Management of male factor infertility: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS)

Endorsing Organization: Italian Society of Embryology, Reproduction, and Research (SIERR)

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Abstract
Infertility affects 15–20% of couples and male factors are present in about half of the cases. For many aspects related to the diagnostic and therapeutic approach of male factor infertility there is no general consensus and the clinical approach is not uniform. In the present document by the Italian Society of Andrology and Sexual Medicine (SIAMS), endorsed by the Italian Society of Embryology, Reproduction, and Research (SIERR), we propose evidence-based recommendations for the diagnosis, treatment and management of male factor infertility to improve patient and couple care. These Guidelines are based on two principal aspects: they are couple-oriented and place high value in assessing, preventing and treating risk factors for infertility. Components of the initial evaluation should include at minimum medical history, physical examination, and semen analysis. Semen microbiological examination, endocrine assessment, and imaging are suggested in most men and recommended when specific risk factors for infertility exist or first step analyses showed abnormalities. Full examination including genetic tests, testicular cytology/histology, or additional tests on sperm is clinically oriented and based on the results of previous investigations. For treatment purposes, the identification of the specific cause and the pathogenetic mechanism is advisable. At least, distinguishing pre-testicular, testicular, and post-testicular forms is essential. Treatment should be couple-oriented, including lifestyle modifications, etiologic therapies, empirical treatments, and ART on the basis of best evidence and with a gradual approach. These Guidelines also highlighted that male infertility and in particular testicular function might be a mirror of general health of a man.

Keywords: assisted reproduction, azoospermia, male infertility, oligozoospermia, sperm, testis

1. Introduction

Infertility, defined as the inability to conceive after one year of unprotected intercourse, affects approximately 15–20% of couples in Western countries and male factors are present in about half of the cases [1]. The clinical approach to male factor infertility is not uniform, and sometimes the diagnostic approach is limited to semen analysis. Treatment of the male partner might not be performed at all, directly addressing the couple to assisted reproduction techniques (ART). Similarly, too often a definition of "idiopathic infertility" is used, but it is quite evident that this term should be reserved to cases in which there is no identifiable cause after a detailed and meticulous diagnostic process.

Indeed, not only male infertility can be caused by a variety of aetiologies and pathophysiologic mechanisms, but also it might reflect the general health of a man [2]. Furthermore, only a correct and extensive diagnostic process could direct further therapy and influence prognosis. Nevertheless, infertility is not a matter of the woman or the man, and the clinical approach should be personalized and should consider different aspects of that particular couple.

In fact, a typical malpractice is to focus the attention just to one of the partners (usually the female), whereas both partners should be evaluated simultaneously. The fertility potential of each partner and/or specific conditions affecting a member of the couple influence the clinical and treatment approach. For example, mild reduction of sperm number and/or quality could be compatible with natural fertility when the female is at her top fertility capability (young, no infertility causes and risk factors) or might be a cofactor of infertility when the fertility potential of the partner is reduced (e.g., older, presence of infertility causes and risk factors).

Furthermore, the identification of subjects with signs of spermatogenic and endocrine testicular impairment is important for their follow up and prognosis, independently from their ability to conceive naturally or by ART.

Guidelines and expert opinion papers for the diagnostic evaluation of male infertility exist, but there is no general consensus for many aspects related to diagnosis and treatment [1, 3-11]. The aim of this article is to summarize the position of the Italian Society of Andrology and Sexual Medicine (Società Italiana di Andrologia e Medicina della Sessualità, SIAMS) for the diagnosis and treatment of male factor infertility. The Guideline has been endorsed by the Italian Society of Embryology, Reproduction, and Research (SIERR).

Statements

- Male factors are present in about half of the cases of couple infertility, either alone or in combination with female causes.
- The diagnostic and therapeutic workup for the male partner of an infertile couple should consider the fertility potential and risk factors of both partners.
- The results of diagnostic tests should be interpreted in the context of the couple and not as independent predictor of (in)fertility.
- Gynaecologists and andrologists should proceed jointly in deciding the clinical approach of the infertile couple.
- If the couple is referred for evaluation before 12 months of regular and unprotected intercourse have passed, the diagnostic evaluation could be postponed only when no risk factors for infertility in both partners (including age) are present.
- The aim of investigation is to have a diagnosis based on etiologic and pathophysiologic mechanisms, which will guide the appropriate treatment and has prognostic value for the fertility outcome of the couple.

2. Methods

SIAMS commissioned an expert task force to provide an updated guideline on male factor infertility. Following scrutiny and discussion of the best evidence from published literature available, the authors generated a series of consensus recommendations according to the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system [12]. The strength of recommendations and the quality of the evidence are expressed in four levels: ØOOO denotes "very low-quality evidence", ØØOO "low quality", ØØØO "moderate quality" and ØØØØ "high quality". In addition, the number '1' denotes a strong recommendation and is expressed with the phrase 'we recommend', whereas the number '2'

denotes a weaker recommendation and it is expressed with the phrase 'we suggest'. Statements in which evidence is not supported by specific studies and/or might represent standards of good clinical practice have been enunciated as "Expert Opinion" based on clinical experience of the members of the task force. The strength also reflects the confidence that authors have that patient and couples with infertility who receive recommended care will be better off. Although male infertility and spermatogenic function might be associated with endocrine disorders of the testis, these Guidelines do not refer specifically to management of male hypogonadism (low testosterone), and the corresponding SIAMS Guidelines should be followed to this regard.
According to SIAMS rulings, these Guidelines have been prepared by a team of experts on the topic coordinated by the senior author and two members of the Guideline Committee of the Society, then sent to the SIAMS Executive Committee and to the Directors of all SIAMS Excellence Centres for revisions and/or approval. Guidelines have then been announced by mail and published for two weeks on the Society's website, siams.info, so that all SIAMS Members could provide further comments and suggest additional minor revisions. Following this last step, the present manuscript has been submitted to the Journal of Endocrinological Investigation.

3. First step analysis

3.1. Risk factor assessment: history and physical examination

Recommendations

- We recommend a first level andrological assessment (history, general physical and genital examination) in all male partners of couples referred for fertility evaluation (Expert Opinion)
- We recommend taking a careful medical, reproductive and familial history with focus on specific causes of infertility (Supplementary table 1) and genetic/environmental/lifestyle risk factors for infertility (table 1) (Expert Opinion)

Evidence

History and physical examination should be part of the initial evaluation of men referred for fertility evaluation. They allow the identification of important aspects that might guide further evaluations. A general physical examination is important mainly to assess signs of hypoandrogenism and anthropometric measures. Physical examination of the penis and the scrotum is essential to assess the testes and epididymes for volume consistency and nodules/cysts, the efferent ducts for total or partial absence, and the spermatic veins for varicocele. Seminiferous tubules contribute to over 80% of the total testis volume, hence a positive linear correlation between testis size and sperm count has been described [13]. The combined evaluation of testis volume (by Prader orchidometer) and of testis consistency is clinically useful. For instance, very small and firm testes are typically found in patients with Klinefelter syndrome [14]. Small and soft testes is a typical finding in central hypogonadism. Testicular hypotrophy with impaired sperm production is a risk factor for testicular germ cell tumour (TGCT) [15] and it might be associated with hypogonadism (low testosterone). An enlarged epididymis might indicate a partial or a total obstruction of the seminal tract. An absent vas deferens at palpation might indicate agenesis. Varicocele is assessed by visualizing and palpating dilated testicular veins within the spermatic cord after Valsalva manoeuvre. Sexual function and frequency of intercourse should be evaluated, as erectile dysfunction (ED), low libido, and ejaculatory dysfunction are frequent findings in men of infertile couples and might represent both the cause and consequence of the infertility status.

Values

Careful medical, reproductive, sexual, and familial history and physical exam are essential starting points to orient towards the underlying aetiology and to undertake additional targeted diagnostic steps. The identification of risk factors impacts on patient's management and might ameliorate reproductive potential (e.g. anabolic substance abuse, smoking, obesity, etc).

3.2. Semen analysis

Recommendations

- We recommend performing semen analysis as a first level investigation in all male partners of couples referred for fertility evaluation (Expert Opinion)
- We recommend confirming results of semen analysis by performing at least one repetition, especially in case of abnormal results (1, ØØØO)
- We suggest completing routinely semen analysis with a vitality test, especially in samples with low motility (2, ØØOO)
- We recommend performing semen analysis in specialized laboratories with trained personnel following World Health Organization (WHO) recommendations (Expert Opinion)
- We recommend performing semen analysis in specialized laboratories participating to both internal and external quality programs (1, ØØØO)
- We recommend including semen analysis in an appropriate and thorough diagnostic process before considering treatments (Expert Opinion)

Evidence

Semen analysis is necessary to investigate the fertility potential of the male partner of a couple and it is essential for the assessment of male health in relation to andrological pathologies that need to be monitored or treated [2, 16, 17] and it must be considered a first level laboratory investigation. Due to the high intra-individual variability of semen analyses, results should be confirmed with at least one repetition. WHO suggests examining two or three semen samples to properly acquire semen characteristics of the male [18]. In fact, variability of semen samples can be due to preanalytic (abstinence, seasonality, etc.) and analytic variables (casual and systematic errors) and due to biological variability [19]. The sperm parameters are a widely used proxy to estimate the individual's potential fertility [20] and, to avoid limitations due to the lack of standardization, semen analysis should be performed in specialized laboratories, strictly following the latest WHO recommendations [18].

The assessment of seminal fluid should include both a macroscopic (volume, pH, appearance, liquefaction, viscosity) and a microscopic evaluation (gamete cellular component: concentration and total sperm number, progressive/non-progressive and total motility, abnormal forms; non-gamete cellular components: leukocytes, red blood cells, epithelial cells, other round cells such as spermatogonia and spermatids) [18, 21]. Azoospermia can be diagnosed only after the semen sample is centrifuged and the pellet is thoroughly examined. If spermatozoa are found, we can distinguish cryptozoospermia from azoospermia. Furthermore, if azoospermia is confirmed, it is necessary to prepare smears for analysis of spermatogenetic elements.

Sperm vitality test should be routinely performed on all samples, and it is particularly informative in semen samples with reduced motility [18]. This test confirms the functional status of spermatozoa through dye exclusion (dead cells with damaged membrane allow entry of membrane-impermeant stains) and, thus, it is important to distinguish between dead and live cells since only viable cells are potentially able to fertilize the oocyte in an assisted reproduction cycle.

It should be stressed that WHO reference ranges are presented as percentiles, as semen parameters are generally not normally distributed. The 5^{th} percentile is indicated as the lower reference value for each sperm parameter, defining oligozoospermia, asthenozoospermia and teratozoospermia when total sperm number is <39 million/ej., progressive motility <30% and normal forms <4%, respectively.

Values

We place a high value on the recommendations of the semen analyses as the first line in the assessment of the fertility potential of the male partners of infertile couples. Nonetheless, several factors affecting its reliability need to be considered:

- laboratory personnel should be trained and experienced; seminologists must take into consideration that during the various phases of semen analyses (pre-analytical, analytical and post-analytical), many factors can interfere with the reliability of the analysis and a thorough medical history might suggest other causes of semen parameters variability [22-24]
- sperm parameters present a wide intra-individual variability and, thus, at least one repetition of a semen analysis might be needed for confirmation - as a spermatogenic cycle lasts about 3 months, this

is the optimal time interval needed for semen analysis repetition, unless acute illnesses and/or pharmacological treatment have occurred during that interval.

Remarks
Semen analysis should be inserted in an appropriate diagnostic process, correctly performed and interpreted, before it could provide information for medical or surgical therapies and/or to direct the couple towards ART. Seminologists should follow internal and external quality control programs [25-27].

4. Second step analysis
4.1. Additional tests on semen and sperm analysis
4.1.1. Urine post-ejaculation
Recommendation
- We recommend investigating the presence of spermatozoa in the urine post-ejaculation in azoospermic men with low semen volume (< 1.0 ml) or in subjects with aspermia, whenever medical history suggests the presence of retrograde ejaculation (1, ØØØO)

Evidence
In some men, especially after prostate or bladder neck surgery, semen might flow backwards into the bladder at ejaculation, resulting in low or very low ejaculate volume or aspermia. To confirm (or to exclude) retrograde ejaculation, a sample of post-ejaculatory urine can be analyzed for the presence of spermatozoa. A systematic review investigated 28 studies and reported successful isolation of sperm from urine and subsequent utilization in ART, with per-cycle pregnancy rates ranging from 20% to 50% in the largest case series [28].

Values
We place a high value on the recommendation of investigating the presence of spermatozoa analyzing urine post-ejaculation, particularly after urological surgery for bladder outlet obstruction. Alkalinization of the urine through diet and/or by ingestion of sodium bicarbonate might increase the chance that spermatozoa passing into the urine will retain their motility. These spermatozoa, in fact, can be retrieved from the urine and used for ART.

Remarks
The clinician should propose this test to men with an ejaculate volume <1.0 ml, after ruling out forms of hypogonadism or congenital bilateral absence of vas deferens (CBAVD). The possibility of an incomplete collection or a minimal abstinence interval (<1 day) should also be investigated.

4.1.2 Antisperm antibodies
Recommendations
- We suggest testing for antisperm antibodies in selected cases (2, ØØØO)

Evidence
Antisperm antibodies (ASA) might interfere with physiological reproduction at multiple levels, affecting sperm number and motility [29], the ability to pass through female genital secretions and the fusion of male and female gametes. Immunological causes of infertility are present in 4-10% of infertile couples and in 1%–2.5% of fertile men [30, 31]. It might be useful to consider testing semen samples with peculiar characteristics such as sperm agglutinations and hypomotility and infertile normozoospermic patients if other causes of infertility have been excluded. Since male gametes and immune cells are kept separate by Sertoli cells tight junctions (forming the so-called blood-testis barrier), any pathology undermining this barrier's integrity might facilitate the contact of the immune cells with sperm antigens, triggering the immune response [32, 33]. No difference in the reproductive outcome of *in vitro* fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) have been detected in the presence of ASA [33].

Values

We place a moderate value on ASA investigations. In the work up of the infertile male the identification of ASA can direct the patient with this specific semen alterations towards assisted reproduction.

Remarks
Inflammation, traumas, testicular torsions, and many other andrological diseases have been associated with ASA formation, requiring investigation of these cases thoroughly.

4.1.3 Additional tests on sperm
Recommendations
- We suggest against routine sperm DNA integrity investigation in male partners of infertile couples (2, ØØOO)

Evidence
Sperm DNA strand breaks might occur physiologically during chromatin condensation and reorganization and other non-reversible DNA strand breaks can be induced by numerous factors [21, 34, 35] (Supplementary table 2). Investigation of sperm DNA integrity could be helpful during the counselling of infertile couples, especially if the male partner is more susceptible to sperm DNA damage (recurrent pregnancy loss, diabetes, antineoplastic treatments, male genital tract infections, varicocele, exposure to toxicants) [36-39].

