnancy has been induced, patients can return to testosterone substitution.

Secondary hypogonadotropic hypogonadism can be caused by some drugs, hormones and anabolic steroids.

7.3 Hypergonadotropic hypogonadism: aetiology, diagnosis and therapeutic management

Common conditions associated with hypergonadotropic hypogonadism in younger men include injury to, and loss of, the testicles (e.g. after bilateral testicular cancer) (Table 9). Men with Klinefelter's syndrome are at risk for hypogonadism (5) with ageing. Recently, it was reported that men with infertility problems are at higher risk for developing hypogonadism (6). Those undergoing extensive testicular biopsy in the context of IVF/ICSI will almost certainly have an exacerbated risk (7).

Hypergonadotropic hypogonadism may occur spontaneously in the elderly, in patients with erectile dysfunction (8), and after luteinizing hormone releasing hormone (LHRH) treatment or surgical castration for prostatic cancer (9). All these conditions are not clinically significant for infertile men. Hypogonadism may be associated with osteoporosis (10).

The laboratory diagnosis of hypergonadotropic hypogonadism is based on decreased serum

testosterone and increased LH levels (2). Testosterone levels should be evaluated in view of the concentration of the serum concentration of sex hormone binding globulin (SHBG). Based on levels of total testosterone and SHBG, free and bioavailable testosterone can be calculated (www.issam.ch). Due to diurnal variation, blood sampling for testosterone assessment should be done before 10 am. The existing guidelines for androgen replacement are mainly based on total testosterone levels and testosterone supplementation is only indicated in men with levels consistently lower than normal (< 12 nmol/L [300 ng/dL]). Injectable, oral and transdermal testosterone preparations are available for clinical use (2). The best preparation is the one that maintains serum testosterone levels as close to physiological concentrations as possible (11).

7.4 CONCLUSIONS

- There is general agreement that patients with primary or secondary hypogonadism should receive testosterone substitution therapy.

7.5 RECOMMENDATIONS

- Effective drug therapy is available to achieve fertility in men with hypogonadotropic hypogonadism (4) (grade A recommendation).
- Male infertility may be accompanied by hypogonadism (6) (grade B recommendation).

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8. CRYPTORCHIDISM

8.1 Introduction

Cryptorchidism is the most frequent congenital abnormality of the male genitalia and is found in 2-5% newborn boys. This figure is dependent on the gestational age, with premature boys having a higher frequency of cryptorchidism, and age post partum. At the age of 3 months, the incidence is reduced 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 and both disrupted endocrine regulation and several gene defects may be involved. For a normal descent of the testes, a normal hypothalamo-pituitary-gonadal axis is needed. Although most boys with maldescended testes show no endocrine abnormalities after birth, endocrine disruption in early pregnancy can potentially affect gonadal development and normal descent. It has been postulated that cryptorchidism may be the consequence of testicular dysgenesis, a developmental disorder of the gonads due to environmental and/or genetic influences early in pregnancy. Testicular dysgenesis syndrome can result in maldescence, hypospadias, reduced fertility and an increased risk for malignant development (1).

8.2 Incidence of cryptorchidism

The Caucasian population has a three-fold higher incidence of cryptorchidism compared to African-Americans. Even between Caucasians, significant differences in the risk of this malformation was found, the condition being significantly more common among Danish compared to Finish newborns (2). Premature babies reveal a much higher incidence than fullterm babies. Scorer examined more than 3,000 newborns in London. The incidence of cryptorchidism in boys weighing > 2,500 g was 2.7%, whereas in premature boys weighing < 2,500 g the corresponding number was 21%. At the age of 3 months, spontaneous descence occurred in most boys and the incidence rate declined to 0.9 and 1.7%, respectively (3).

8.3 Testicular descent and maldescent

The process of testicular descent has two distinct phases: a) transabdominal; and b) inguinal.

During 'transabdominal descence', development of the gubernaculum and genitoinguinal ligament plays an important role. The antiMüllerian hormone additionally regulates the transabdominal descence of the testis. Induction of the gubernaculum is dependent on 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). There are other gene families, e.g. the homeobox (HOX) genes and GREAT gene, which are important for the development of genital organs and may be associated with testicular maldescence (6,7).

8.4 Hormonal control of testicular descent

Maldescence can be caused by two hormonal factors: hypogonadism and androgen insensitivity. Also, the increasing incidence of reproductive abnormalities in human males may be explained by an increased oestrogen exposure during gestation (8). Some pesticides and synthetic chemicals are known to act as hormonal modulators, often possessing oestrogenic activity (xeno-oestrogens) (9). The oestrogenic and antiandrogenic properties of these chemicals may cause hypospadias, cryptorchidism, reduction of sperm density, and an increase in the incidence of testicular tumours in animal models by receptor-mediated mechanisms or direct toxic effects (10).

8.5 Pathophysiological effects in maldescended testes

8.5.1 Degeneration of germ cells

It is established that the degeneration of germ cells in maldescended testes becomes apparent after the first year. Depending on the different position of the testis, the degenerative changes are variable (11). During the second year of life, the number of germ cells clearly starts to decline. In 10-45% of affected patients, a 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, but hormone treatment with either hCG or GnRH analogues can be considered, particularly in cases where testes are located in the high scrotal position (12).

8.5.2 Relationship with fertility

Semen parameters are often impaired in men with a history of cryptorchidism (13). It has been suggested that surgical treatment performed during the first or second year of life has a positive effect on subsequent fertility (14). However, definitive proof of the protective effect of early orchidopexy is lacking. Paternity in men with a history of unilateral cryptorchidism is almost equal (89.7%) to paternity in men without cryptorchidism (93.7%). In men with unilateral cryptorchidism, paternity seems independent of the age of orchidopexy, preoperative

testicular location and testicular size (15). However, it cannot be excluded that a history of unilateral cryptorchidism may result in a reduced fertility potential, i.e. not affecting paternity but causing a prolonged time to 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%.

8.5.3 Germ cell tumours

Cryptorchidism is a risk factor for testicular cancer development and is associated with testicular microcalcification and carcinoma *in situ* (CIS) of the testis. In about 5-10% of testicular cancers, there is a history of cryptorchidism (16). The risk of a germ cell tumour is 3.6-7.4 times higher than in the general population, and 2-6% of men with a history of cryptorchidism will develop a testicular tumour (16). So far, there is a lack of evidence for a protective effect of early orchidopexy (17).

8.6 Treatment of undescended testes

8.6.1 Hormonal treatment

In randomized, controlled trials for the efficacy and side-effects of hCG and GnRH treatment, a large variation in success rates have been reported. The corresponding figures in all randomized trials were 21%, 19% and 4% for GnRH, hCG and placebo, respectively (12). A meta-analysis of 33 studies published between 1958 and 1990 by Pyorala et al. (18) showed that the success rate was best in prescrotal and high scrotal testes. Non-palpable testes rarely descend as a result of hormonal treatment.

The current hormonal protocol for high scrotal testes is three hCG injections given once per week. The dose is 1,500 IU per injection for children at ages 1-3 years, 3,000 IU at ages 4-6 years, and 5,000 IU at ages 6-15 years. The recommended age for this treatment is 12-18 months. In a patient with bilateral impalpable testes, a hCG stimulation test can be performed. The presence of testes is confirmed by a rise in testosterone level. Inhibin B is produced by the Sertoli cells of the testis and can be a good indicator for testicular function in children (19).

Hormonal treatment is generally considered safe, with only a few side-effects. However, some studies have indicated an increased risk of interstitial fibrosis and germ cell apoptosis following hCG treatment (20). Early adverse effects include penile growth, pain in the genital region, pain at the site of injection and psychological changes due to androgen effects.

8.6.2 Surgical treatment

The success rates of surgical treatment is 70-90% in undescended testes (21). When the spermatic cord or vessels are too short to allow proper mobilisation of the testis into the scrotum, a staged orchidopexy (Fowler-Stephenson procedure) can be performed. The applied techniques are open surgery, laparoscopy, or microsurgery.

If not corrected by adulthood, an undescended testis should not be removed. A biopsy at the time of orchidopexy (see page 55) can reveal the presence of CIS and thereby prevent a malignant tumour.

After orchidopexy, vascular damage is the most severe complication and may cause testicular atrophy in 1-2% of cases. In non-palpable testes, the post-operative atrophy rate was 12% in cases where the vascular pedicles were long enough to allow scrotal positioning. Up to 40% post-operative atrophy was reported in cases of staged orchidopexy.

8.7 CONCLUSIONS

- Cryptorchidism is multifactorial in origin and may be caused by genetic factors and endocrine disruption early in pregnancy.
- Cryptorchidism is often associated with testicular dysgenesis and is a risk factor for infertility and germ cell tumours.
- It is still matter of discussion whether early surgical intervention may prevent germ cell loss.
- Paternity in men with unilateral cryptorchidism is almost equal to paternity in men without cryptorchidism.
- In bilateral cases of cryptorchidism, the likelihood of paternity is significantly reduced.

8.7 RECOMMENDATIONS

- The success rate of hormonal treatment of cryptorchidism has only been shown for prescrotal and high scrotal testes.
- Non-palpable testes rarely descend by hormonal treatment (18) (grade B recommendation).
- If corrected in adulthood, a testicular biopsy for detection of CIS is recommended at the time of the orchidopexy to exclude the risk of tumour development (16) (grade B recommendation).

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9. IDIOPATHIC MALE INFERTILITY

9.1 Introduction

Many men presenting with infertility are found to have idiopathic OAT syndrome. No demonstrable cause of male infertility, except for OAT, is found in 40-75% of infertile men. Drug treatments for idiopathic male infertility are discussed.

9.2 Empirical treatments

A wide variety of empirical drug approaches have been used (Table 10). However, there is little scientific evidence for an empirical approach (1). Criteria for the analysis of all therapeutic trials have been re-evaluated. There is consensus that only randomized, controlled trials, with 'pregnancy' as the outcome parameter, can be accepted for efficacy analysis. The use of recombinant human FSH in patients with idiopathic oligozoospermia, but with normal FSH and inhibin B, may be a debatable choice in the future to improve spermatogenesis (1).

Table 10: Empirical therapy of idiopathic oligo-astheno-teratozoospermia (OAT) syndrome‡

Therapeutic approaches	EAU recommendations
<i>Hormonal</i>	
• GnRH	Contradictory results Not controlled trials (2) Further trials are needed
• hCG/hMG	Lack of efficacy Not recommended
• FSH	Efficacy not yet shown Further trials are needed (3)
• Androgens	Lack of efficacy Not recommended
• Anti-oestrogens (clomiphene citrate, tamoxifen-testosterone undecanoate)	Potentially effective (4) Use must be counterbalanced against possible side-effects (5). Further studies are needed
<i>Non-hormonal</i>	
• Kinin-enhancing drugs	Unproven efficacy Clinical trials only
• Bromocriptine	Lack of efficacy Not recommended
• Antioxidants	May benefit selected patients Clinical trials only
• Mast cell blockers	Some efficacy shown Further evaluation needed Clinical trials only
• Alpha-blockers	Lack of efficacy Not recommended
• Systemic corticosteroids	Lack of efficacy Patients with high levels of antisperm antibodies should enter an ART programme
• Magnesium supplementation	Unproven efficacy (4) Not recommended

* ART = assisted reproduction techniques; FSH = follicle-stimulating hormone; GnRH = gonadotrophin-releasing hormone; hCG = human chorionic gonadotrophin; hMG = human menopausal gonadotrophin.

‡Also based in parts on the recommendations of the 'Infertility Guidelines Group' of the Royal College of Obstetricians and Gynaecologists, London 1998.

9.3 RECOMMENDATIONS

- Medical treatment of male infertility can only be advised in cases of hypogonadotropic hypogonadism (1) (grade A recommendation).
- Drugs are usually ineffective in the treatment of idiopathic male infertility (grade B recommendation).

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10. MALE CONTRACEPTION

10.1 Introduction

It is more precise to discuss the 'male contribution to contraception' rather than 'male contraception', because men do not conceive. The 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. Some 45 million unintended pregnancies are terminated each year, with unsafe abortions killing an estimated 68,000 women every year (1).

Three of the four methods of male contraception have been in use for 100s 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 between 3 and 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 have to be effective, reversible, acceptable and cheap.

Biomedical research is attempting to (3):

- Prevent sperm production (through use of androgens, progestogen and GnRH in various combinations)
- Interfere with the ability of sperm to mature and carry out fertilization by using an epididymal approach to create a hostile environment for sperms
- Produce better barrier methods; polyurethane condoms can be used by those with latex allergy, but have higher breakage rates (4)
- Produce an antisperm contraceptive vaccine (5)
- Inhibit sperm-egg interactions.

All these approaches remain experimental, but the method nearest to being generally available clinically is hormonal male contraception. This method is based on the suppression of gonadotrophins and the use of 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 progestogen has resulted in complete suppression of spermatogenesis in all races and provided contraceptive efficacy equivalent to female hormonal methods (7). Phase 3 clinical trials of depot preparations of androgen/progestin combinations are in progress.

10.2 Vasectomy

Vasectomy is an effective method of permanent male surgical sterilization (8). Before the procedure is performed, the couple should be given accurate information about the benefits and risks. An Australian telephone survey found that 9.2% of respondents said they regretted having a vasectomy (9).

10.2.1 Surgical techniques

There are various techniques. The least invasive approach to the vas is the no-scalpel vasectomy approach (10), which is also associated with a low rate of complications (11). The most effective occlusion technique seems to be cauterization of the lumen of the vas and fascial interposition (12-14). Most techniques can be safely performed as an outpatient procedure under local anaesthesia.

10.2.2 Complications

Acute local complications include haematoma, wound infection and epididymitis in up to 5% of all cases (15). Long-term complications, such as chronic testicular pain (16), must be discussed with the patient. Epididymal

tubal damage is common, with the consequent development of sperm granuloma and time-dependent secondary epididymal obstruction setting a limit to vasectomy reversal.