Values and remarks
A clinically relevant cut-off for sperm DNA integrity has not been identified and there is a lack of prospective trials using standardized methods. Sperm DNA damage might be investigated through direct (TUNEL, COMET) and indirect methods (SCSA, SCD), each with specific advantages and disadvantages (table 2)[18]. SCD test has been introduced in many laboratories as commercial kits are available. Nonetheless, results tend to be subjective (inter-individual variability in cell halo measurement) and not so straightforward in their interpretation as this method cannot define a specific type of DNA damage.
The role of sperm DNA integrity is still debated and despite a link with infertility, poor reproductive performance and spontaneous recurrent miscarriage [40, 41], available data are still too limited to routinely perform this investigation [42]. This investigation might nonetheless be informative during the work up of the infertile couple [6], in selected cases outlined above.

4.2. Microbiological/virological analysis
Recommendations
- We recommend performing sperm culture with antibiotic sensitivity testing in infertile patients with suspected urogenital infection/inflammation (Expert Opinion)
- We recommend performing *Chlamydia trachomatis* and Mycoplasmas test by nucleic acid amplification tests (NAATs) in urethral swab or urine of patients with suspected urogenital infection (1, ØØØO)
- We recommend microbiological assessment of the female partner in patients with proven bacterial urogenital infection before starting antibiotic therapy (Expert Opinion)
- We recommend performing *Human Papillomavirus* (HPV) DNA test in the semen samples of infertile patients with asthenozoospermia after having excluded the presence of other seminal infections (1, ØØOO)

Evidence
Prostatitis, vesiculitis, and epididymitis are considered urogenital infection/inflammation [commonly called also male accessory gland infections/inflammations (MAGI)] [43]. The diagnosis of urogenital infection/inflammation is made when oligo-astheno-, and/or terato-zoospermia associate with the following:
- At least one factor A (positive history for urinary infection, epididymitis, and/or sexually-transmitted disease; and/or presence of thickened or tender epididymis, tender vas deferens, and/or abnormal

digital-rectal examination) plus at least one factor B (abnormal prostate fluid expression and/or abnormal urine after prostate massage);
- At least one factor A plus at least one factor C [leukocytospermia, and/or abnormal rheological parameters of the semen (abnormal aspect, increased viscosity, elevated pH), and/or positive sperm culture];
- At least one factor B plus at least one factor C;
- Two or more factors C.

Urogenital infection/inflammation represents a potentially reversible cause of male infertility. The WHO guidelines on the diagnosis of infertile couples indicate sperm culture among the diagnostic tests of urogenital infection/inflammation [43]. However, there is no consensus concerning the other microbiological testing. Some evidence suggests that the Meares-Stamey test could be used for the diagnosis of chronic bacterial prostatitis [44-47].
No consensus has been reached on the microorganisms to be searched. *Chlamydia trachomatis* and Mycoplasmas (e.g. *Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum*, *Ureaplasma parvum*) represent common causes of non-gonococcal urethritis [48]. A systematic review and meta-analysis showed a significant association between male infertility and *Mycoplasma hominis* and *Ureaplasma urealyticum*, but no association was found with *Mycoplasma genitalium* and *Ureaplasma parvum* [49].
Culture, direct immunofluorescence assays, enzyme-linked immunosorbent assays or NAATs have been developed and used for the diagnosis of *Chlamydia trachomatis*. Among these, NAATs have the greatest accuracy on urethral swabs or urine for the diagnosis of *Chlamydia trachomatis* [43, 50-54]. Similarly, NAAT of the urethral swab is the preferred system to search Mycoplasmas [55, 56], due to their typical slow *in vitro* growth [57]. Generally, meatal swabs should be avoided in men for the low content of cellular material, and the urethral swabs should be preferred [58].
The prevalence of HPV infection is higher in infertile patients compared to fertile men (20.4% vs. 11.4%) [59]. A recent meta-analysis of observational studies (including 5203 men) showed that HPV-positive patients have significantly lower conventional sperm parameters and higher miscarriage rate than HPV-negative patients [60]. Other meta-analytic data support the association between HPV infection and astheno-teratozoospermia, high sperm DNA fragmentation, and elevated miscarriage rate in infertile patients undergoing ART [61, 62].

Values
The evidence on the usefulness of sperm culture in the diagnosis of urogenital infection and in identifying its etiology in patients with male factor infertility is of high value and the statement has been issued according to the Good Clinical Practice.
Chlamydia trachomatis and Mycoplasmas analysis by NAAT has higher accuracy on urethral swab compared to urine samples [56, 63-65].
The recommendation on the evaluation of HPV DNA is of low/moderate quality. It has a higher value in the presence of anamnestic or concurrent condylomatosis in patients and/or their partners, HPV infection in the partner, and recurrent pregnancy loss.

Remarks
Only four studies evaluated the effect of HPV infection on pregnancy outcome. Some of the studies included in the meta-analysis by Weinberg and colleagues did not clearly define pregnancy and miscarriage [60].

4.3. Endocrine assessment

Recommendations
- We recommend measuring serum FSH, LH and total testosterone in the fasting morning in all men with oligo-astheno-teratozoospermia (OAT) (any degree) and azoospermia (1, ØØØØ)
- We suggest measuring sex-hormone binding globulin (SHBG) for the calculation of free testosterone in all men with OAT (any degree) and azoospermia (Expert Opinion)

- We recommend against directly measuring free testosterone serum levels (1, ØØØO)
- We recommend measuring serum prolactin in case of hypogonadotropic hypogonadism (1, ØØØØ)
- We recommend interpreting hormonal data along with the clinical presentation, semen analysis, biochemical data and testis volume (Expert Opinion)
- We suggest measuring serum inhibin B only in selected cases (e.g. severe OAT/azoospermia with normal FSH levels) (2, ØOOO)

Evidence

FSH and testosterone are fundamental for spermatogenesis [66]. The routine immunometric measurement of serum testosterone is acceptable, but all kits are inaccurate at low concentrations [67]. The gold standard for testosterone measurement is based on mass spectrometry, but this methodology is not affordable in every laboratory yet. The current methods to measure free testosterone directly in serum are very inaccurate and should be avoided [68, 69]. SHBG measurement and the calculation of free testosterone might overcome this issue [70] especially in cases of borderline or low total testosterone levels.

Hypogonadotropic hypogonadism is associated with low testosterone and low/inadequately normal gonadotropin levels, oligozoospermia, OAT or azoospermia. In such cases, imaging studies and hormonal evaluation of hypothalamus-pituitary function are required in order to indicate or exclude organic causes, such as prolactin-secreting pituitary adenoma [8].

Elevated serum FSH levels (>10 IU/L) are indicative of a primary spermatogenic failure, especially at the pre-meiotic level, since the presence of normally proliferating spermatogonia is generally associated with normal FSH levels. Severe OAT/azoospermia with normal FSH and testis volume, low semen volume and low pH are suggestive of distal genital tract obstruction involving seminal vescicles, a suspicion corroborated by normal levels of inhibin B and by completing the diagnostic workup with scrotal/transrectal ultrasound exam. An obstructive condition should be distinguished from a meiotic or late spermatogenic arrest and this cannot be done on the basis of hormonal measurements only [71]. In fact, serum FSH levels might be normal in case of OAT/severe OAT/azoospermia due to meiotic or post-meiotic spermatogenic arrest. Low inhibin B levels (<100 pg/ml) suggest insufficient spermatogonial proliferation but are not fully informative of the histological picture [72-74].

Values

We place very high value on the endocrine assessment of OAT and azoospermia, since it allows the distinction between primary and secondary spermatogenic failure and the recognition of hypogonadism, providing the indication for a rational infertility treatment in case of secondary hypogonadism. Moreover, hormonal measurements could be useful for the decision whether to treat or not infertile men with empirical approaches. Hormonal measurements alone, however, do not allow an etiological diagnosis in the vast majority of cases and should always be interpreted together with the complete clinical, biochemical and instrumental data set.

Remarks

Men with impaired semen parameters tend to have lower testosterone levels than men with normal semen analysis [75, 76]. Elevated FSH serum levels suggest Sertoli cell only syndrome, severe hypospermatogenesis or an early block of the spermatogenetic process. In case of low testosterone with low-normal gonadotropin levels and/or elevated serum prolactin, the workup should continue with the complete assessment of the pituitary function.

4.4. Imaging

4.4.1. Scrotal US

Recommendations

- We recommend testicular ultrasound (US) as part of routine investigation of the male partner of infertile couples (1, ØOOO)
- We recommend testicular US in men with azoospermia and OAT and men with risk factors for infertility and testicular cancer (1, ØØØØ)

Evidence
Testicular US is a non-invasive diagnostic test performed in men with fertility problems, scrotal pain, genital inflammation, palpable scrotal abnormalities.
Scrotal US evaluation, in conjunction with semen analysis and as an adjunct to physical examination, should be performed in the initial assessment of men of infertile couples. The testicular US is the gold standard for scrotal investigation by assessing reproductive, inflammatory and neoplasia related features. In particular, it can detect alterations in volume, echotexture and vascularization of the testis and epididymis that could be associated with sperm abnormalities or inflammation (orchitis, epididymitis) [77]. It is important in assessing testicular volume, which is generally overestimated by Prader orchidometer [78, 79]. Reduced testicular volume (<12 ml) [80] is associated with impaired semen parameters [81], reduced fertility [82] and hypogonadism [83]. Various testicular US parameters can be combined to predict sperm and testosterone level impairment [84]. In addition, scrotal US can detect lesions within the testis and epididymis suggesting benign or malignant findings [85, 86]. Furthermore, scrotal US provides information on epididymal and deferential abnormalities or agenesis, often correlated with obstructive infertility [77, 87].
Finally, colour Doppler US (CDUS) is able to detect and stage varicocele, which could exert a negative impact on sperm parameters [77, 85]. Supplementary table 3 reports the main information given by testicular US.

Values
Testicular volume is tightly associated with both sperm and hormonal parameters. Scrotal US has a relevant role in testis volume assessment, when Prader orchidometer is unreliable. Recently, reference values in healthy fertile men have been defined by a multicentre study of the European Academy of Andrology [88]. Scrotal US might detect signs of testicular dysgenesis, often related to impaired spermatogenesis and to a higher risk of malignancy, or testicular lesions suggestive of malignancy.

Remarks
We recommend using scrotal US in everyday clinical practice in the diagnostic work-up of infertile men with oligozoospermia or azoospermia. Importantly, infertility is a risk factor for testicular cancer [15]. Furthermore, infertile men with testicular microcalcification were found to have a ~ 18-fold higher prevalence of testicular cancer [89, 90].

4.4.2. Trans-rectal ultrasound
Recommendations
- We recommend performing trans-rectal ultrasound (TRUS) prostate-vesicular scan in patients with suspected distal ductal obstruction or abnormalities (1, ØØØO)
- We suggest considering TRUS prostate-vesicular scan in infertile patients with urogenital infection/inflammation to assess its extension and prostate and seminal vesicles echo-pattern (2, ØØOO)

Evidence
TRUS of the prostate and seminal vesicles allows the identification of congenital (e.g. atresia/stenosis, midline prostatic cysts, or congenital cysts) or acquired (e.g. infection/inflammation, calcifications, or iatrogenic) causes of ejaculatory ducts obstruction and other abnormalities, which affect 1-5% of male infertile patients [91].
In patients with obstructive azoospermia, prostate-vesicular TRUS plays a role in the decision-making (for example suggesting sperm retrieval by testicular sperm extraction) [77].
A low volume of prostate and seminal vesicles suggests hypogonadism, even if the clinical impact of the prostate-vesicular TRUS in the management of male hypogonadism is low [77].
Prostate-vesicular TRUS provides information on echo-pattern abnormalities (e.g. hypo- or hyperechogenicity, calcifications, hyperemia, etc.) of both glands in infertile patients with urogenital infection/inflammation [43, 77, 92-96]. This might be useful to distinguish between uncomplicated

(prostatitis alone) and complicated (prostate-vesiculitis or prostate-vesicular-epididymitis), unilateral or bilateral, and hypertrophic-congestive or fibro-sclerotic forms of urogenital infection/inflammation, which may impact differently on sperm parameters [97-101]. This evidence attributes to prostate-vesicular TRUS a possible prognostic value. However, its role in the decision-making of infertile patients with urogenital infection/inflammation has not been fully shown by prospective studies [77]. Supplementary table 4 highlights the main features of urogenital infection/inflammation detected by TRUS.

Values
The evidence supporting the usefulness of prostate-vesicular TRUS in patients with suspected obstruction or other anomalies of the seminal tracts is of high quality and is released according to a systematic review. The evidence supporting the usefulness of prostate-vesicular TRUS in the management of infertile patients with urogenital infection/inflammation is of low quality.

Remarks
The quality of the evidence is limited by the lack of population cohort studies evaluating the usefulness of prostate-vesicular TRUS in patients with urogenital infection/inflammation and, prospectively, its clinical impact in the management of infertility in these patients.

5. Third step analysis

5.1. Testicular cytology/histology

Recommendations

- We suggest performing testicular fine needle aspiration cytology (when available) to distinguish obstructive from non-obstructive azoospermia (2 ØØOO)
- We recommend against performing testicular histologic analysis by open biopsy with the sole diagnostic purpose, unless it is associated with cryopreservation of sperm for future ICSI (Expert Opinion)

Evidence
Azoospermia and severe oligozoospermia might be caused by different alterations of spermatogenesis or might be the result of obstruction/sub-obstruction of the seminal tract. In cases of azoospermia, clear distinction between obstructive and non-obstructive forms is fundamental for further clinical and therapeutic approach. History, testicular volume, semen characteristics, imaging, and endocrine assessment in most cases allow distinguishing the two forms [102]. However, the gold standard is histopathology analysis of the testes [4, 8]. Fine needle aspiration cytological analysis has been proposed as an alternative to standard biopsy in the evaluation of azoospermic and severely oligozoospermic men [103]. It allows the classification of spermatogenic alteration in Sertoli cell-only syndrome, hypospermatogenesis, germ cell maturation arrest. The identification of the specific testicular alteration might also have implication for treatment, as for example FSH treatment is better suggested when oligozoospermia is caused by hypospermatogenesis without maturation arrest [104-106].
Testicular histology by open biopsy is the gold standard for histopathology of the testis, but it is not recommended for diagnostic purposes in infertile men [4].