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 of any systemic disease after vasectomy. In a meta-analysis, Bernal-Delgado et al. could not detect an increased rate of prostate cancer in men who underwent vasectomy (17).

10.2.3 Vasectomy failure

Using an effective occlusion technique, the risk of recanalization after vasectomy should be less than 1% (12). No motile spermatozoa should be detected 3 months after vasectomy. Persistent motility is a sign of vasectomy failure and the need to repeat the procedure. A 'special clearance' can be given to men who continue to produce non-motile spermatozoa up to 1 year after vasectomy (18). Patients should be informed preoperatively that long-term re-canalization, although rare, may occur (19).

10.2.4 Counselling

Counselling has to address the following items concerning vasectomy.

- It should be considered irreversible.
- It has a low complication rate. However, because vasectomy is an elective operation, even small risks should be explained as men may wish to consider these before giving their consent.
- It has a low, but existing, failure rate.
- Couples should be advised to continue with other effective contraception until clearance is achieved.
- All available data indicate that vasectomy is safe and not associated with any serious, long-term side-effects (15) (level of evidence: 2a).
- Fascial interposition and cauterization seem to give a higher efficacy (12-14) (level of evidence: 2a).

10.3 Vasectomy reversal

A wide range of surgical success rates has been published for vasectomy reversal (up to 90%), depending on the time elapsed after vasectomy, type of vasectomy (e.g. open-ended or sealed), type of reversal (vasovasostomy or vasoepididymostomy) and whether reversal was unilateral or bilateral. Although there have been no randomized, controlled trials that compare macrosurgery (loops) and microsurgery, there is consensus that microsurgical techniques with the help of magnification and smaller suture materials should be applied (20).

10.3.1 Length of time since vasectomy

Vasovasostomy results have shown patency rates (up to 90%) superior to pregnancy rates. The longer the interval from vasectomy to reversal, the lower the pregnancy rates. Belker et al. (21) reported results in 1,469 men who had undergone microsurgical vasectomy reversal. Patency and pregnancy rates, respectively, were 97% and 76% 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 or longer.

10.3.2 Epididymovasostomy

The chance of secondary epididymal obstruction increases with time after vasectomy. If this has occurred, epididymovasostomy is needed to reverse the vasectomy (see section 5 Obstructive azoospermia).

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

Calculations of cost per delivery for vasectomy reversal versus sperm retrieval-ICSI under a wide variety of initial assumptions clearly indicate that vasectomy reversal is associated with a considerably lower cost per delivery and higher delivery rates (22,23). Sperm retrieval and ICSI must yield a 81% pregnancy rate per cycle to achieve equal costs to vasectomy reversal.

10.4 CONCLUSIONS

- The most cost-effective approach to treatment of post-vasectomy infertility is microsurgical reversal. This also has the highest chance of delivery.
- Couples can have a family after successful vasectomy reversal. There is no need for hormonal treatment of the female partner, with its associated risks of ovarian hyperstimulation and multiple pregnancies.
- MESA/TESE and ICSI should be reserved for failed surgery.

10.5 RECOMMENDATIONS

- All available data indicate vasectomy is safe and not associated with any serious, long-term side-effects (15) (grade A recommendation).
- Fascial interposition and cauterization seem to give a higher efficacy (12-14) (grade B recommendation).
- Consultation has to include information about the surgical method, risk of failure, irreversibility, need for post-procedure contraception until clearance, and the risk of complications.
- Other methods of male contraception have high failure rates or are still experimental (e.g. hormonal approach).
- Microsurgical vasectomy reversal is a low-risk and (cost-)effective method of restoring fertility (grade B recommendation).
- Sperm aspiration together with ICSI is a second-line option for selective cases and in cases of failed vaso-vasostomy.

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11. MALE ACCESSORY GLAND INFECTIONS (MAGIS)

11.1 Introduction

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

11.2 Urethritis

Infectious, sexually acquired urethritis may be caused by a variety of pathogens, most commonly by *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Neisseria gonorrhoea* (4). Non-infectious causes of urethritis include

irritations due to allergic reactions, trauma and manipulations. Urethral discharge and bladder voiding problems are the predominant symptoms of acute urethritis.

11.2.1 Diagnosis and treatment

Diagnosis is based on the analysis of urethral smear and first-voided urine (VB1). Evidence of ≥ 4 granulocytes per microscopic high-power field (x1000) in an urethral smear, or of 15 granulocytes per microscopic field (x400) in the smear of the sediment of 3 mL VB1, has been considered pathognomonic (4). In urethritis, defined by inflammatory discharge, semen analysis for disorders of male fertility is not possible as the anterior urethra is full of infectious and inflammatory material that hampers any useful analysis (5).

The impact of urethritis on semen quality and fertility has not really been proven due to contamination of the ejaculate with inflammatory material from the urethra.

The negative influence of sexually transmitted micro-organisms on sperm function is still a matter of debate (1,6,7). Urethral strictures and ejaculatory disturbances have been claimed to impair male fertility (2), as has the development of obstruction (8), either as normal urethral stricture or lesion in the posterior urethra in the area of the verumontanum, both of which may lead to ejaculatory disturbances and central obstruction of the seminal pathway (2).

Treatment of sexually transmitted diseases is standardized by the guidelines of the Centers of Disease Control and Prevention in Atlanta (GA, USA) (9). As the aetiology of acute urethritis is usually unknown at the time of diagnosis, empirical therapy directed against potential pathogens is suggested. This involves giving a single dose of a fluoroquinolone followed by a 2-week regimen of doxycycline. Treatment is effective both for gonococcal and (co-existing) chlamydial/ureaplasma infections.

11.3 Prostatitis

Prostatitis represents the most common urological diagnosis in men under the age of 50 years (10).

Traditionally, the disease has been classified into four clinical entities:

- Acute bacterial prostatitis (ABP) and prostatic abscess as a sequela/complication of ABP
- Chronic bacterial prostatitis (CBP)
- Non- or abacterial prostatitis (NBP)
- Prostatodynia.

To improve the definition and understanding of the prostatitis syndrome, a new classification system has been proposed by the National Institute of Health (NIH) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Washington DC, USA (10) (Table 11).

Table 11: New NIH/NIDDK classification of the prostatitis syndrome (10).

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

* CPPS = Chronic pelvic pain syndrome.

11.3.1 Microbiology

Acute bacterial prostatitis (NIH I), CBP (NIH II) and more significantly, prostatic abscesses are clinically relevant, but uncommon, diseases. The commonest causes of bacterial prostatitis are gram-negative bacteria, predominantly strains of *Escherichia coli* (11). However, the role of gram-positive bacteria in bacterial prostatitis is controversial. Although enterococci may cause bacterial prostatitis and associated recurrent urinary tract infection (UTI), the importance of other gram-positive bacteria in chronic prostatitis is doubtful (11), as is that of *C. trachomatis* and *Mycoplasma*, particularly *U. urealyticum*, (11-15). Hidden bacteria may be aetiologically involved in patients with chronic idiopathic prostatitis after exclusion of typical bacterial infection (16).

Detection of bacteria by molecular techniques has not been evaluated definitively.

11.3.2 *Diagnosis*

Symptom evaluation must be done by means of standardized scores, especially the new NIH symptom score (17). Other investigative procedures include laboratory diagnosis of CBP using the four-specimen test for bacterial localization (10,11). The test measures sequential quantitative bacteriological cultures of the urethra, bladder urine and prostatic secretions, both in expressed prostatic excretion (EPS) and urine after prostatic massage (12). Simplified techniques compare bacterial and leukocyte counts in the urine before and after prostatic massage (18). Screening of bladder voiding and imaging analysis of the prostate gland are clinical procedures that need to be integrated.

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

11.3.3 *Ejaculate analysis*

An ejaculate analysis (see section 2 Investigations) helps to clarify whether the prostate is part of a generalized infection of the accessory sex glands (MAGI) and provides information about sperm quality. In addition, leukocyte analysis allows differentiation between inflammatory and non-inflammatory CPPS (NIH IIa vs. NIH IIIb).

11.3.4 *Microbiological findings*

After exclusion of urethritis and bladder infection, $\geq 10^3$ peroxidase-positive white blood cells per mL ejaculate are indicative of an inflammatory process. In these cases, a culture should be performed for common urinary tract pathogens, particularly gram-negative bacteria.

A concentration of $\geq 10^3$ cfu/mL of urinary tract pathogens in the ejaculate is considered a significant bacteriospermia. Usually, various micro-organisms are cultured from the genital tract of men seen in infertility clinics with more than one strain of bacteria in most cases (1). The time when samples are obtained influences the positive rate of micro-organisms in semen and the frequency of isolation of different strains (19).

Despite modern DNA detection techniques, the ideal diagnostic test for *C. trachomatis* in semen has not yet been established (14). In contrast to serological findings in women, antibody tests for *C. trachomatis* in seminal plasma are not indicative if no type-specific methods are used (14).

By analogy with *Mycoplasma*, *U. urealyticum* appears to be pathogenic in high concentrations only ($\geq 10^3$ cfu/mL ejaculate). No more than about 10% of samples analyzed for ureaplasma exceed this concentration (20). Normal colonization of the urethra hampers the clarification of 'mycoplasmal-associated' urogenital infections using samples such as the ejaculate (15).

11.3.5 *White blood cells*

The clinical significance of an increased concentration of white blood cells (WBC) or leukocytes in the ejaculate is highly controversial (21). It seems to be generally accepted that 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 most authors consider leukocytospermia to be a sign of inflammation, it is not necessarily associated with bacterial or viral infections (7). This is in accordance with earlier findings that elevated leukocyte numbers are not a natural cause of male infertility (22).

According to WHO classification, $> 1 \times 10^6$ WBC per mL have been defined as leukospermia. Only two studies have analyzed alterations of WBC in the ejaculate of patients with proven prostatitis (23,24). Both studies demonstrated a higher number of leukocytes compared to men without inflammation (CPPS, type NIH IIIb).

11.3.6 *Sperm quality*

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

11.3.7 *Seminal plasma alterations*

Seminal plasma elastase is accepted as a biochemical indicator of polymorphonuclear lymphocyte activity in the ejaculate (1,28,29), with a suggested cut-off level of about 600 ng/mL (1). Various cytokines are involved in inflammation and may influence sperm function, and several studies have investigated the association between interleukin concentration, leukocytes and sperm function (30-32). No differences were found among the subgroups defined on the basis of progressive motility, percentage of abnormal forms and diagnosis of prostatitis. The prostate seems to be the main site of origin of interleukin-6 (IL-6) in the seminal plasma.

Although it is accepted that cytokines, especially IL-6, must play an important role in the male accessory gland inflammatory process (33), elevated cytokine levels do not depend on the number of leukocytes in EPS (34).

11.3.8 Glandular secretory dysfunction

Infections of the sex glands can impair their excretory function. Decreased quantities of citric acid, phosphatase, fructose, zinc and alpha-glutamyltransferase activity have been evaluated as disturbed prostatic secretory parameters (1). Reduced fructose concentration is an indicator of impaired vesicular function (20,35).

11.3.9 Sperm antibodies

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

11.3.10 Reactive oxygen species

It is generally accepted that reactive oxygen species may be increased in chronic urogenital infections associated with increased leukocyte numbers (40). However, the biological significance in prostatitis remains unclear (1).

11.3.11 Therapy

Treatment of chronic prostatitis is normally targeted at relieving symptoms (10,41). Andrologically, therapy for altered semen composition in male adnexitis is aimed at:

- Reduction or eradication of micro-organisms in prostatic secretions and semen
- Normalization of inflammatory parameters, such as leukocytes and secretory parameters
- Possible improvement of sperm parameters to counteract fertility impairment (42).

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

Only antibiotic therapy of CBP (NIH II) has provided symptomatic relief, eradication of micro-organisms and a decrease in cellular and humoral inflammatory parameters in urogenital secretions. The use of alpha-blockers for symptom relief is controversial.

Although antibiotic procedures may improve sperm quality (42), there is no convincing evidence that treatment of chronic prostatitis increases the probability of conception (1,43).

11.4 Orchitis and epididymo-orchitis

11.4.1 Introduction

Orchitis is an inflammatory lesion of the testis associated with a predominantly WBC exudate inside and outside the seminiferous tubules, potentially resulting in tubular sclerosis. The inflammation causes pain and swelling. Chronic inflammatory alterations in the seminiferous tubules disrupt the normal process of spermatogenesis and cause alterations both in sperm number and quality (44).

It is generally accepted that orchitis may also be an important cause of spermatogenetic arrest (45), which may be reversible in most cases. Testicular atrophy may develop as a result of tubular sclerosis (45).

11.4.2 Diagnosis

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

11.4.3 Ejaculate analysis

Ejaculate analysis, including leukocyte analysis, indicates persistent inflammatory activity. In many cases, especially in acute epididymo-orchitis, transiently decreased sperm counts and reduced forward motility can be observed (44,46). Obstructive azoospermia due to complete obstruction is considered a rare complication.

Mumps orchitis may result in bilateral testicular atrophy (45) and testicular azoospermia. When granulomatous orchitis is suspected, sperm-bound autoantibodies occur.

11.4.4 Therapy

Only the therapy of acute bacterial epididymo-orchitis and of specific granulomatous orchitis is standardized (45) (Table 12). Several regimens are thought to improve the inflammatory lesion. Unfortunately, therapies using corticosteroids and non-steroidal antiphlogistic substances, such as diclofenac, indomethacin, and acetylsalicylic acid have not been evaluated for their andrological outcome (47). A further therapeutic trial involves using GnRH treatment to prevent the deleterious effects of inflammation on spermatogenesis (49). In

mumps orchitis, systemic interferon alpha-2b therapy has been reported to prevent testicular atrophy and azoospermia (50). In idiopathic granulomatous orchitis, surgical removal of the testis appears to be the therapy of choice.