Values and remarks
The evidence regarding testicular fine needle aspiration cytology as a diagnostic procedure in infertile males is low and this technique is available only in a few centres. However, it is a minimally invasive, office-based, procedure in which testicular material is retrieved from both testes with a fine needle, with or without local anaesthesia. Other than diagnostic information, it might have prognostic value for subsequent sperm retrieval by testicular sperm extraction (TESE) and response to FSH treatment. Testicular histology by open biopsy is an invasive procedure, which is not recommended with the sole purpose of diagnosis [4], but it should be associated with cryopreservation of sperm, in order not to repeat testicular sperm retrieval at time of ICSI [107].

5.2. Genetic testing

Recommendations

- We recommend targeted genetic testing in infertile men in order to identify the aetiology and to assess the risk of transmission to the descendants (1, ØØØØ)
- We recommend karyotype analysis in men with sperm concentration ≤10 mill./ml and affected by primary testicular disturbances and/or with positive family history for chromosomal anomalies or for recurrent abortion (1, ØØØO)
- We recommend: i) screening for Yq microdeletions in men with a sperm concentration ≤5 mill./ml and affected by primary testicular disturbances (1, ØØØO); ii) defining the extension of *AZFa* and *AZFb* deletions since the distinction between partial and complete deletions is highly relevant for the prognosis of TESE outcome (1, ØØØØ)
- We recommend screening of mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in patients with congenital uni-or bilateral absence of vas deferens (1, ØØØØ)
- We suggest screening of a panel of candidate genes in patients with congenital hypogonadotropic hypogonadism (HH) (2, ØØOO)
- We suggest sequencing of androgen receptor (AR) gene in suspected mild forms of androgen resistance (2, ØØØØ)
- We recommend providing genetic counselling to all couples with a genetic abnormality (1, ØØØØ).

Evidence

The risk of genetic anomalies correlates inversely with sperm count and shows the highest frequency in non-obstructive azoospermic men (NOA) [108]. Structural chromosomal anomalies are 10 times higher in severe OAT patients in respect to the general population [108] and might lead to abortion or congenital anomalies in the offspring. *AZF* deletions (in cases spermatozoa can be found in the ejaculate or retrieved by TESE) will be obligatorily transmitted to the male offspring. Y chromosome microdeletion screening in azoospermic men prior to TESE has a prognostic value, i.e. in case of complete *AZFa* and *AZFb* deletions the likelihood of sperm recovery is virtually zero [109]. Recessive *CFTR* mutations cause congenital absence of vas deferens (CAVD) without unilateral renal agenesis in over 90% of cases [110].

Values

We place high value to the recommendations given that genetic testing is a prerequisite for an appropriate management of the infertile couple [109, 111]. Besides diagnosing the cause of infertility, it might have relevance for decision making on the feasibility of testis biopsy. Some genetic factors have consequences not only on reproductive but also on general health (Klinefelter syndrome, *CFTR* mutations, Kallmann syndrome, etc) of the carrier and his descendants [112].

Remarks

The distinction between primary testicular impairment and congenital obstructive/subobstructive forms is crucial, as for example karyotype and Y chromosome microdeletions analysis should be performed in the first cases and *CFTR* mutations in the second.

In case of CAVD the screening for *CFTR* mutations in the female partner is mandatory before TESE/ICSI, due to the high frequency of healthy carriers in the general population. Mutation screening in candidate genes for HH or in the *AR* gene should be performed based on the clinical assessment. Long term follow-up is requested in Klinefelter patients and in all men with hypoandrogenism.

A growing number of novel monogenic causes of primary testicular failure (quantitative or qualitative defects) are under validation, but not yet available for routine testing [108, 113].

6. General health of infertile men

Recommendations

- We suggest attentioning cardiometabolic and bone health in infertile men with azoospermia and oligozoospermia, particularly when primary testicular damage and/or alteration of the LH-T axis exists (2 ØOOO)
- We suggest regularly following up of infertile men with signs of primary testicular damage and/or alteration of the LH-T axis, independently from the outcome of fertility treatments (Expert Opinion)

Evidence

Observational and prospective, longitudinal studies showed that infertile men have more comorbidities and increased mortality than controls [2, 82, 83, 114-118]. Infertile men with poor semen quality have been described to be at risk for metabolic syndrome and osteoporosis [2, 83, 119], are at higher risk of hospitalization, in particular for cardiovascular diseases and diabetes mellitus, and have higher long-term morbidity [117, 118]. Infertile men are also at risk for clinical and subclinical hypogonadism [2, 81, 119], and their clinical sequelae. A large study on more than 5,000 men showed that low sperm count is associated with poorer metabolic, cardiovascular, and bone health. Importantly, although hypogonadism is mainly involved in this association, a low sperm count in itself seems to be a marker of general male health [2]. A recent systematic review confirmed the interconnections between a man's fertility and overall health [120].

Values

We suggest paying attention to the cardiometabolic and bone health of men with infertility, as testicular function might be considered as a mirror of general health. Attention should be particularly given to infertile men at risk for future clinical hypogonadism and these patients should be regularly followed up.

Remarks

The population of infertile males has a unique opportunity for health assessment and disease prevention. When appropriate diagnostic workup is performed in infertile men, patients might benefit from the identification of clinically significant comorbidities and risk factors. Therefore, the diagnosis of male infertility might allow a window into future comorbidity and/or mortality. The early identification of hypogonadism, metabolic derangements, and osteopenia/osteoporosis allows for more adequate management, follow up, treatment, and lifestyle modifications.

7. Approach to treatment

7.1. Lifestyle

Recommendations

- We recommend life-style changes (including decrease alcohol intake, weight loss, increased physical activity, smoking cessation) in men with infertility to improve general health (1, ØØØO)

Evidence

Moderate alcohol consumption has an impact on aspects of health including infertility: higher alcohol intake can result in a detrimental effect on male fertility [121]. Alcohol consumption seems to have a negative impact on sperm morphology, motility and oxidative stress [121, 122]. Heavy alcohol consumption has also a negative impact on male sexual performance and testosterone levels, which can be restored by alcohol cessation.

Obesity can have a significant impact on male fertility in addition to other potential risks (e.g. cardiovascular disease, diabetes) [123]. Several studies demonstrated that an increase in body mass index (BMI) is correlated with a decrease in sperm concentration and motility [124]. Sperm DNA damage is also increased in obese men [125]. A relationship also exists between obesity and ED [126]. Therefore, an ideal weight might provide a way for men to restore or increase fertility, and weight loss aids in ameliorating seminal and hormonal parameters [127, 128]. Similarly, physical activity is suggested to be beneficial, though too much exercise might be detrimental.

Men who smoke tend to have decreased total sperm count, sperm concentration, motility, normal morphology, semen volume and fertilizing capacity [129]. Smoking could reduce the mitochondrial activity in spermatozoa further leading to a decreased fertilization capacity [130]. Furthermore, smoking impacts on DNA integrity of the sperm and on the amount of seminal leukocytes, which might lead to reactive oxygen species (ROS) generation.

Other lifestyles might affect a man’s fertility, although with lower evidence. Slight variations of the scrotal temperature are able to damage spermatogenesis: an increase of 1°C is correlated to a 14% drop in spermatogenesis [131], and exposure to high temperature leads to increase in apoptosis [132]. Men tend to have a decline in semen parameters during infertility treatment and during this period they might experience anxiety or depression disturbances [133]. Stress increases adrenergic activation, leading to

more vasoconstriction in the testis that results in lower testosterone level and decrease spermatogenesis [134]. Finally, occupational exposure to heavy metals, organic solvents and ionizing radiation might negatively affect semen quality [135]. These factors, together or independently, might result in failure to conceive, and might also result in the conception of non-viable embryos during IVF [136].

Values
The lifestyle factors discussed above might potentially impact male fertility. A healthy lifestyle might benefit fertility and maximize fertility treatment outcome.

Remarks
Changes in lifestyle might improve not only semen quality, but also general health.

7.2. Non-hormonal treatment

7.2.1. Nutraceuticals/antioxidants

Recommendations

- We recommend against treatment with nutraceuticals/antioxidants in unselected infertile men to increase sperm parameters (Expert Opinion)
- We suggest considering the use of nutraceuticals/antioxidants in selected patients with idiopathic oligozoospermia and/or asthenozoospermia and/or clearly sign of high oxidative stress, since in some cases they might improve sperm parameters (2, ØOOO)
- We cannot recommend either for or against the use of nutraceuticals/antioxidants to increase the pregnancy rate (Expert Opinion)

Evidence
The Cochrane review on the use of antioxidants for the treatment of male infertility showed among couples attending fertility clinics an increase of the live-birth rate (OR 1.79, n=750) and the clinical pregnancy rate (OR 2.97, n=786) in the treated-arm compared to placebo. However, no effect was found when studies at high risk of bias were not included in the analysis [137]. Overall, 18 different oral compounds were assessed.
Several meta-analyses support the use of antioxidants to improve sperm parameters. In particular, selenium, coenzyme Q10 (CoQ10), and ω3 fatty acids seem effective in increasing sperm count [138, 139], selenium, carnitines, CoQ10, zinc, and ω3 fatty acids seem to improve sperm motility [138-142], CoQ10, selenium, ω3 fatty acids, and carnitines might ameliorate the percentage of spermatozoa with normal morphology [138, 139, 142]. However, these studies show a high degree of heterogeneity, due to the different therapeutic schemes used (antioxidant molecule(s) and doses, treatment length). In contrast, a meta-analysis did not report any effect of supplementation with folate compared to placebo on sperm parameters [143]. A systematic review and meta-analysis of 61 studies [59 of which were randomized controlled trials (RCTs)] recently confirmed the efficacy of up to a six-month-long therapeutic regimen with a specific antioxidant on conventional sperm parameters (data for pregnancy rate were limited) [142]. L-carnitine (1-3 g daily) improved significantly sperm concentration and motility, whereas L-carnitine plus L-acetylcarnitine (for 2 months) only ameliorated motility compared to placebo, and Coenzyme Q10 (CoQ10) (200-300 mg daily) significantly improved sperm concentration, motility, and morphology. A more recent RCT in unselected men with OAT showed no effect of a combination of antioxidants on sperm parameters and pregnancy rate [144]. A large multicentre RCT on couple planning infertility treatment (irrespectively on male factors or semen alterations) showed no effect of folic acid and zinc supplementation by male partners for 6 months on semen parameters and birth rates [145].
Oxidative stress can damage sperm DNA integrity and meta-analyses have shown that higher sperm DNA fragmentation is associated with worse pregnancy outcome [146-148]. Therefore, antioxidants could be considered for the treatment of male idiopathic infertility with proven sperm DNA damage. However, the Cochrane review found that the effect on sperm DNA fragmentation in unselected men was not significant [137]. Similarly, a combination of antioxidants did not improve sperm DNA fragmentation in unselected men with high DNA fragmentation [144].

Values
The data supporting the use of antioxidants/nutraceuticals to improve pregnancy outcome are of low quality. A higher value might be placed after the exclusion of microbial urogenital infection and in the presence of documented increased sperm DNA fragmentation as indirect parameter of increased oxidative stress.

Remarks
The evidence reported mainly derives from RCTs. However, it is limited by the low total number of events, by extremely high heterogeneity in inclusion and exclusion criteria, and by the different therapeutic schemes used in the various studies. If nutraceuticals/antioxidants have a role in increasing semen parameters and pregnancy rates, selection of patients is fundamental [149]. For example, many studies did not exclude microbial urogenital infection [137, 142]. This might limit the reliability of their findings since microbial inflammation negatively impacts sperm DNA fragmentation [149, 150]. There is an urgent need for RCTs in selected patients showing high ROS levels in their semen in order to verify whether antioxidants may have a rational in these men.

7.2.2. Treatment of urogenital infection/inflammation
Recommendations
- We recommend antibiotic treatment in patients with bacterial urogenital infection and their partner to cure infection (1, ØØØO)
- We suggest considering the use of antibiotics in infertile patients with bacterial urogenital infection to improve sperm parameters (2, ØOOO)
- We cannot recommend either for or against the use of antibiotics in infertile patients with urogenital infection to increase the pregnancy rate and sperm DNA fragmentation (Expert Opinion)

Evidence
Bacterial urogenital infection alters sperm parameters and might lead to male infertility [151-153]. Antibiotics should be prescribed to patients with urogenital infection and their partners only when germs are present and in a concentration considered pathogenic. Evidence from clinical trials [97, 154-156] and one prospective observational study [151] supports the concept that antibiotic administration might improve sperm quality in infertile patients with urogenital infection, although no difference on sperm quality was reported in a single study [157]. One prospective study reported the amelioration of sperm DNA fragmentation following antibiotic treatment [151] but there is a lack of properly sized and designed trials evaluating this aspect. Therefore, no specific recommendation of the effects of antibiotic therapy on sperm DNA fragmentation can be made. Similarly, few properly designed studies have so far been published on the impact of antibiotic therapy on pregnancy rate in infertile patients with urogenital infection. One study supports an improvement of pregnancy rate after antibiotic therapy [156], but no effect was found in two clinical trials [155, 158].
Some studies suggest the use of non-steroidal anti-inflammatory (NSAI) drugs (e.g. salicylates, fenamic acids, profens, Cox-2 inhibitors, arylacetics, etc.), glucocorticoids, and fibrinolytic agents (e.g. serratiopeptidase, bromelin, escin) after germ eradication in bacterial and abacterial urogenital infection. A randomized prospective study showed a higher efficacy of NSAI drugs plus carnitine given with a sequential scheme than their combination or their single administration (NSAI or carnitine) on sperm motility and viability [159]. However, the evidence is still insufficient to make a recommendation on this aspect.

Values
Data supporting the use of antibiotics in patients with bacterial and abacterial urogenital infection are of low quality.

Remarks
Few RCTs have evaluated the effects of antibiotic treatment in bacterial urogenital infection and overall they include a limited number of patients. Only one RCT has so far been performed on the treatment of

abacterial urogenital infection and the effects on the pregnancy outcome in patients with urogenital inflammation have been rarely reported [160].