Table 12: Treatment of epididymo-orchitis

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

11.5 Epididymitis

11.5.1 Introduction

Inflammation of the epididymis causes pain and swelling that is located unilaterally and usually with acute onset. The testicle is involved in the inflammatory process in most cases known as epididymo-orchitis.

Among sexually active men under 35 years of age epididymitis is most often caused by *C. trachomatis* or *N. gonorrhoea* (Table 12) (51,52). Sexually transmitted epididymitis is usually accompanied by urethritis. Non-sexually transmitted epididymitis is associated with UTI. This type occurs more often in men aged over 35 years, those who have recently undergone urinary tract instrumentation or surgery, and those who have anatomical abnormalities (52).

11.5.2 Diagnosis

In acute epididymitis, inflammation and swelling usually begin in the tail of the epididymis, and may spread to involve the rest of the epididymis and testicular tissue (46). Although men with epididymitis due to sexually transmitted micro-organisms always have a history of sexual activity, exposure may have occurred months prior to onset. The microbial aetiology of epididymitis is usually easy to determine by gram-stained examination of both a urethral smear for urethritis and of a midstream urine specimen for gram-negative bacteriuria (51,52). Intracellular gram-negative diplococci on the smear correlate with the presence of *N. gonorrhoea*. Only WBCs on urethral smear are indicative of non-gonorrhoeal urethritis; *C. trachomatis* will be isolated in approximately two-thirds of these patients (53).

11.5.3 Ejaculate analysis

Ejaculate analysis according to WHO criteria, including leukocyte analysis, may indicate persistent inflammatory activity. In many cases, transiently decreased sperm counts and forward motility are observed (46,48,51). Ipsilateral low-grade orchitis (54,55) has been discussed as the cause of this slight impairment in sperm quality (Table 13) (56).

Development of stenosis in the epididymal duct, reduction of sperm count and azoospermia are more important in the follow up of bilateral epididymitis (see section 5 Obstructive azoospermia). The extent of azoospermia after epididymitis remains unclear.

Table 13: Acute epididymitis and impact on sperm parameters

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

11.5.4 Treatment

Antibiotic therapy is indicated before culture results are available (Table 12). Treatment of epididymitis will result in:

- Microbiological cure of infection
- Improvement of clinical signs and symptoms
- Prevention of potential testicular damage
- Prevention of transmission to others
- Decrease of potential complications, e.g. infertility or chronic pain.

Patients who have epididymitis known or suspected to be caused by *N. gonorrhoea* or *C. trachomatis* should be instructed to refer sex partners for evaluation and treatment (60).

11.6 CONCLUSIONS

- Urethritis and prostatitis are not always associated with male subfertility or infertility. In many cases, basic ejaculate analysis does not reveal a link between accessory sex gland infection and impaired sperm characteristics.
- Furthermore, antibiotic treatment often only eradicates micro-organisms; it has no positive effect on inflammatory alterations and/or cannot reverse functional deficits and anatomical dysfunctions.

11.7 RECOMMENDATIONS

- As the aetiology of acute urethritis is unknown in most cases at the time of diagnosis, empirical therapy is suggested with a single dose of a fluoroquinolone, followed by a 2-week regimen of doxycycline. Treatment is effective both for gonococcal and (co-existing) chlamydial/ureaplasma infections (9) (grade B recommendation).
- Only antibiotic therapy of (chronic) bacterial prostatitis has proved to be efficacious in providing symptomatic relief, eradication of micro-organisms, and a decrease in cellular and humoral inflammatory parameters in urogenital secretions (61-64) (grade B recommendation).
- Although antibiotic procedures for MAGIs may provide improvement in sperm quality, therapy does not necessarily enhance the probability of conception (1,43) (grade B recommendation).
- Patients who have epididymitis known or suspected to be caused by *N. gonorrhoea* or *C. trachomatis* should be instructed to refer sex partners for evaluation and treatment (60) (grade B recommendation).

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12. GERM CELL MALIGNANCIES AND TESTICULAR MICROCALCIFICATIONS

12.1 Germ cell malignancies and male infertility

Testicular germ cell cancer (TGCC) is the most common malignancy in Caucasian males aged between 15 and 40 years and affects about 1% of subfertile males. The lifetime risk of TGCC varies between ethnic groups and from country to country. The highest annual incidence occurs in Caucasians, varying from 10 per 100,000 in, for example, Denmark and Norway to 2 per 100,000 in Finland and the Baltic countries. It is generally accepted that seminomas and non-seminomas are always preceded by CIS, and that CIS will eventually progress to an invasive cancer if not treated (1,2).

The most convincing evidence for a general decline in male reproductive health in humans is the increase in testicular cancer noted over the recent past in several Western countries (3). The incidence of testicular cancer has increased in almost all countries which have reliable cancer registers – at least in the Caucasian population (4). It has also been observed that both cryptorchidism and hypospadias are associated with an increased risk of testicular cancer, based on the observation that men with cryptorchidism and/or hypospadias are overrepresented among patients with testicular cancer.

Dysgenetic testes have an increased risk of developing testicular cancer in adulthood. These cancers seem to arise from premalignant gonocytes or CIS cells (5). Testicular microlithiasis can be associated with both germ cell tumours and CIS of the testis.

12.2 Testicular germ cell cancer and reproductive function.

Men with TGCT have decreased semen quality, even prior to a cancer diagnosis (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 prior to orchidectomy should therefore be considered (see section 14 Semen cryopreservation). Treatment of TGCT may imply an additional impairment of semen quality (7).

As well as spermatogenic failure, TGCT patients have Leydig cell dysfunction, even in the contralateral testis (8). The risk of hypogonadism may therefore be increased in men treated for TGCT. Obtaining pretreatment levels of testosterone, SHBG, LH and oestradiol, may help in disclosing post-treatment hypogonadism. Long-term follow up of TGCT men with low normal androgen levels should be considered as they may be at risk for developing hypogonadism due to age-related decrease in testosterone production (9).

12.3 Testicular microlithiasis

Microcalcifications inside the testicular parenchyma can be found in 0.6-9% of men referred for testicular ultrasound (10-13). Although the true incidence in the general population is unknown, it is probably a rare condition. However, ultrasound findings of testicular microlithiasis are common in men with germ cell tumours, cryptorchidism, testicular dysgenesis, male infertility, testicular torsion and atrophy, Klinefelter's syndrome, hypogonadism, male pseudohermaphroditism, varicocele, epididymal cysts, pulmonary microlithiasis and non-Hodgkin's lymphoma. The incidence seems to be higher with high-frequency ultrasound machines (14).

The relationship between testicular microlithiasis (TM) and infertility is unclear. It probably relates to dysgenesis of the testis, with degenerate cells being sloughed inside an obstructed seminiferous tubule and failure of the Sertoli cells to phagocytose the debris. Subsequently, calcification occurs.

Testicular microcalcification is a condition found in testis at risk for malignant development. The reported incidence of TM in men with germ cell malignancy is 6-46% (15-17), and it has therefore been suggested that TM should be considered premalignant. However, it remains to be established whether TM is present before development of invasive TGCT, and whether TM might be an indicator for the preinvasive stage of TGCTs, known as carcinoma *in situ* (CIS). Testicular biopsies of men with TM have found a higher prevalence of CIS, especially in men with bilateral microlithiasis (18). On the other hand, TM is found most often in men with a benign testicular condition and the microcalcifications itself are not malignant.

Further investigation of the association between TM and CIS would require testicular biopsies to be carried out in large series of men without signs of a TGCT. Available data, however, indicate that a finding of TM in high-risk patients (e.g. patients referred due to infertility and/or cryptorchidism) warrants follow up by repeated ultrasound and/or testicular biopsy for detection of CIS.

12.4 RECOMMENDATIONS

- It is recommended that either a testicular biopsy or a follow-up scrotal ultrasound should be performed in men with TM and a history of male infertility, cryptorchidism or testicular cancer and in men with atrophic testis to rule out CIS of the testis (17,18) (grade B recommendation).
- It is important to encourage and educate these patients about self-examination, since this may result in early detection of germ cell tumours.
- In case of suspicious findings on physical examination or ultrasound in patients with TM and associated lesions, a surgical exploration with testicular biopsy or orchidectomy should be considered.
- Testicular biopsy, follow-up scrotal ultrasound or the routine use of biochemical tumour markers, abdominal and pelvic computed tomography scanning does not seem to be justified for men with isolated TM without associated risk factors (male infertility, cryptorchidism, testicular cancer, atrophic testis) (11) (grade B recommendation).
- Men with TGCT are at increased risk of developing hypogonadism, which should be considered in follow up of these patients (9) (grade B recommendation).

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13. DISORDERS OF EJACULATION

13.1 Definition

Disorders of ejaculation are uncommon, but important causes of male infertility. Several heterogeneous dysfunctions belong to this group, and may be of either organic or functional origin.

13.2 Classification and aetiology

13.2.1 Anejaculation

Anejaculation is the complete absence of an antegrade or retrograde ejaculation. It is caused by a failure of emission of semen from the seminal vesicles, the prostate and the ejaculatory ducts into the urethra (1). True anejaculation is usually associated with a normal orgasmic sensation. Occasionally (e.g. in incomplete spinal cord injuries), this sensation may be altered or decreased. True anejaculation is always connected with central or peripheral nervous system dysfunctions or with drugs (2) (Table 14).

Table 14: Aetiologies of anejaculation

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

13.2.2 Anorgasmia

Anorgasmia is the inability to reach orgasm. It may give rise to anejaculation. The cause is usually psychological. Anorgasmia is very often a primary condition. Some patients report sporadic events of nocturnal emission or of ejaculation occurring during great emotional excitement unrelated to sexual activity (3).

13.2.3 Delayed ejaculation

Delayed ejaculation is the condition in which abnormal stimulation of the erected penis is necessary to obtain an orgasm with ejaculation (1). It may be considered a slight form of anorgasmia: both can be found alternately in the same patient. The causes of delayed ejaculation may be psychological or organic, such as incomplete spinal cord lesion (3), iatrogenic penile nerve damage (4) or pharmacological (antidepressants, antihypertensives, antipsychotics).

13.2.4 Retrograde ejaculation

Retrograde ejaculation is the total, or sometimes partial, absence of an antegrade ejaculation due to semen passing backwards through the bladder neck into the bladder. Patients experience a normal or decreased orgasmic sensation, except in paraplegia. Partial antegrade ejaculation must not be confused with the secretion of bulbo-urethral glands. The causes of retrograde ejaculation can be subdivided as shown in Table 15.

Table 15: Aetiology of retrograde ejaculation

Neurogenic causes	Pharmacological causes
• Spinal cord injury	• Antihypertensives
• Cauda equina lesions	• Alpha1-adrenoceptor antagonists
• Multiple sclerosis	• Antipsychotics
• Autonomic neuropathy (juvenile diabetes)	• Antidepressants
• Retroperitoneal lymphadenectomy	
• Sympathectomy	
• Colorectal and anal surgery	

Urethral causes	Bladder neck incompetence
• Ectopic ureterocele	• Congenital defects/dysfunction of hemitrigone
• Urethral stricture	• Bladder extrophy
• Urethral valves or verumontaneum hyperplasia	• Bladder neck resection
• Congenital dopamine beta-hydroxylase deficiency	• Prostatectomy

13.2.5 Asthenic ejaculation

Asthenic ejaculation, also defined as partial ejaculatory incompetence or 'éjaculation baveuse' (5), is characterized 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, while in asthenic ejaculation due to urethral obstruction, these contractions are present. Asthenic ejaculation is generally due to the neurogenic or urethral pathologies already listed in Table 15. Asthenic ejaculation does not usually alter semen quality.

13.2.6 Premature ejaculation

Premature ejaculation is the inability to control ejaculation for a 'sufficient' length of time during vaginal

penetration. Although a universally accepted definition of 'sufficient' length of time does not exist, some patients are unable to delay ejaculation beyond a few coital thrusts, or even after vaginal penetration. Premature ejaculation may be strictly organic (e.g. prostatitis-related) or 'psychogenic' (i.e. neurobiologically based), primary or acquired, partner-related or non-selective, and can be associated with erectile dysfunction. Premature ejaculation does not impair fertility, provided intravaginal ejaculation occurs.

13.2.7 Painful ejaculation

Painful ejaculation is usually an acquired condition, often related to lower urinary tract symptoms (6). It sometimes causes moderate sexual dysfunction. The painful sensation may be felt in the perineum, or urethra and urethral meatus (7). It can be caused by ejaculatory duct obstruction, all types of chronic prostatitis/chronic pelvic pain syndrome, 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, neuropathies, traumas, urogenital infections, previous surgery and medications. Particular attention must be paid to the characteristics of micturition and ejaculation (presence of nocturnal emission, ejaculatory ability in given circumstances, primary or acquired disorder) as well as to psychosexual aspects (education, features of affective relationship, pre-existent psychological traumas, previous psychological therapies).

13.3.2 Physical examination

A genital and rectal examination 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 will determine if there is total or partial retrograde ejaculation.

13.3.4 Microbiological examinations

Initial, mid-stream urine, EPS and/or urine after prostatic massage are cultured for evidence of prostatic infection. In cases of increased leukocytes in semen, semen culture is also suggested (8).

13.3.5 Optional diagnostic work up

This may include:

- Neurophysiological tests (bulbocavernosus evoked response and dorsal nerve somatosensory evoked-potentials)
- Tests for autonomic neuropathies (i.e. appreciation of temperature regulation in the feet)
- Psychosexual evaluation
- Videocystometry
- Cystoscopy
- Transrectal ultrasonography
- Uroflowmetry
- Vibratory stimulation of the penis.

13.4 Treatment

The treatment of infertility due to disorders of ejaculation is rarely aetiological, and generally consists of retrieving spermatozoa to be used in assisted reproduction techniques (ART). In decision-making, the following aspects must be considered:

- Age of patient and of his partner
- Psychological problems in the patient and his partner
- Couple's willingness and acceptance of the different fertility procedures
- Associated pathologies
- Psychosexual counselling.