7.3. Hormonal treatment

Recommendations

- We recommend against treatment with androgens in men considering parenthood (1, ØØØØ)
- We suggest treatment with FSH in selected men with oligozoospermia and/or asthenozoospermia and FSH plasma levels <8 IU/L and no signs of obstruction of the seminal tract to increase sperm quantity and quality and pregnancy rate (natural and by ART) (2, ØØOO)
- We recommend against FSH treatment for improving pregnancy rate without a specific couple-oriented diagnostic workup (1, ØØØØ)
- We recommend combined FSH and hCG treatment in men with hypogonadotropic hypogonadism, including congenital forms, to induce spermatogenesis (1, ØØØØ)
- We cannot recommend either for or against anti-oestrogens (tamoxifen or clomiphene) and aromatase inhibitors to increase sperm quantity and quality and pregnancy rate (Expert Opinion)

Evidence

A quantitatively and qualitatively normal spermatogenesis requires the combined effects of FSH and testosterone. As a consequence, different hormonal treatments have been proposed to replace and correct hormonal deficiencies (such in case of hypogonadotropic hypogonadism) and/or to force spermatogenesis in an empirical way in patients with normal hormonal levels (such as in case of idiopathic OAT), including gonadotropins, anti-oestrogens, and aromatase inhibitors. The evidence is strong that treatment of hypogonadotropic hypogonadism with gonadotropins is effective in stimulating spermatogenesis and increasing pregnancy rate [113]. A large number of studies showed that FSH treatment is rational and effective in increasing sperm number and improving sperm quality (in particular sperm motility and DNA fragmentation) and pregnancy rate in men of infertile couples affected by idiopathic oligozoospermia and/or asthenozoospermia with FSH plasma levels within the normal range (<8 IU/L) [104, 161, 162]. Few RCTs have been performed in this field, but meta-analysis confirmed the efficacy of FSH treatment in this selected group of infertile men [163-165]. Purified and recombinant FSH (originators and biosimilars) seem to have comparable results [166]. FSH treatment has also been shown to decrease sperm DNA fragmentation in RCT [167].

Anti-oestrogens (tamoxifen or clomiphene) and aromatase inhibitors are used off-label in infertile men to increase gonadotropin secretion from the pituitary and to decrease the conversion of testosterone to oestradiol respectively. Two meta-analyses showed that anti-oestrogens might be effective in increasing sperm parameters and pregnancy rate in infertile patients with idiopathic infertility, but the evidence is of low quality and few RCTs have been performed [168, 169]. A meta-analysis showed that aromatase inhibitors increase sperm parameters, but the evidence is of low quality and data of pregnancy are not available [170].

Values

We place high value in selecting the correct conditions that might benefit from FSH treatment to maximize the rate of responders. We place high value in considering this treatment in a couple-oriented manner (therefore including female age and female factors of infertility), considering that treatment should be done for at least 3-4 months before an effect on semen parameters is evident.

Remarks

The scheme of FSH treatments (dose, duration) should be personalized, however RCTs on this topic have not been performed and usually a fixed scheme (for example 150 U three times per weeks for 3-4 months) is used. Anti-oestrogens and aromatase inhibitors are not registered for treatment of male infertility in many countries, including Italy.

7.4. Varicocele

Recommendations

- We recommend evaluating both partners, together with the options available, to determine the approach to varicocele treatment (1, ØØOO)
- We suggest treating varicocele in infertile couples in which the male partner has abnormal semen parameters and the female partner has normal fertility or a potentially treatable cause of infertility and time to conception is not a concern (2, ØOOO)
- We suggest only monitoring in cases with subclinical varicocele (2, ØØØO)

Evidence

Varicocele is clinically defined as a palpable elongated, dilated and tortuous testicular pampiniform plexus of veins in the spermatic cord. A subclinical varicocele is not detected on physical examination but only identified on CDUS.

Varicocele was reported in 15-20% of the normal adult male population [171] and the European Academy of Andrology showed that varicocele detected by CDUS was found in 37.2% of healthy, fertile men and was not associated with seminal or hormonal parameters, but men with severe (grade IV and V) varicocele had higher LH levels [88]. This prevalence is similar to that reported in primary infertile men [172, 173]. However, varicocele is the most commonly described diagnosis in men presenting with infertility [174, 175].

Although the association between varicocele and male fertility is not clear, varicocele might have negative effects on semen quality and sperm function and might be associated with progressive decline in testicular function and testicular volume. Increased scrotal temperature, hypoxia and reflux of toxic metabolites have been described as the causes of spermatogenic damage associated with increased oxidative stress (OS) and sperm DNA damage [38].

Meta-analyses show semen quality improvement (including decreased sperm DNA damage) following palpable varicocele repair by surgery with respect to no-surgery [38, 176]. There is some evidence that treatment of varicocele in men from couples with otherwise unexplained infertility might improve the chance of pregnancy, although there is no evidence whether any treatment compared to no treatment in subfertile men might be of benefit on live birth rates [177].

Varicocele repair results in an increase in testosterone levels [178] and testicular volume in young men [179]. Therefore, although not included in the primary aim of the present guidelines, we would consider varicocele repair in young males with progressive testicular failure and/or decrease of seminal parameters. Subclinical varicocele should only be monitored because no significant post-operative fertility improvement has been described [180].

Values and remarks

We place high value in considering possible varicocele treatment in a couple-oriented manner. We value the recommendation to avoid the treatment of subclinical varicocele. We place a relatively high value to the suggestion regarding varicocele repair in case of progressive failure of testicular function and monitoring only cases with subclinical varicocele. Whether treatment of varicocele might increase the pregnancy rates during IVF is still debatable [181].

7.5. Approach to NOA

Recommendations

- We recommend preferring the use of TESE to testicular sperm aspiration (TESA) in NOA patients (1, ØØØØ)
- We suggest choosing between micro-TESE (mTESE) and conventional-TESE (cTESE) case by case and according to ART centre set-up, where the use of an operating microscope is available and according to the surgeon's expertise (2, ØØØØ)

Evidence

NOA is characterized by severely compromised spermatogenesis, resulting in the absence of spermatozoa in the ejaculate. Methods of surgical sperm retrieval (SR) approaches include cTESE, mTESE, and percutaneous TESA. In a meta-analysis including 117 studies, no differences in SR rates between cTESE and mTESE was found [182]. Conversely, another meta-analysis including 15 studies reported that SR rates

using mTESE were significantly higher (52%) than cTESE (35%) and cTESE had double probability of SR compared to TESA (56% *vs* 28) [183]. Similarly, in a systematic review of studies comparing cTESE and mTESE, the latter had an overall SR rate ranging from 42.9 to 63.0% compared to 16.7-45.0% of cTESE [184].

Values
We place a moderate value on suggesting the use of mTESE compared to cTESE, considering the lower accessibility of the technique, requiring the use the operating microscope and microsurgical expertise.

Remarks
SR rates depend on different factors, including patient characteristics, surgeon's and embryologist's experience, and laboratory technique.

7.6. Approach to obstructive azoospermia (OA)
Recommendations
- We suggest choosing between percutaneous (testicular/epididymal) and surgical sperm retrieval in OA case by case and according to ART centre set-up (2, ØØOO)
- We suggest microsurgery in men with OA amenable to reconstruction and according to the surgeon's expertise (2, ØØØØ)

Evidence
OA results in the absence of sperm and spermatogenetic cells in the ejaculate. Obstruction could be proximal or distal, and congenital or acquired. Although most proximal OA can be treated with microsurgery reconstruction, surgical SR and ART represent a valid opportunity. SR rates in OA are excellent whatever it is the cause of the obstruction [185]. The use of testicular or epididymal surgically retrieved sperm has been shown to result in similar fertilization rates (34% to 80%) and clinical pregnancy rates (17% to 65%) [186-188]. These data are corroborated also by a meta-analysis and a Cochrane review that found no differences using testicular versus epididymal sperm in OA, in terms of fertilization rates, clinical pregnancy or live birth rates [187, 189]. Moreover, fresh sperm retrieved from the vas, testis, or epididymis showed similar viability compared to frozen-thawed ones and no differences in terms of fertilization and clinical pregnancy rates [190-192]. Fresh or frozen sperm retrieved from testis or epididymis in OA compared to those from ejaculated of normozoospermic men, affect the fertilization rates but do not affect euploidy rate in the embryos [193]. OA and sub-obstructive oligozoospermia might also be the result of midline prostatic cysts, which can be successfully treated with trans-rectal ultrasonically-guided cyst aspiration [194].

Values
We place high value to the recommendation of using a sperm retrieval technique in men with OA undergoing ART due to the high SR rates independently of the surgical technique selected.

Remarks
Since there is no clear evidence of superiority of any technique, the choice of the SR technique should be performed considering patients and ART centre characteristics and experience.

7.7. Sperm selection
Recommendations
- We recommend performing sperm selection by swim-up or density gradient separation in preparation to ART (1, ØØØØ)

Evidence
Ejaculated male gametes require a maturation process to acquire the ability of interacting with the oocyte and acquire their fertilization potential. Sperm capacitation is thus essential for *in vivo* fertilization and its imitation *in vitro* is the foundation of ART. In fact, sperm processing is necessary to separate spermatozoa

from seminal plasma, non-sperm cells and cellular debris, in order to yield a final preparation containing a high percentage of viable motile cells with no morphological abnormalities [18]. Numerous studies have tried to evaluate different sperm selection methods used in ART, in relation to the recovery of motile spermatozoa or the percentage of pregnancy [195]. Most studies did not find differences in the percentage of fertilization, implantation and pregnancy between swim-up and density gradient separation [196-203].

Values

We place a high value suggesting sperm selection techniques in preparation to ART. Their aim is the selection of motile spermatozoa to improve the outcome of assisted reproduction and, when inserted in the work up of the infertile couple, it can direct towards the most appropriate fertilization method. Swim-up and density gradient separation are considered the "Gold Standard" among sperm selection techniques [204]. In particular, following WHO indications, swim-up can be used when sperm parameters are almost normal, while in case of OAT density-gradient separation can be used to retrieve a higher sperm concentration.

Remarks

The choice of sperm selection method must be tailored on the characteristics of the semen. To date, however, there is still a great variability in both assessment of sperm parameters and sperm preparation, making comparisons extremely difficult [204].

7.8. ART

Recommendations

- We recommend ART for male factors in case other treatments are not indicated or not effective (1, ØØØØ)
- We suggest against alternative sperm selection techniques for ART (2, ØOOO)

Evidence

Sperm selection and micromanipulation, such as ICSI, is useful for treating male factor infertility. Several additional sperm selection methods have been introduced for ART such as, high magnification ICSI, named IMSI (intracytoplasmic morphologically selected sperm injection) (table 3). A recent Cochrane meta-analysis including 13 RCTs comparing 1256 couples undergoing IMSI and 1519 couples undergoing ICSI [205] reported no differences between the two techniques. Low quality evidence was reported for IMSI *vs* ICSI regarding live birth and very-low quality evidence with respect to miscarriage. There was no indication that IMSI influenced congenital abnormality rate.

Another Cochrane meta-analysis focused on advanced sperm selection techniques [Hyaluronic acid-ICSI, Magnetic-activated cell sorting (MACS), Zeta sperm selection] *vs* standard IVF or ICSI [206]. The authors included 8 RCTs (4147 women) and found very-low quality evidence. No one of the abovementioned additional techniques showed significant differences compared to ICSI in terms of live birth, clinical pregnancy and miscarriage.

The use of microfluidics for sperm sorting has been proposed to improve the SR and reduce ROS production [207]. However, RCTs comparing this method with standard methods are still ongoing and only few *proof-of-concept* studies have been published.

Values

We placed a high value to recommendation regarding ART in male factor infertility when other treatments are not indicated or not effective. ART should not be the first choice without a comprehensive diagnostic work-up of the couple and considering all the available treatments.

Remarks

ART represents a valid option for treating some conditions as severe OAT, cryptozoospermia, NOA, OA. The choice of additional advanced techniques for sperm retrieval and selection is based on low quality evidence.

8. Preservation of fertility

Recommendations

- We recommend offering sperm cryopreservation before medical and surgical treatments inducing infertility/sterility (1, ØØØØ)
- We suggest offering sperm cryopreservation in OAT patients with active desire of fatherhood in case of progressive deterioration of semen quality (2, ØØØØ)

Evidence

Sperm cryopreservation is widely used to maintain these reproductive cells in a vital state at cryogenic temperatures (-196°C). Cryoprotectants and adequate cryopreservation methods (rapid or slow freezing procedures) prevent freezing damage and preserve the male gametes in a state of "suspended animation". The role of sperm banks is both to preserve the patient's fertility before starting gonadotoxic treatments and to access ART. There are many major indications for sperm cryopreservation, in particular conditions where either medical or surgical treatments might interfere with spermatogenesis, genome integrity or ejaculation mechanisms (table 4).

Other indications for sperm cryopreservation are allowing access to ART for patients with spinal cord injury [208, 209] and patients with severe alteration of spermatogenesis [210]. The latter might also present variations of semen quality that might result in azoospermia, either transient or permanent, and therefore cryopreserved semen could represent the only chance of fertility. Patients with NOA and Klinefelter syndrome might benefit from cryopreservation from TESE/microTESE [14, 182, 211], whereas patients with OA might cryopreserve sperm from percutaneous and surgical sperm retrieval from the testis or epididymis.

Sperm vitrification is emerging as a promising tool to preserve fertility in cases of very low number of spermatozoa. Due to scanty and conflicting evidence however, it should still be considered an experimental procedure [212].

Values

We place a high value on recommending sperm cryopreservation for men with conditions requiring medical and surgical treatments inducing infertility/sterility since most of these pathologies have good prognosis and these long-surviving patients will face long-term consequences of treatments, including transient or permanent damage to spermatogenesis. For example, azoospermia can be present in up to 3-6% of patients with testicular cancer two years after chemo-radiotherapy [213]. Several gonadotoxic regimens (like BEACOPP for lymphomas) might be associated with longer recovering time for spermatogenesis and permanent azoospermia as doses and/or cycles increase [214]. In parallel, sperm DNA damage can be detected for up to two years after the end of the treatment. Such damage is more marked in advanced stages and is also influenced by the treatment type and dose. Nonetheless, several antineoplastic regimens can be compatible with at least a partial recovery of spermatogenesis, and cancer patients in fertile age might find in sperm cryopreservation a strong psychological support to deal with the various stages of treatment protocols [215].

Remarks

To date, rapid sperm freezing is still the gold standard in sperm cryopreservation, offering excellent results in terms of post-thawing cell viability and reproductive outcomes [216, 217]. The cumulative rate of patients who achieved fatherhood with cryopreserved semen is close to 50% [218]. Thus, clinicians are encouraged to discuss post-treatment fertility and possible use of ART, as well as sperm cryopreservation strategies.

9. Conclusion

We provided the first SIAMS and SIERR Guidelines on the clinical and therapeutic approach to male factor infertility. These Guidelines are based on two principal aspects: they are couple-oriented and place high value in assessing, preventing and treating risk factors for infertility.

Components of the initial evaluation in men without known risk factors for infertility should include at minimum medical history, physical examination, and semen analysis. Semen microbiological examination,

endocrine assessment, and imaging are suggested in most men and recommended when specific risk factors for infertility exist or first step analyses showed abnormalities. Full examination including genetic tests, testicular cytology/histology, or additional tests on sperm is clinically oriented and based on the results of previous investigations.

For treatment purpose, the identification of the specific cause and the pathogenetic mechanism is advisable. At least, distinguishing male factor infertility in pre-testicular (hypothalamic-pituitary disorders), testicular (primary damage of the testes), and post-testicular (obstructive and subobstructive forms) is essential. Treatment should be couple-oriented, and could include lifestyle modifications, etiologic therapies, empirical treatments, and ART on the basis of best evidence and with a gradual approach. Importantly, early detection of clinical condition at risk for decline in fertility potential and preservation of fertility in circumstances able to decrease fertility in men are of crucial importance. These Guidelines also highlighted that male infertility and in particular testicular function might be a mirror of general health of a man.

Many areas, especially regarding the best therapeutic approach for male infertility, are still nebulous. We plead multicentre RCTs aimed at increasing the level of evidence for some therapeutic interventions and identifying novel therapeutic strategies for subgroups of patients with infertility. In general, we advocate for more attention and information on reproductive health among the general population and medical personnel.

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Table 1. Risk factors with potential negative effect on male reproduction. The presence of these factors should be explored during medical history taking. Major risk factors are those with a proven relevance to male infertility. Minor risk factors are those with low evidence and a non-obligate impact on reproductive function. The presence of major risk factors implies the need for a comprehensive andrological diagnostic workup and take action for changing modifiable risk factors.

Major risk factors	Minor risk factors
Modifiable	
● Anabolic steroid abuse ● High alcohol consumption (>20 units per week) ● Recent complain of decreased libido/erectile dysfunction	● Cigarette smoking ● Marijuana use ● Obesity/high energy diets ● Genital heat stress ● Environmental/occupational exposure to potential toxic agents (not always modifiable)
Unmodifiable	
● Family history for infertility (including premature ovarian insufficiency in mother or sister), known genetic factors (chromosomal anomalies, cystic fibrosis), testis tumour ● Pubertal disorders ● History of cryptorchidism, testicular torsion or trauma, orchitis, reproductive tract infections, testis cancer ● Testicular hypotrophy ● Known genetic factors (e.g., chromosomal anomalies, cystic fibrosis, thalassemia) ● Cytotoxic treatment (chemo/radiotherapy) ● Medications interfering with the central or local regulation of testicular function ● Surgical intervention at the inguinal/scrotal level followed by decrease of testis volume or hydrocele (including intervention for varicocele) ● Systemic/chronic diseases (kidney or liver insufficiency, haemochromatosis, sarcoidosis, etc)	● Family history for malformations or repeated abortions ● Advanced age (>45 years) ● Infertility with previous partner

Table 2. Techniques for sperm DNA integrity evaluation.

Method	Description	Advantages	Disadvantages
SCSA (sperm chromatin structure assay)	A cytofluorimetric method through acidic denaturation and acridine orange staining	● Standardized protocol ● Statistically robust cytofluorimetric analyses ● Defined DNA Fragmentation Index (DFI) threshold	● Indirect test ● No indication on the type of chromatin fragmentation
COMET (SCGE, single cell gel electrophoresis)	A test-detecting the "tail" obtained after lysis and gel electrophoresis of spermatozoa in an alkaline or neutral environment	● Direct test ● Requires few cells for analysis (fluorescence microscope) ● Evaluates both single(SSBs) and double strand DNA breaks (DSBs)	● Threshold values are not well defined ● Time consuming
TUNEL (terminal deoxynucleotidyl transferase–mediated fluorescein–dUTP nick end labeling	A test investigating DNA breaks highlighted by marked nucleotides inserted with the enzime TdT. The evaluation can be performed with either fluorescence microscope or cytofluorimetric analysis.	● Direct test ● Commercial kits available ● Evaluates both SSBs and DSBs	● Not well-defined thresholds ● Unstandardized assay protocol
SCD (sperm chromatin dispersion)	A test measuring sperm cells sensitivity/resistance to DNA denaturation	● Commercial kit available ● Quick test ● No complex equipment needed	● Indirect test ● Results are sensitive to technician's experience ● Interpretation of results might be difficult ● It provides no information on the type of chromatin fragmentation

Table 3. Recommendations and level of evidence for additional sperm selection methods in ART performed for male factor infertility.

Method	Level of evidence	Comments
IMSI (intracytoplasmic morphologically selected sperm injection)	ØOOO	The current low quality evidence from RCTs does not support or refute the clinical use of IMSI. There is uncertainty and very low-quality evidence that IMSI might increase the chances of a clinical pregnancy.
Hyaluronic acid (HA)-ICSI	ØOOO	There is uncertainty that HA-ICSI might reduce miscarriage compared with ICSI. HA-ICSI *vs* ICSI effects on live birth was 25% ICSI *vs* 24.5-31% HA-ICSI. Miscarriage per woman was 7% ICSI *vs* 3-6% HA-ICSI and per clinical pregnancy 20% ICSI *vs* 9-16% HA-ICSI. No significant differences were reported for clinical pregnancy, 37% ICSI *vs* 34-40% HA-ICSI
MACS (magnetic-activated cell sorting)	ØOOO	Very low quality evidence in RCTs reporting live birth, miscarriages and clinical pregnancy
Zeta sperm selection	ØOOO	Very low quality evidence in RCTs reporting live birth, miscarriages and clinical pregnancy
Microfluidic chip sperm sorting	ØOOO	Very low quality evidence in retrospective cohort studies. Few RCTs still ongoing.

Table 4. Main indications to sperm cryopreservation due to possible iatrogenic damage to spermatogenesis.

Indication	Description
Cancer and cancer treatments	Systemic and local effects from cancer, as well as chemo and radiotherapy, might interfere with either spermatogenesis or genome integrity. Surgical treatments might also entail iatrogenic damage to ejaculatory mechanisms [34, 213, 214, 219-224]
Autoimmune and inflammatory diseases	Patients affected by autoimmune/chronic inflammatory diseases often experience impaired reproductive function related both to the disease itself and its treatment (cyclophosphamide, alkylating agents and other immunosuppressant agents) [225, 226]
Urological diseases	Surgical treatment of many non-oncological urological pathologies (bladder outlet obstruction, benign prostatic hyperplasia, etc.) might cause permanent damage to ejaculatory mechanisms [28]

Figure legends

Figure 1. Diagnostic flow-chart for male factor infertility. Numbers indicates the corresponding recommendations in the text.
Abbreviations: AR, androgen receptor; ASA, antisperm antibodies; CAVD, congenital absence of vas deferens; CDUS, colour Doppler ultrasound; CFTR: cystic fibrosis transmembrane conductance regulator; FNAC, fine needle aspiration cytology; FSH: follicle-stimulating hormone; HH, hypogonadotropic hypogonadism; HPV: Human Papillomavirus; LH: luteinizing hormone; OAT, oligo-astheno-teratozoospermia; SHBG: sex hormone-binding globulin; TRUS, trans-rectal ultrasound; UGI, urogenital infection/inflammation; US, ultrasound.

Figure 2. Therapeutic flow-chart for male factor infertility. Numbers indicates the corresponding recommendations in the text.
Abbreviations: ART, assisted reproduction technique; ASA, antisperm antibodies; FSH: follicle-stimulating hormone; hCG: human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; HPV: Human Papillomavirus; OAT, oligo-astheno-teratozoospermia; PESA, percutaneous sperm aspiration; TESA: testicular sperm aspiration; TESE: testicular sperm extraction; UGI, urogenital infection/inflammation.

References

1. Tournaye H, Krausz C, Oates RD. Concepts in diagnosis and therapy for male reproductive impairment. Lancet Diabetes Endocrinol. 2017;5(7):554-64. doi: 10.1016/S2213-8587(16)30043-2.
2. Ferlin A, Garolla A, Ghezzi M, Selice R, Palego P, Caretta N, et al. Sperm Count and Hypogonadism as Markers of General Male Health. Eur Urol Focus. 2019. doi: 10.1016/j.euf.2019.08.001.
3. Association MIBPPCotAU, Medicine PCotASfR. Report on optimal evaluation of the infertile male. Fertil Steril. 2006;86(5 Suppl 1):S202-9. doi: 10.1016/j.fertnstert.2006.08.029.
4. Salonia A, Bettocchi C, Carvalho J, Corona G, Jones T, Kadjoglu A, et al. EAU Guidelines on Sexual and Reproductive Health. European Association of Urology 2020; 2020.
5. (UK) NIfHaCE. Addendum to Clinical Guideline 156, Fertility problems: assessment and treatment. 2016.
6. Medicine PCotASfR. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril. 2015;103(3):e18-25. doi: 10.1016/j.fertnstert.2014.12.103.
7. Esteves SC, Chan P. A systematic review of recent clinical practice guidelines and best practice statements for the evaluation of the infertile male. Int Urol Nephrol. 2015;47(9):1441-56. doi: 10.1007/s11255-015-1059-0.
8. Colpi GM, Francavilla S, Haidl G, Link K, Behre HM, Goulis DG, et al. European Academy of Andrology guideline Management of oligo-astheno-teratozoospermia. Andrology. 2018;6(4):513-24. doi: 10.1111/andr.12502.
9. Ferlin A, Foresta C. Infertility: Practical Clinical Issues for Routine Investigation of the Male Partner. J Clin Med. 2020;9(6). doi: 10.3390/jcm9061644.
10. Schlegel PN, Sigman M, Collura B, De Jonge CJ, Eisenberg ML, Lamb DJ, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. Fertil Steril. 2021;115(1):54-61. doi: 10.1016/j.fertnstert.2020.11.015.
11. Schlegel PN, Sigman M, Collura B, De Jonge CJ, Eisenberg ML, Lamb DJ, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part II. Fertil Steril. 2021;115(1):62-9. doi: 10.1016/j.fertnstert.2020.11.016.
12. Swiglo BA, Murad MH, Schünemann HJ, Kunz R, Vigersky RA, Guyatt GH, et al. A case for clarity, consistency, and helpfulness: state-of-the-art clinical practice guidelines in endocrinology using the grading of recommendations, assessment, development, and evaluation system. J Clin Endocrinol Metab. 2008;93(3):666-73. doi: 10.1210/jc.2007-1907.
13. Nieschlag E. Clinical trials in male hormonal contraception. Contraception. 2010;82(5):457-70. doi: 10.1016/j.contraception.2010.03.020.
14. Zitzmann M, Aksglaede L, Corona G, Isidori AM, Juul A, T'Sjoen G, et al. European academy of andrology guidelines on Klinefelter Syndrome Endorsing Organization: European Society of Endocrinology. Andrology. 2021;9(1):145-67. doi: 10.1111/andr.12909.
15. Hanson BM, Eisenberg ML, Hotaling JM. Male infertility: a biomarker of individual and familial cancer risk. Fertil Steril. 2018;109(1):6-19. doi: 10.1016/j.fertnstert.2017.11.005.
16. Barratt CLR, Björndahl L, De Jonge CJ, Lamb DJ, Osorio Martini F, McLachlan R, et al. The diagnosis of male infertility: an analysis of the evidence to support the development of global WHO guidance-challenges and future research opportunities. Hum Reprod Update. 2017;23(6):660-80. doi: 10.1093/humupd/dmx021.
17. Tournaye H, Krausz C, Oates RD. Novel concepts in the aetiology of male reproductive impairment. Lancet Diabetes Endocrinol. 2017;5(7):544-53. doi: 10.1016/S2213-8587(16)30040-7.
18. Ford WC. Comments on the release of the 5th edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. Asian J Androl. 2010;12(1):59-63. doi: 10.1038/aja.2008.57.
19. Leushuis E, van der Steeg JW, Steures P, Repping S, Bossuyt PM, Blankenstein MA, et al. Reproducibility and reliability of repeated semen analyses in male partners of subfertile couples. Fertil Steril. 2010;94(7):2631-5. doi: 10.1016/j.fertnstert.2010.03.021.
20. Ombelet W, Dhont N, Thijssen A, Bosmans E, Kruger T. Semen quality and prediction of IUI success in male subfertility: a systematic review. Reprod Biomed Online. 2014;28(3):300-9. doi: 10.1016/j.rbmo.2013.10.023.