13.5 Aetiological treatments

If possible, stop any pharmacological treatments that are interfering with ejaculation. Tamsulosin can be

administered during antidepressant treatment (9). Treatment should be given for urogenital infections (i.e. in cases of painful ejaculation) (8). Selective serotonin re-uptake inhibitors (SSRIs) should be given for premature ejaculation, which appears to be related to serotonin levels (10). If possible, any underlying urethral pathology or metabolic disorder (e.g. diabetes) should be corrected. Psychotherapy is usually not very effective.

13.6 Symptomatic treatments

13.6.1 Premature ejaculation

This can be treated with topical anaesthetics to increase intravaginal ejaculation latency time or with SSRIs (e.g. paroxetine, fluoxetine).

13.6.2 Retrograde ejaculation

In the absence of spinal cord injury, anatomical anomalies of the urethra, or pharmacological agents, an attempt must be made with drug treatment 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

- Ephedrine sulfate, 10-15 mg 4 times a day (12)
- Midodrin, 5 mg 3 times a day (13)
- Brompheniramine maleate, 8 mg twice a day (14)
- Imipramine, 25-75 mg 3 times a day (15)
- Desipramine, 50 mg every second day (16)

Sperm collection from post-orgasmic urine for use in ART is suggested if:

- Drug treatment is ineffective or intolerable due to side-effects
- When 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 alkalinized (pH 7.2-7.8) and osmolarity must be 200-300 mOsmol/kg. The patient is asked to have intercourse or to masturbate. Within 10 minutes after ejaculation, urine must be voided and centrifuged, and the pellet resuspended in 0.5 mL Tyrode's or Ham's F-10 medium and immediately inseminated (17). As an alternative, a catheter may be applied to the bladder and 10-50 mL Tyrode's or Ham's F-10 medium instilled into the bladder. The patient must ejaculate, and a second catheterization is performed immediately to retrieve spermatozoa. The latter treatment minimizes the contact between spermatozoa and urine (18). If the biological sperm preparation is not of sufficient quality for intrauterine insemination, the couple must undergo in-vitro reproductive procedures (i.e. ICSI) with fresh or cryopreserved spermatozoa.

13.6.3 Anejaculation

Drug treatment for anejaculation due to lymphadenectomy and neuropathy is not very effective. Nor is psychosexual therapy in anorgasmic subjects. In all these cases and in spinal cord injured men, vibrostimulation (i.e. the application of a vibrator to the penis) is first-line therapy.

In anejaculation, vibrostimulation evokes the ejaculation reflex (19). It requires an intact lumbosacral spinal cord segment. Complete 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 themselves in their own home. Intravaginal insemination via a 10 mL syringe during ovulation can be performed. 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 is an electric stimulation of the periprostatic nerves via a probe inserted into the rectum, which seems unaffected by reflex arc integrity. Anaesthesia is required except in cases of complete spinal cord injury. In 90% of patients, electrostimulation induces ejaculation, which is retrograde in one-third of them. Semen quality is often poor and most couples must resort to IVF programmes (21).

When electroejaculation fails or cannot be performed, sperm retrieval from the seminal ducts may be achieved by sperm aspiration from the vas deferens (22) (see section 5 Obstructive azoospermia) or seminal tract washout (23).

When there is a failure to retrieve sperm, epididymal obstruction or testicular failure must be suspected. TESE can then be performed (8,24). Anejaculation following either surgery for testicular cancer or total mesorectal excision may be prevented by monolateral lymphadenectomy or autonomic nerve preservation (24), respectively.

13.7 CONCLUSIONS

- Ejaculation disorders can be treated with a wide range of drugs and physical stimulation trials with a high level of efficacy.

13.8 RECOMMENDATIONS

- If present, aetiological treatments for ejaculatory disorders should be offered first, before sperm collection and ART is performed.
- Premature ejaculation can successfully be treated with either topical anaesthetic creams or SSRIs (22).
- Both vibrostimulation and electroejaculation are effective methods for sperm retrieval in men with spinal cord injury.

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14. SEMEN CRYOPRESERVATION

14.1 Definition

Cryopreservation is the storage of biological material at low subzero temperatures, such as -80°C or -196°C (the boiling point of liquid nitrogen). At these low temperatures, biochemical processes of cell metabolism are slowed or interrupted, while at -196°C, the biochemical reactions that lead to cell death are effectively stopped.

14.2 Introduction

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

14.3 Indications for storage

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

- Prior to potentially sterilising cancer chemotherapy or radiotherapy (2).
- Prior to any potentially sterilising chemotherapy for any other non-malignant disease, e.g. Behçet's disease.
- Prior to surgery that will, or may, interfere with fertility, e.g. bladder neck surgery in a younger man. Removal of the second testicle in a man with bilateral testicular malignancy, etc.
- For men with progressive decrease in semen quality due to diseases carrying an associated risk of subsequent azoospermia (i.e. pituitary macroadenomas, craniopharyngiomas, empty sella syndrome, chronic nephropathies, uncontrolled diabetes mellitus, multiple sclerosis).
- For men with paraplegia, after sperm have been obtained by electroejaculation.
- For men with psychogenic anejaculation, after sperm have been obtained either by electroejaculation or a sperm retrieving procedure.
- After gonadotrophin treatment has induced spermatogenesis in men with hypogonadotropic hypogonadism.
- For men with NOA, the chance of finding sperm using micro-TESE is approximately 60-70%. Cryopreservation can be used to separate sperm collection from ICSI and thus to avoid unnecessary hyperstimulation of the female partner. It may also be used to avoid repeated sperm retrieval procedures.
- In any situation where sperm have been obtained by a sperm retrieving procedure, e.g. after failed vasectomy reversal, or in some cases of epididymal obstruction not amenable to surgery.
- For storage of sperm prior to vasectomy. This service is offered by a limited number of clinics as an insurance policy against change of mind or circumstances.
- For storage of donor sperm. Cryopreservation and a 3-6 months' quarantine period reduce the risk of transmission of infection from sperm donors. In most countries, fresh sperm are no longer used.

14.4 Precautions and techniques

14.4.1 Freezing and thawing process

Available techniques are not yet optimal as damage occurs to cells during cryopreservation and during prolonged storage. Most damage occurs during freezing and thawing. The major causes of damage during freezing are ice crystal formation and cell dehydration causing cell wall disruption and intracellular organelles. Sperm morphology, motility and vitality decrease significantly after thawing, while cryopreservation increases the damage done to sperm DNA (3-5,6). Further damage may be caused by contamination of samples with micro-organisms and high levels of superoxide radicals (7,8). To reduce ice crystal formation, a cryopreservation solution is added prior to freezing. There are various commercially available cryopreservation solutions, most of which contain varying proportions of glycerol and albumen. After freezing, the tissues are immersed in liquid nitrogen.

Various techniques have been developed to try to reduce damage caused by freezing and thawing. Rapid method (9,10): The sample is held in the vapour phase for 10 min before being plunged into liquid nitrogen.

Slow method (11): The sample is gradually cooled in the vapour phase for about 40 min.