21. Paoli D, Pecora G, Pallotti F, Faja F, Pelloni M, Lenzi A, et al. Cytological and molecular aspects of the ageing sperm. Hum Reprod. 2019;34(2):218-27. doi: 10.1093/humrep/dey357.
22. Semet M, Paci M, Saïas-Magnan J, Metzler-Guillemain C, Boissier R, Lejeune H, et al. The impact of drugs on male fertility: a review. Andrology. 2017;5(4):640-63. doi: 10.1111/andr.12366.
23. Hanson BM, Aston KI, Jenkins TG, Carrell DT, Hotaling JM. The impact of ejaculatory abstinence on semen analysis parameters: a systematic review. J Assist Reprod Genet. 2018;35(2):213-20. doi: 10.1007/s10815-017-1086-0.
24. Verón GL, Tissera AD, Bello R, Beltramone F, Estofan G, Molina RI, et al. Impact of age, clinical conditions, and lifestyle on routine semen parameters and sperm kinematics. Fertil Steril. 2018;110(1):68-75.e4. doi: 10.1016/j.fertnstert.2018.03.016.
25. Gandini L, Menditto A, Chiodo F, Lenzi A. From the European Academy of Andrology. Italian pilot study for an external quality control scheme in semen analysis and antisperm antibiotics detection. Int J Androl. 2000;23(1):1-3. doi: 10.1046/j.1365-2605.2000.00208.x.
26. Mallidis C, Cooper TG, Hellenkemper B, Lablans M, Uckert F, Nieschlag E. Ten years' experience with an external quality control program for semen analysis. Fertil Steril. 2012;98(3):611-6.e4. doi: 10.1016/j.fertnstert.2012.05.006.
27. Filimberti E, Degl'Innocenti S, Borsotti M, Quercioli M, Piomboni P, Natali I, et al. High variability in results of semen analysis in andrology laboratories in Tuscany (Italy): the experience of an external quality control (EQC) programme. Andrology. 2013;1(3):401-7. doi: 10.1111/j.2047-2927.2012.00042.x.
28. Mehta A, Sigman M. Management of the dry ejaculate: a systematic review of aspermia and retrograde ejaculation. Fertil Steril. 2015;104(5):1074-81. doi: 10.1016/j.fertnstert.2015.09.024.
29. Cui D, Han G, Shang Y, Liu C, Xia L, Li L, et al. Antisperm antibodies in infertile men and their effect on semen parameters: a systematic review and meta-analysis. Clin Chim Acta. 2015;444:29-36. doi: 10.1016/j.cca.2015.01.033.
30. Barbonetti A, Castellini C, D'Andrea S, Cordeschi G, Santucci R, Francavilla S, et al. Prevalence of anti-sperm antibodies and relationship of degree of sperm auto-immunization to semen parameters and post-coital test outcome: a retrospective analysis of over 10 000 men. Hum Reprod. 2019;34(5):834-41. doi: 10.1093/humrep/dez030.
31. Chiu WW, Chamley LW. Clinical associations and mechanisms of action of antisperm antibodies. Fertil Steril. 2004;82(3):529-35. doi: 10.1016/j.fertnstert.2003.09.084.
32. Paoli D, Gilio B, Piroli E, Gallo M, Lombardo F, Dondero F, et al. Testicular tumors as a possible cause of antisperm autoimmune response. Fertil Steril. 2009;91(2):414-9. doi: 10.1016/j.fertnstert.2007.11.084.
33. Zini A, Fahmy N, Belzile E, Ciampi A, Al-Hathal N, Kotb A. Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis. Hum Reprod. 2011;26(6):1288-95. doi: 10.1093/humrep/der074.
34. Paoli D, Pallotti F, Lenzi A, Lombardo F. Fatherhood and Sperm DNA Damage in Testicular Cancer Patients. Front Endocrinol (Lausanne). 2018;9:506. doi: 10.3389/fendo.2018.00506.
35. Muratori M, Marchiani S, Tamburrino L, Baldi E. Sperm DNA Fragmentation: Mechanisms of Origin. Adv Exp Med Biol. 2019;1166:75-85. doi: 10.1007/978-3-030-21664-1_5.
36. Carlini T, Paoli D, Pelloni M, Faja F, Dal Lago A, Lombardo F, et al. Sperm DNA fragmentation in Italian couples with recurrent pregnancy loss. Reprod Biomed Online. 2017;34(1):58-65. doi: 10.1016/j.rbmo.2016.09.014.
37. Roessner C, Paasch U, Kratzsch J, Glander HJ, Grunewald S. Sperm apoptosis signalling in diabetic men. Reprod Biomed Online. 2012;25(3):292-9. doi: 10.1016/j.rbmo.2012.06.004.
38. Wang YJ, Zhang RQ, Lin YJ, Zhang RG, Zhang WL. Relationship between varicocele and sperm DNA damage and the effect of varicocele repair: a meta-analysis. Reprod Biomed Online. 2012;25(3):307-14. doi: 10.1016/j.rbmo.2012.05.002.
39. Moskovtsev SI, Mullen JB, Lecker I, Jarvi K, White J, Roberts M, et al. Frequency and severity of sperm DNA damage in patients with confirmed cases of male infertility of different aetiologies. Reprod Biomed Online. 2010;20(6):759-63. doi: 10.1016/j.rbmo.2010.03.002.
40. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod. 2012;27(10):2908-17. doi: 10.1093/humrep/des261.

41. Simon L, Zini A, Dyachenko A, Ciampi A, Carrell DT. A systematic review and meta-analysis to determine the effect of sperm DNA damage on. Asian J Androl. 2017;19(1):80-90. doi: 10.4103/1008-682X.182822.
42. Pacey A. Is sperm DNA fragmentation a useful test that identifies a treatable cause of male infertility? Best Pract Res Clin Obstet Gynaecol. 2018;53:11-9. doi: 10.1016/j.bpobgyn.2018.09.003.
43. Purvis K, Christiansen E. Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. Int J Androl. 1993;16(1):1-13. doi: 10.1111/j.1365-2605.1993.tb01146.x.
44. Bundrick W, Heron SP, Ray P, Schiff WM, Tennenberg AM, Wiesinger BA, et al. Levofloxacin versus ciprofloxacin in the treatment of chronic bacterial prostatitis: a randomized double-blind multicenter study. Urology. 2003;62(3):537-41. doi: 10.1016/s0090-4295(03)00565-x.
45. Giannarini G, Mogorovich A, Valent F, Morelli G, De Maria M, Manassero F, et al. Prulifloxacin versus levofloxacin in the treatment of chronic bacterial prostatitis: a prospective, randomized, double-blind trial. J Chemother. 2007;19(3):304-8. doi: 10.1179/joc.2007.19.3.304.
46. Safarinejad MR. Once-daily high-dose pindolol for paroxetine-refractory premature ejaculation: a double-blind, placebo-controlled and randomized study. J Clin Psychopharmacol. 2008;28(1):39-44. doi: 10.1097/jcp.0b013e31816073a5.
47. Busetto GM, Giovannone R, Ferro M, Tricarico S, Del Giudice F, Matei DV, et al. Chronic bacterial prostatitis: efficacy of short-lasting antibiotic therapy with prulifloxacin (Unidrox®) in association with saw palmetto extract, lactobacillus sporogens and arbutin (Lactorepens®). BMC Urol. 2014;14:53. doi: 10.1186/1471-2490-14-53.
48. Maeda S, Deguchi T, Ishiko H, Matsumoto T, Naito S, Kumon H, et al. Detection of Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum (biovar 1) and Ureaplasma urealyticum (biovar 2) in patients with non-gonococcal urethritis using polymerase chain reaction-microtiter plate hybridization. Int J Urol. 2004;11(9):750-4. doi: 10.1111/j.1442-2042.2004.00887.x.
49. Huang C, Zhu HL, Xu KR, Wang SY, Fan LQ, Zhu WB. Mycoplasma and ureaplasma infection and male infertility: a systematic review and meta-analysis. Andrology. 2015;3(5):809-16. doi: 10.1111/andr.12078.
50. La Vignera S, Condorelli RA, Vicari E, Salmeri M, Morgia G, Favilla V, et al. Microbiological investigation in male infertility: a practical overview. J Med Microbiol. 2014;63(Pt 1):1-14. doi: 10.1099/jmm.0.062968-0.
51. Magid DJ, Stiffman M, Anderson LA, Irwin K, Lyons EE. Adherence to CDC STD guideline recommendations for the treatment of Chlamydia trachomatis infection in two managed care organizations. Sex Transm Dis. 2003;30(1):30-2. doi: 10.1097/00007435-200301000-00007.
52. Prevention CfDCa. Recommendations for the laboratory-based detection of Chlamydia trachomatis and Neisseria gonorrhoeae--2014. MMWR Recomm Rep. 2014;63(RR-02):1-19.
53. Zakher B, Cantor AG, Pappas M, Daeges M, Nelson HD. Screening for gonorrhea and Chlamydia: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 2014;161(12):884-93. doi: 10.7326/M14-1022.
54. Lunny C, Taylor D, Hoang L, Wong T, Gilbert M, Lester R, et al. Self-Collected versus Clinician-Collected Sampling for Chlamydia and Gonorrhea Screening: A Systemic Review and Meta-Analysis. PLoS One. 2015;10(7):e0132776. doi: 10.1371/journal.pone.0132776.
55. Jensen JS, Björnelius E, Dohn B, Lidbrink P. Use of TaqMan 5' nuclease real-time PCR for quantitative detection of Mycoplasma genitalium DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. J Clin Microbiol. 2004;42(2):683-92. doi: 10.1128/JCM.42.2.683-692.2004.
56. Gaydos CA, Manhart LE, Taylor SN, Lillis RA, Hook EW, Klausner JD, et al. Molecular Testing for Mycoplasma genitalium in the United States: Results from the AMES Prospective Multicenter Clinical Study. J Clin Microbiol. 2019;57(11). doi: 10.1128/JCM.01125-19.
57. Hamasuna R, Osada Y, Jensen JS. Isolation of Mycoplasma genitalium from first-void urine specimens by coculture with Vero cells. J Clin Microbiol. 2007;45(3):847-50. doi: 10.1128/JCM.02056-06.
58. Jordan SJ, Schwebke JR, Aaron KJ, Van Der Pol B, Hook EW. Meatal Swabs Contain Less Cellular Material and Are Associated with a Decrease in Gram Stain Smear Quality Compared to Urethral Swabs in Men. J Clin Microbiol. 2017;55(7):2249-54. doi: 10.1128/JCM.00423-17.
59. Lyu Z, Feng X, Li N, Zhao W, Wei L, Chen Y, et al. Human papillomavirus in semen and the risk for male infertility: a systematic review and meta-analysis. BMC Infect Dis. 2017;17(1):714. doi: 10.1186/s12879-017-2812-z.

60. Weinberg M, Sar-Shalom Nahshon C, Feferkorn I, Bornstein J. Evaluation of human papilloma virus in semen as a risk factor for low sperm quality and poor in vitro fertilization outcomes: a systematic review and meta-analysis. Fertil Steril. 2020;113(5):955-69.e4. doi: 10.1016/j.fertnstert.2020.01.010.
61. Cao X, Wei R, Zhang X, Zhou J, Lou J, Cui Y. Impact of human papillomavirus infection in semen on sperm progressive motility in infertile men: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2020;18(1):38. doi: 10.1186/s12958-020-00604-0.
62. Moreno-Sepulveda J, Rajmil O. Seminal human papillomavirus infection and reproduction: a systematic review and meta-analysis. Andrology. 2021;9(2):478-502. doi: 10.1111/andr.12948.
63. Dize L, Agreda P, Quinn N, Barnes MR, Hsieh YH, Gaydos CA. Comparison of self-obtained penile-meatal swabs to urine for the detection of C. trachomatis, N. gonorrhoeae and T. vaginalis. Sex Transm Infect. 2013;89(4):305-7. doi: 10.1136/sextrans-2012-050686.
64. Chernesky MA, Jang D, Portillo E, Smieja M, Gilchrist J, Ewert R, et al. Self-collected swabs of the urinary meatus diagnose more Chlamydia trachomatis and Neisseria gonorrhoeae infections than first catch urine from men. Sex Transm Infect. 2013;89(2):102-4. doi: 10.1136/sextrans-2012-050573.
65. Coorevits L, Traen A, Bingé L, Van Dorpe J, Praet M, Boelens J, et al. Identifying a consensus sample type to test for Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Trichomonas vaginalis and human papillomavirus. Clin Microbiol Infect. 2018;24(12):1328-32. doi: 10.1016/j.cmi.2018.03.013.
66. Plant TM, Marshall GR. The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. Endocr Rev. 2001;22(6):764-86. doi: 10.1210/edrv.22.6.0446.
67. Taieb J, Mathian B, Millot F, Patricot MC, Mathieu E, Queyrel N, et al. Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children. Clin Chem. 2003;49(8):1381-95. doi: 10.1373/49.8.1381.
68. Shea JL, Wong PY, Chen Y. Free testosterone: clinical utility and important analytical aspects of measurement. Adv Clin Chem. 2014;63:59-84. doi: 10.1016/b978-0-12-800094-6.00002-9.
69. Goldman AL, Bhasin S, Wu FCW, Krishna M, Matsumoto AM, Jasuja R. A Reappraisal of Testosterone's Binding in Circulation: Physiological and Clinical Implications. Endocr Rev. 2017;38(4):302-24. doi: 10.1210/er.2017-00025.
70. Keevil BG, Adaway J. Assessment of free testosterone concentration. J Steroid Biochem Mol Biol. 2019;190:207-11. doi: 10.1016/j.jsbmb.2019.04.008.
71. Bergmann M, Behre HM, Nieschlag E. Serum FSH and testicular morphology in male infertility. Clin Endocrinol (Oxf). 1994;40(1):133-6. doi: 10.1111/j.1365-2265.1994.tb02455.x.
72. von Eckardstein S, Simoni M, Bergmann M, Weinbauer GF, Gassner P, Schepers AG, et al. Serum inhibin B in combination with serum follicle-stimulating hormone (FSH) is a more sensitive marker than serum FSH alone for impaired spermatogenesis in men, but cannot predict the presence of sperm in testicular tissue samples. J Clin Endocrinol Metab. 1999;84(7):2496-501. doi: 10.1210/jcem.84.7.5855.
73. Kumanov P, Nandipati K, Tomova A, Agarwal A. Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. Fertil Steril. 2006;86(2):332-8. doi: 10.1016/j.fertnstert.2006.01.022.
74. Barbotin AL, Ballot C, Sigala J, Ramdane N, Duhamel A, Marcelli F, et al. The serum inhibin B concentration and reference ranges in normozoospermia. Eur J Endocrinol. 2015;172(6):669-76. doi: 10.1530/EJE-14-0932.
75. Andersson AM, Jørgensen N, Frydelund-Larsen L, Rajpert-De Meyts E, Skakkebaek NE. Impaired Leydig cell function in infertile men: a study of 357 idiopathic infertile men and 318 proven fertile controls. J Clin Endocrinol Metab. 2004;89(7):3161-7. doi: 10.1210/jc.2003-031786.
76. Ferlin A, Vinanzi C, Selice R, Garolla A, Frigo AC, Foresta C. Toward a pharmacogenetic approach to male infertility: polymorphism of follicle-stimulating hormone beta-subunit promoter. Fertil Steril. 2011;96(6):1344-9.e2. doi: 10.1016/j.fertnstert.2011.09.034.
77. Lotti F, Maggi M. Ultrasound of the male genital tract in relation to male reproductive health. Hum Reprod Update. 2015;21(1):56-83. doi: 10.1093/humupd/dmu042.
78. Dogra VS, Gottlieb RH, Oka M, Rubens DJ. Sonography of the scrotum. Radiology. 2003;227(1):18-36. doi: 10.1148/radiol.2271001744.
79. Goede J, van der Voort-Doedens LM, Sijstermans K, Hack WW. The volume of retractile testes. J Urol. 2011;186(5):2050-4. doi: 10.1016/j.juro.2011.07.020.