Using a programmable automatic freezing machine, which is pre-set to cool at a rate of between 1-10°C/min.

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

The likelihood of sperm survival decreases with increased storage time and if there is repeated freezing and thawing. The maximum viable storage time for human sperm is not known but many laboratory or regulatory authorities apply a storage time limit of up to 10 years (12). However, longer storage times will sometimes be needed, e.g. for a 17-year-old male who has had sperm stored prior to chemotherapy for testicular cancer.

14.4.2 Cryopreservation of very small numbers of sperm

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

14.4.3 Testing for infections and preventing cross-contamination

Sperm storage in straws is widely used. Large numbers of straws are stored in canisters with the straws bathed in a pool of liquid nitrogen. Micro-organism contamination of the pool of liquid nitrogen results in

contamination of the outside of all the straws. The most widely used safeguard is to accept samples for storage only from patients whose semen samples have been tested for infection and shown to be safe. Donor samples should be tested for viral infections (hepatitis B and C, HIV) and sexually transmitted infections (*C. trachomatis*, *gonorrhoea*, syphilis).

Until the test results are known, samples must be stored in an individual quarantine vessel (15) (<http://www.hfea.gov.uk/cps/rde/xchg/SID-3F57D79B-1F88B22E/hfea/hs.xsl/576.html>). Some laboratories use the additional safeguard of double-wrapping the straws before freezing, but this is an extra cost. It may also interfere with the freezing process, reducing the quality of the samples on thawing. In some centres, there is also cytomegalovirus (CMV) testing with separate storage facilities for CMV-negative and CMV-positive samples.

There is a considerable ethical problem about how to store samples prior to cancer chemotherapy for a man who is hepatitis- or HIV-positive. Very few clinics have separate storage facilities for HIV-positive samples. However, the success of antiretrovirals is increasing the number of HIV-positive men who may wish to store sperm. There is also concern about HIV transmission to children conceived using HIV-positive sperm as sperm-washing techniques may fail in about 5%.

14.4.4 *Fail-safe precautions to prevent loss of stored materials*

Any laboratory undertaking 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 prior to sterilising cancer chemotherapy because in such cases there may be no opportunity to obtain further sperm. The level of precaution will depend on the cost and resources available to the laboratory, but if possible the following safeguards should be in place. All in-use storage vessels should be fitted with an alarm system activated by a rising temperature or liquid nitrogen leakage. The alarm system should alert a laboratory staff member, according to a 24-hour 365-day rota. Ideally, there should be a spare storage container into which samples can be transferred following a vessel failure.

14.4.5 *'Orphan' samples*

In malignancy and some other situations, years may pass before stored samples are required. Inevitably, during this time, the owners of some samples may become untraceable or die, leaving behind 'orphan' samples for which the owner can no longer be contacted. The duty of the laboratory and the legal ownership of these samples can create considerable problems.

It is best to obtain consent about what to do with the sample in the event of death or untraceability from the owner of the sample at the time of, or very shortly after, storage. Some countries legally require such consent. Choices available for the owner of the sample depend on the laws of the country and may or may not be appropriate in all situations. They include:

- A request that the sample should be destroyed
- Use of the sample by their wife or partner
- Use of the sample in research
- Donation of the sample to help another infertile couple.

14.5 **Biological aspects**

Cryopreservation induces deterioration of the seminal quality. After the sample has been thawed, motility (16) and morphology (17,18) appear worsened, including mitochondrial acrosomal and sperm tail damage (19). Recent studies confirm some correlation between these parameters. Sperm freezing decreases motility by 31%, morphology by 37%, and mitochondrial activity by 36% (9). Motility seems to be best correlated with IVF capacity of the thawed sample. Further improvement can be achieved by selecting out the subpopulation of sperm with the best motility and DNA integrity and freezing these sperm in seminal plasma, making this the optimal procedure (13).

14.6 **CONCLUSIONS**

- The purpose of sperm cryopreservation is to secure future pregnancies by assisted reproduction techniques.
- Cryopreservation techniques are far from optimal and future efforts are needed to improve the outcome of sperm banking.
- Cryopreservation should be compulsorily explained and suggested in case of specific pathologies or before making a patient undergo surgery, chemotherapy or radiotherapy that might damage his reproductive integrity.
- If testicular biopsies are indicated, carrying out sperm cryopreservation as part of the same procedure is strongly advised.

14.7 RECOMMENDATIONS

- 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.
- If cryopreservation is not locally available, this should be discussed with the patient, including advice about the possibility of visiting, or transferring to, the nearest cryopreservation unit before therapy starts.
- Consent for cryopreservation should include a record of the man's wishes for his samples if he dies or is otherwise untraceable.
- Precautions should be taken to prevent transmission of viral, sexually transmitted or any other infection by cryostored materials from donor to recipient and also to prevent contamination of stored samples. These include testing of the patient, the use of rapid testing and quarantining of the samples until the test results are known. Samples from hepatitis-positive or HIV-positive men should not be stored in the same container as samples from men who have been tested and are free from infection.

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15. LEVELS OF EVIDENCE AND GRADES OF GUIDELINE RECOMMENDATIONS

Table 17: Levels of evidence (1)

Level	Type of evidence
1a	Evidence obtained from meta-analysis of randomized trials
1b	Evidence obtained from at least one randomized 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

Table 18: Grades of guideline recommendations (1)

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

15.1 REFERENCES

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16. ABBREVIATIONS USED IN THE TEXT

This list is not comprehensive for the most common abbreviations

ABP	acute bacterial prostatitis
ART	assisted reproduction techniques
CAG	cytosine-adenine-guanosine
CBAVD	congenital bilateral absence of the vas deferens
CBP	chronic bacterial prostatitis
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator gene
CIS	carcinoma <i>in situ</i>
CMV	cytomegalovirus
CPPS	chronic pelvic pain syndrome
CFTR	cystic fibrosis transmembrane conductance regulator
EPS	expressed prostatic excretion
FISH	(multicolour) fluorescent <i>in situ</i> hybridization
HIV	human immunodeficiency virus
FSH	follicle-stimulating hormone
GnRH	gonadotrophin-releasing hormone
hCG	human chorionic gonadotrophin
hMG	human menopausal gonadotrophin
IBT	immunobead test
ICSI	intracytoplasmic sperm injection
IL-6	interleukin-6
IVF	in-vitro fertilization
LH	luteinizing hormone
MAGI	male accessory gland infection
MAR	mixed antiglobulin reaction
MESA	microsurgical epididymal sperm aspiration
NBP	non- or abacterial prostatitis
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institute of Health
NOA	non-obstructive azoospermia
OA	obstructive azoospermia
OAT	oligo-astheno-teratozoospermia [syndrome]
SHBG	sex hormone binding globulin
SSRIs	selective serotonin reuptake inhibitors
STS	sequence tagged sites
TESE	testicular sperm extraction
TEFNA	testicular fine needle aspiration
TGCT	testicular germ cell tumour
TM	testicular microlithiasis
TRUS	transurethral ultrasound
TURED	transurethral resection of the ejaculatory ducts
WBC	white blood cells
VB1	first-voided urine