80. Isidori AM, Giannetta E, Lenzi A. Male hypogonadism. Pituitary. 2008;11(2):171-80. doi: 10.1007/s11102-008-0111-9.
81. Jørgensen N, Joensen UN, Toppari J, Punab M, Erenpreiss J, Zilaitiene B, et al. Compensated reduction in Leydig cell function is associated with lower semen quality variables: a study of 8182 European young men. Hum Reprod. 2016;31(5):947-57. doi: 10.1093/humrep/dew021.
82. Ventimiglia E, Capogrosso P, Boeri L, Serino A, Colicchia M, Ippolito S, et al. Infertility as a proxy of general male health: results of a cross-sectional survey. Fertil Steril. 2015;104(1):48-55. doi: 10.1016/j.fertnstert.2015.04.020.
83. Ventimiglia E, Capogrosso P, Colicchia M, Boeri L, Serino A, Castagna G, et al. Metabolic syndrome in white European men presenting for primary couple's infertility: investigation of the clinical and reproductive burden. Andrology. 2016;4(5):944-51. doi: 10.1111/andr.12232.
84. Pozza C, Kanakis G, Carlomagno F, Lemma A, Pofi R, Tenuta M, et al. Testicular ultrasound score: A new proposal for a scoring system to predict testicular function. Andrology. 2020;8(5):1051-63. doi: 10.1111/andr.12822.
85. Isidori AM, Pozza C, Gianfrilli D, Giannetta E, Lemma A, Pofi R, et al. Differential diagnosis of nonpalpable testicular lesions: qualitative and quantitative contrast-enhanced US of benign and malignant testicular tumors. Radiology. 2014;273(2):606-18. doi: 10.1148/radiol.14132718.
86. Pozza C, Gianfrilli D, Fattorini G, Giannetta E, Barbagallo F, Nicolai E, et al. Diagnostic value of qualitative and strain ratio elastography in the differential diagnosis of non-palpable testicular lesions. Andrology. 2016;4(6):1193-203. doi: 10.1111/andr.12260.
87. Lotti F, Corona G, Castellini G, Maseroli E, Fino MG, Cozzolino M, et al. Semen quality impairment is associated with sexual dysfunction according to its severity. Hum Reprod. 2016;31(12):2668-80. doi: 10.1093/humrep/dew246.
88. Lotti F, Frizza F, Balercia G, Barbonetti A, Behre HM, Calogero AE, et al. The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: Scrotal ultrasound reference ranges and associations with clinical, seminal, and biochemical characteristics. Andrology. 2021;9(2):559-76. doi: 10.1111/andr.12951.
89. Bieniek JM, Juvet T, Margolis M, Grober ED, Lo KC, Jarvi KA. Prevalence and Management of Incidental Small Testicular Masses Discovered on Ultrasonographic Evaluation of Male Infertility. J Urol. 2018;199(2):481-6. doi: 10.1016/j.juro.2017.08.004.
90. Barbonetti A, Martorella A, Minaldi E, D'Andrea S, Bardhi D, Castellini C, et al. Testicular Cancer in Infertile Men With and Without Testicular Microlithiasis: A Systematic Review and Meta-Analysis of Case-Control Studies. Front Endocrinol (Lausanne). 2019;10:164. doi: 10.3389/fendo.2019.00164.
91. Singh R, Hamada AJ, Bukavina L, Agarwal A. Physical deformities relevant to male infertility. Nat Rev Urol. 2012;9(3):156-74. doi: 10.1038/nrurol.2012.11.
92. Di Trapani D, Pavone C, Serretta V, Cavallo N, Costa G, Pavone-Macaluso M. Chronic prostatitis and prostatodynia: ultrasonographic alterations of the prostate, bladder neck, seminal vesicles and periprostatic venous plexus. Eur Urol. 1988;15(3-4):230-4. doi: 10.1159/000473440.
93. Doble A, Carter SS. Ultrasonographic findings in prostatitis. Urol Clin North Am. 1989;16(4):763-72.
94. Lotti F, Corona G, Mancini M, Filimberti E, Degli Innocenti S, Colpi GM, et al. Ultrasonographic and clinical correlates of seminal plasma interleukin-8 levels in patients attending an andrology clinic for infertility. Int J Androl. 2011;34(6 Pt 1):600-13. doi: 10.1111/j.1365-2605.2010.01121.x.
95. Lotti F, Maggi M. Interleukin 8 and the male genital tract. J Reprod Immunol. 2013;100(1):54-65. doi: 10.1016/j.jri.2013.02.004.
96. La Vignera S, Calogero AE, Arancio A, Castiglione R, De Grande G, Vicari E. Transrectal ultrasonography in infertile patients with persistently elevated bacteriospermia. Asian J Androl. 2008;10(5):731-40. doi: 10.1111/j.1745-7262.2008.00425.x.
97. Vicari E. Seminal leukocyte concentration and related specific reactive oxygen species production in patients with male accessory gland infections. Hum Reprod. 1999;14(8):2025-30. doi: 10.1093/humrep/14.8.2025.
98. La Vignera S, Condorelli RA, Di Mauro M, D'Agata R, Vicari E, Calogero AE. Seminal vesicles and diabetic neuropathy: ultrasound evaluation. J Androl. 2011;32(5):478-83. doi: 10.2164/jandrol.110.011676.

99. La Vignera S, Vicari E, Condorelli R, D'Agata R, Calogero AE. Hypertrophic-congestive and fibro-sclerotic ultrasound variants of male accessory gland infection have different sperm output. J Endocrinol Invest. 2011;34(10):e330-5. doi: 10.1007/BF03346729.
100. La Vignera S, Vicari E, Condorelli RA, D'Agata R, Calogero AE. Male accessory gland infection and sperm parameters (review). Int J Androl. 2011;34(5 Pt 2):e330-47. doi: 10.1111/j.1365-2605.2011.01200.x.
101. La Vignera S, Condorelli RA, Cannarella R, Giacone F, Mongioi' L, Scalia G, et al. High rate of detection of ultrasound signs of prostatitis in patients with HPV-DNA persistence on semen: role of ultrasound in HPV-related male accessory gland infection. J Endocrinol Invest. 2019;42(12):1459-65. doi: 10.1007/s40618-019-01069-8.
102. Oates R. Evaluation of the azoospermic male. Asian J Androl. 2012;14(1):82-7. doi: 10.1038/aja.2011.60.
103. Bettella A, Ferlin A, Menegazzo M, Ferigo M, Tavolini IM, Bassi PF, et al. Testicular fine needle aspiration as a diagnostic tool in non-obstructive azoospermia. Asian J Androl. 2005;7(3):289-94. doi: 10.1111/j.1745-7262.2005.00043.x.
104. Barbonetti A, Calogero AE, Balercia G, Garolla A, Krausz C, La Vignera S, et al. The use of follicle stimulating hormone (FSH) for the treatment of the infertile man: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS). J Endocrinol Invest. 2018;41(9):1107-22. doi: 10.1007/s40618-018-0843-y.
105. Foresta C, Bettella A, Merico M, Garolla A, Plebani M, Ferlin A, et al. FSH in the treatment of oligozoospermia. Mol Cell Endocrinol. 2000;161(1-2):89-97. doi: 10.1016/s0303-7207(99)00228-2.
106. Foresta C, Selice R, Ferlin A, Garolla A. Recombinant FSH in the treatment of oligozoospermia. Expert Opin Biol Ther. 2009;9(5):659-66. doi: 10.1517/14712590902932673.
107. Esteves SC. Novel concepts in male factor infertility: clinical and laboratory perspectives. J Assist Reprod Genet. 2016;33(10):1319-35. doi: 10.1007/s10815-016-0763-8.
108. Krausz C, Riera-Escamilla A. Genetics of male infertility. Nat Rev Urol. 2018;15(6):369-84. doi: 10.1038/s41585-018-0003-3.
109. Krausz C, Hoefsloot L, Simoni M, Tüttelmann F, Andrology EAo, Network EMGQ. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. Andrology. 2014;2(1):5-19. doi: 10.1111/j.2047-2927.2013.00173.x.
110. Bieth E, Hamdi SM, Mieusset R. Genetics of the congenital absence of the vas deferens. Hum Genet. 2021;140(1):59-76. doi: 10.1007/s00439-020-02122-w.
111. Ferlin A, Dipresa S, Delbarba A, Maffezzoni F, Porcelli T, Cappelli C, et al. Contemporary genetics-based diagnostics of male infertility. Expert Rev Mol Diagn. 2019;19(7):623-33. doi: 10.1080/14737159.2019.1633917.
112. Krausz C, Riera-Escamilla A. Monogenic Forms of Male Infertility. Exp Suppl. 2019;111:341-66. doi: 10.1007/978-3-030-25905-1_16.
113. Krausz C, Riera-Escamilla A, Moreno-Mendoza D, Holleman K, Cioppi F, Algaba F, et al. Genetic dissection of spermatogenic arrest through exome analysis: clinical implications for the management of azoospermic men. Genet Med. 2020;22(12):1956-66. doi: 10.1038/s41436-020-0907-1.
114. Jensen TK, Jacobsen R, Christensen K, Nielsen NC, Bostofte E. Good semen quality and life expectancy: a cohort study of 43,277 men. Am J Epidemiol. 2009;170(5):559-65. doi: 10.1093/aje/kwp168.
115. Salonia A, Matloob R, Gallina A, Abdollah F, Saccà A, Briganti A, et al. Are infertile men less healthy than fertile men? Results of a prospective case-control survey. Eur Urol. 2009;56(6):1025-31. doi: 10.1016/j.eururo.2009.03.001.
116. Eisenberg ML, Li S, Behr B, Cullen MR, Galusha D, Lamb DJ, et al. Semen quality, infertility and mortality in the USA. Hum Reprod. 2014;29(7):1567-74. doi: 10.1093/humrep/deu106.
117. Eisenberg ML, Li S, Cullen MR, Baker LC. Increased risk of incident chronic medical conditions in infertile men: analysis of United States claims data. Fertil Steril. 2016;105(3):629-36. doi: 10.1016/j.fertnstert.2015.11.011.
118. Latif T, Kold Jensen T, Mehlsen J, Holmboe SA, Brinth L, Pors K, et al. Semen Quality as a Predictor of Subsequent Morbidity: A Danish Cohort Study of 4,712 Men With Long-Term Follow-up. Am J Epidemiol. 2017;186(8):910-7. doi: 10.1093/aje/kwx067.

119. Bobjer J, Bogefors K, Isaksson S, Leijonhufvud I, Åkesson K, Giwercman YL, et al. High prevalence of hypogonadism and associated impaired metabolic and bone mineral status in subfertile men. Clin Endocrinol (Oxf). 2016;85(2):189-95. doi: 10.1111/cen.13038.
120. Del Giudice F, Kasman AM, Ferro M, Sciarra A, De Berardinis E, Belladelli F, et al. Clinical correlation among male infertility and overall male health: A systematic review of the literature. Investig Clin Urol. 2020;61(4):355-71. doi: 10.4111/icu.2020.61.4.355.
121. Ricci E, Al Beitawi S, Cipriani S, Candiani M, Chiaffarino F, Viganò P, et al. Semen quality and alcohol intake: a systematic review and meta-analysis. Reprod Biomed Online. 2017;34(1):38-47. doi: 10.1016/j.rbmo.2016.09.012.
122. La Vignera S, Condorelli RA, Balercia G, Vicari E, Calogero AE. Does alcohol have any effect on male reproductive function? A review of literature. Asian J Androl. 2013;15(2):221-5. doi: 10.1038/aja.2012.118.
123. Rastrelli G, Lotti F, Reisman Y, Sforza A, Maggi M, Corona G. Metabolically healthy and unhealthy obesity in erectile dysfunction and male infertility. Expert Rev Endocrinol Metab. 2019;14(5):321-34. doi: 10.1080/17446651.2019.1657827.
124. Jensen TK, Andersson AM, Jørgensen N, Andersen AG, Carlsen E, Petersen JH, et al. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. Fertil Steril. 2004;82(4):863-70. doi: 10.1016/j.fertnstert.2004.03.056.
125. Chavarro JE, Toth TL, Wright DL, Meeker JD, Hauser R. Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. Fertil Steril. 2010;93(7):2222-31. doi: 10.1016/j.fertnstert.2009.01.100.
126. Corona G, Mannucci E, Schulman C, Petrone L, Mansani R, Cilotti A, et al. Psychobiologic correlates of the metabolic syndrome and associated sexual dysfunction. Eur Urol. 2006;50(3):595-604; discussion doi: 10.1016/j.eururo.2006.02.053.
127. Håkonsen LB, Thulstrup AM, Aggerholm AS, Olsen J, Bonde JP, Andersen CY, et al. Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. Reprod Health. 2011;8:24. doi: 10.1186/1742-4755-8-24.
128. Redman LM. Physical activity and its effects on reproduction. Reprod Biomed Online. 2006;12(5):579-86. doi: 10.1016/s1472-6483(10)61183-2.
129. Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette Smoking and Semen Quality: A New Meta-analysis Examining the Effect of the 2010 World Health Organization Laboratory Methods for the Examination of Human Semen. Eur Urol. 2016;70(4):635-45. doi: 10.1016/j.eururo.2016.04.010.
130. Calogero AE, Bagdy G, D'Agata R. Mechanisms of stress on reproduction. Evidence for a complex intra-hypothalamic circuit. Ann N Y Acad Sci. 1998;851:364-70. doi: 10.1111/j.1749-6632.1998.tb09010.x.
131. Durairajanayagam D, Agarwal A, Ong C. Causes, effects and molecular mechanisms of testicular heat stress. Reprod Biomed Online. 2015;30(1):14-27. doi: 10.1016/j.rbmo.2014.09.018.
132. Aitken RJ, Baker MA. Oxidative stress, sperm survival and fertility control. Mol Cell Endocrinol. 2006;250(1-2):66-9. doi: 10.1016/j.mce.2005.12.026.
133. Li Y, Lin H, Cao J. Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. Fertil Steril. 2011;95(1):116-23. doi: 10.1016/j.fertnstert.2010.06.031.
134. Gollenberg AL, Liu F, Brazil C, Drobnis EZ, Guzick D, Overstreet JW, et al. Semen quality in fertile men in relation to psychosocial stress. Fertil Steril. 2010;93(4):1104-11. doi: 10.1016/j.fertnstert.2008.12.018.
135. Holmes D. Endocrine disruptors cause toxic fallout. Lancet Diabetes Endocrinol. 2013;1(2):93-4. doi: 10.1016/S2213-8587(13)70057-3.
136. Mínguez-Alarcón L, Chavarro JE, Gaskins AJ. Caffeine, alcohol, smoking, and reproductive outcomes among couples undergoing assisted reproductive technology treatments. Fertil Steril. 2018;110(4):587-92. doi: 10.1016/j.fertnstert.2018.05.026.
137. Smits RM, Mackenzie-Proctor R, Yazdani A, Stankiewicz MT, Jordan V, Showell MG. Antioxidants for male subfertility. Cochrane Database Syst Rev. 2019;3:CD007411. doi: 10.1002/14651858.CD007411.pub4.
138. Buhling K, Schumacher A, Eulenburg CZ, Laakmann E. Influence of oral vitamin and mineral supplementation on male infertility: a meta-analysis and systematic review. Reprod Biomed Online. 2019;39(2):269-79. doi: 10.1016/j.rbmo.2019.03.099.

139. Salas-Huetos A, Rosique-Esteban N, Becerra-Tomás N, Vizmanos B, Bulló M, Salas-Salvadó J. The Effect of Nutrients and Dietary Supplements on Sperm Quality Parameters: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. Adv Nutr. 2018;9(6):833-48. doi: 10.1093/advances/nmy057.
140. Hosseini B, Nourmohamadi M, Hajipour S, Taghizadeh M, Asemi Z, Keshavarz SA, et al. The Effect of Omega-3 Fatty Acids, EPA, and/or DHA on Male Infertility: A Systematic Review and Meta-analysis. J Diet Suppl. 2019;16(2):245-56. doi: 10.1080/19390211.2018.1431753.
141. Lafuente R, González-Comadrán M, Solà I, López G, Brassesco M, Carreras R, et al. Coenzyme Q10 and male infertility: a meta-analysis. J Assist Reprod Genet. 2013;30(9):1147-56. doi: 10.1007/s10815-013-0047-5.
142. Omar MI, Pal RP, Kelly BD, Bruins HM, Yuan Y, Diemer T, et al. Benefits of Empiric Nutritional and Medical Therapy for Semen Parameters and Pregnancy and Live Birth Rates in Couples with Idiopathic Infertility: A Systematic Review and Meta-analysis. Eur Urol. 2019;75(4):615-25. doi: 10.1016/j.eururo.2018.12.022.
143. Irani M, Amirian M, Sadeghi R, Lez JL, Latifnejad Roudsari R. The Effect of Folate and Folate Plus Zinc Supplementation on Endocrine Parameters and Sperm Characteristics in Sub-Fertile Men: A Systematic Review and Meta-Analysis. Urol J. 2017;14(5):4069-78.
144. Steiner AZ, Hansen KR, Barnhart KT, Cedars MI, Legro RS, Diamond MP, et al. The effect of antioxidants on male factor infertility: the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial. Fertil Steril. 2020;113(3):552-60.e3. doi: 10.1016/j.fertnstert.2019.11.008.
145. Schisterman EF, Sjaarda LA, Clemons T, Carrell DT, Perkins NJ, Johnstone E, et al. Effect of Folic Acid and Zinc Supplementation in Men on Semen Quality and Live Birth Among Couples Undergoing Infertility Treatment: A Randomized Clinical Trial. JAMA. 2020;323(1):35-48. doi: 10.1001/jama.2019.18714.
146. Chen Q, Zhao JY, Xue X, Zhu GX. The association between sperm DNA fragmentation and reproductive outcomes following intrauterine insemination, a meta analysis. Reprod Toxicol. 2019;86:50-5. doi: 10.1016/j.reprotox.2019.03.004.
147. Deng C, Li T, Xie Y, Guo Y, Yang QY, Liang X, et al. Sperm DNA fragmentation index influences assisted reproductive technology outcome: A systematic review and meta-analysis combined with a retrospective cohort study. Andrologia. 2019;51(6):e13263. doi: 10.1111/and.13263.
148. McQueen DB, Zhang J, Robins JC. Sperm DNA fragmentation and recurrent pregnancy loss: a systematic review and meta-analysis. Fertil Steril. 2019;112(1):54-60.e3. doi: 10.1016/j.fertnstert.2019.03.003.
149. Calogero AE, Aversa A, La Vignera S, Corona G, Ferlin A. The use of nutraceuticals in male sexual and reproductive disturbances: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS). J Endocrinol Invest. 2017;40(12):1389-97. doi: 10.1007/s40618-017-0699-6.
150. Calogero AE, Duca Y, Condorelli RA, La Vignera S. Male accessory gland inflammation, infertility, and sexual dysfunctions: a practical approach to diagnosis and therapy. Andrology. 2017;5(6):1064-72. doi: 10.1111/andr.12427.
151. Gallegos G, Ramos B, Santiso R, Goyanes V, Gosálvez J, Fernández JL. Sperm DNA fragmentation in infertile men with genitourinary infection by Chlamydia trachomatis and Mycoplasma. Fertil Steril. 2008;90(2):328-34. doi: 10.1016/j.fertnstert.2007.06.035.
152. Cai T, Wagenlehner FM, Mazzoli S, Meacci F, Mondaini N, Nesi G, et al. Semen quality in patients with Chlamydia trachomatis genital infection treated concurrently with prulifloxacin and a phytotherapeutic agent. J Androl. 2012;33(4):615-23. doi: 10.2164/jandrol.111.013961.
153. Ahmadi MH, Mirsalehian A, Sadighi Gilani MA, Bahador A, Talebi M. Asymptomatic Infection With Mycoplasma hominis Negatively Affects Semen Parameters and Leads to Male Infertility as Confirmed by Improved Semen Parameters After Antibiotic Treatment. Urology. 2017;100:97-102. doi: 10.1016/j.urology.2016.11.018.
154. Mićić S. Kallikrein and antibiotics in the treatment of infertile men with genital tract infections. Andrologia. 1988;20(1):55-9.
155. Comhaire FH, Rowe PJ, Farley TM. The effect of doxycycline in infertile couples with male accessory gland infection: a double blind prospective study. Int J Androl. 1986;9(2):91-8. doi: 10.1111/j.1365-2605.1986.tb00871.x.

156. Vicari E. Effectiveness and limits of antimicrobial treatment on seminal leukocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. Hum Reprod. 2000;15(12):2536-44. doi: 10.1093/humrep/15.12.2536.
157. Merino G, Carranza-Lira S. Infection and male infertility: effect of different antibiotic regimens on semen quality. Arch Androl. 1995;35(3):209-12. doi: 10.3109/01485019508987872.
158. Harrison RF, de Louvois J, Blades M, Hurley R. Doxycycline treatment and human infertility. Lancet. 1975;1(7907):605-7. doi: 10.1016/s0140-6736(75)91885-1.
159. Vicari E, La Vignera S, Calogero AE. Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculoepididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds. Fertil Steril. 2002;78(6):1203-8. doi: 10.1016/s0015-0282(02)04350-9.
160. Brunner RJ, Demeter JH, Sindhwani P. Review of Guidelines for the Evaluation and Treatment of Leukocytospermia in Male Infertility. World J Mens Health. 2019;37(2):128-37. doi: 10.5534/wjmh.180078.
161. Santi D, Crépieux P, Reiter E, Spaggiari G, Brigante G, Casarini L, et al. Follicle-stimulating Hormone (FSH) Action on Spermatogenesis: A Focus on Physiological and Therapeutic Roles. J Clin Med. 2020;9(4). doi: 10.3390/jcm9041014.
162. Simoni M, Brigante G, Rochira V, Santi D, Casarini L. Prospects for FSH Treatment of Male Infertility. J Clin Endocrinol Metab. 2020;105(7). doi: 10.1210/clinem/dgaa243.
163. Santi D, Granata AR, Simoni M. FSH treatment of male idiopathic infertility improves pregnancy rate: a meta-analysis. Endocr Connect. 2015;4(3):R46-58. doi: 10.1530/EC-15-0050.
164. Attia AM, Abou-Setta AM, Al-Inany HG. Gonadotrophins for idiopathic male factor subfertility. Cochrane Database Syst Rev. 2013(8):CD005071. doi: 10.1002/14651858.CD005071.pub4.
165. Cannarella R, La Vignera S, Condorelli RA, Mongioì LM, Calogero AE. FSH dosage effect on conventional sperm parameters: a meta-analysis of randomized controlled studies. Asian J Androl. 2020;22(3):309-16. doi: 10.4103/aja.aja_42_19.
166. De Rocco Ponce M, Foresta C, Rago R, Dal Lago A, Balercia G, Calogero AE, et al. Use of Biosimilar Follicle-Stimulating Hormone in Asthenozoospermic Infertile Patients: A Multicentric Study. J Clin Med. 2020;9(5). doi: 10.3390/jcm9051478.
167. Simoni M, Santi D, Negri L, Hoffmann I, Muratori M, Baldi E, et al. Treatment with human, recombinant FSH improves sperm DNA fragmentation in idiopathic infertile men depending on the FSH receptor polymorphism p.N680S: a pharmacogenetic study. Hum Reprod. 2016;31(9):1960-9. doi: 10.1093/humrep/dew167.
168. Chua ME, Escusa KG, Luna S, Tapia LC, Dofitas B, Morales M. Revisiting oestrogen antagonists (clomiphene or tamoxifen) as medical empiric therapy for idiopathic male infertility: a meta-analysis. Andrology. 2013;1(5):749-57. doi: 10.1111/j.2047-2927.2013.00107.x.
169. Cannarella R, Condorelli RA, Mongioì LM, Barbagallo F, Calogero AE, La Vignera S. Effects of the selective estrogen receptor modulators for the treatment of male infertility: a systematic review and meta-analysis. Expert Opin Pharmacother. 2019;20(12):1517-25. doi: 10.1080/14656566.2019.1615057.
170. Del Giudice F, Busetto GM, De Berardinis E, Sperduti I, Ferro M, Maggi M, et al. A systematic review and meta-analysis of clinical trials implementing aromatase inhibitors to treat male infertility. Asian J Androl. 2020;22(4):360-7. doi: 10.4103/aja.aja_101_19.
171. Alsaikhan B, Alrabeeah K, Delouya G, Zini A. Epidemiology of varicocele. Asian J Androl. 2016;18(2):179-81. doi: 10.4103/1008-682X.172640.
172. Freeman S, Bertolotto M, Richenberg J, Belfield J, Dogra V, Huang DY, et al. Ultrasound evaluation of varicoceles: guidelines and recommendations of the European Society of Urogenital Radiology Scrotal and Penile Imaging Working Group (ESUR-SPIWG) for detection, classification, and grading. Eur Radiol. 2020;30(1):11-25. doi: 10.1007/s00330-019-06280-y.
173. Bertolotto M, Freeman S, Richenberg J, Belfield J, Dogra V, Huang DY, et al. Ultrasound evaluation of varicoceles: systematic literature review and rationale of the ESUR-SPIWG Guidelines and Recommendations. J Ultrasound. 2020;23(4):487-507. doi: 10.1007/s40477-020-00509-z.
174. Redmon JB, Carey P, Pryor JL. Varicocele--the most common cause of male factor infertility? Hum Reprod Update. 2002;8(1):53-8. doi: 10.1093/humupd/8.1.53.

175. Fallara G, Cazzaniga W, Boeri L, Capogrosso P, Candela L, Pozzi E, et al. Male factor infertility trends throughout the last 10 years: Report from a tertiary-referral academic andrology centre. Andrology. 2021;9(2):610-7. doi: 10.1111/andr.12947.
176. Esteves SC, Santi D, Simoni M. An update on clinical and surgical interventions to reduce sperm DNA fragmentation in infertile men. Andrology. 2020;8(1):53-81. doi: 10.1111/andr.12724.
177. Persad E, O'Loughlin CA, Kaur S, Wagner G, Matyas N, Hassler-Di Fratta MR, et al. Surgical or radiological treatment for varicoceles in subfertile men. Cochrane Database Syst Rev. 2021;4:CD000479. doi: 10.1002/14651858.CD000479.pub6.
178. La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Effects of varicocelectomy on sperm DNA fragmentation, mitochondrial function, chromatin condensation, and apoptosis. J Androl. 2012;33(3):389-96. doi: 10.2164/jandrol.111.013433.
179. Sansone A, Fegatelli DA, Pozza C, Fattorini G, Lauretta R, Minnetti M, et al. Effects of percutaneous varicocele repair on testicular volume: results from a 12-month follow-up. Asian J Androl. 2019;21(4):408-12. doi: 10.4103/aja.aja_102_18.
180. Kohn TP, Ohlander SJ, Jacob JS, Griffin TM, Lipshultz LI, Pastuszak AW. The Effect of Subclinical Varicocele on Pregnancy Rates and Semen Parameters: a Systematic Review and Meta-Analysis. Curr Urol Rep. 2018;19(7):53. doi: 10.1007/s11934-018-0798-8.
181. Kirby EW, Wiener LE, Rajanahally S, Crowell K, Coward RM. Undergoing varicocele repair before assisted reproduction improves pregnancy rate and live birth rate in azoospermic and oligospermic men with a varicocele: a systematic review and meta-analysis. Fertil Steril. 2016;106(6):1338-43. doi: 10.1016/j.fertnstert.2016.07.1093.
182. Corona G, Minhas S, Giwercman A, Bettocchi C, Dinkelman-Smit M, Dohle G, et al. Sperm recovery and ICSI outcomes in men with non-obstructive azoospermia: a systematic review and meta-analysis. Hum Reprod Update. 2019;25(6):733-57. doi: 10.1093/humupd/dmz028.
183. Bernie AM, Mata DA, Ramasamy R, Schlegel PN. Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azoospermia: a systematic review and meta-analysis. Fertil Steril. 2015;104(5):1099-103.e1-3. doi: 10.1016/j.fertnstert.2015.07.1136.
184. Deruyver Y, Vanderschueren D, Van der Aa F. Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: a systematic review. Andrology. 2014;2(1):20-4. doi: 10.1111/j.2047-2927.2013.00148.x.
185. Esteves SC, Lee W, Benjamin DJ, Seol B, Verza S, Agarwal A. Reproductive potential of men with obstructive azoospermia undergoing percutaneous sperm retrieval and intracytoplasmic sperm injection according to the cause of obstruction. J Urol. 2013;189(1):232-7. doi: 10.1016/j.juro.2012.08.084.
186. Desai N, AbdelHafez F, Sabanegh E, Goldfarb J. Paternal effect on genomic activation, clinical pregnancy and live birth rate after ICSI with cryopreserved epididymal versus testicular spermatozoa. Reprod Biol Endocrinol. 2009;7:142. doi: 10.1186/1477-7827-7-142.
187. Nicopoullos JD, Gilling-Smith C, Almeida PA, Norman-Taylor J, Grace I, Ramsay JW. Use of surgical sperm retrieval in azoospermic men: a meta-analysis. Fertil Steril. 2004;82(3):691-701. doi: 10.1016/j.fertnstert.2004.02.116.
188. Schlegel PN, Palermo GD, Alikani M, Adler A, Reing AM, Cohen J, et al. Micropuncture retrieval of epididymal sperm with in vitro fertilization: importance of in vitro micromanipulation techniques. Urology. 1995;46(2):238-41. doi: 10.1016/s0090-4295(99)80199-x.
189. Van Peperstraten A, Proctor ML, Johnson NP, Philipson G. Techniques for surgical retrieval of sperm prior to intra-cytoplasmic sperm injection (ICSI) for azoospermia. Cochrane Database Syst Rev. 2008(2):CD002807. doi: 10.1002/14651858.CD002807.pub3.
190. Bachtell NE, Conaghan J, Turek PJ. The relative viability of human spermatozoa from the vas deferens, epididymis and testis before and after cryopreservation. Hum Reprod. 1999;14(12):3048-51. doi: 10.1093/humrep/14.12.3048.
191. Habermann H, Seo R, Cieslak J, Niederberger C, Prins GS, Ross L. In vitro fertilization outcomes after intracytoplasmic sperm injection with fresh or frozen-thawed testicular spermatozoa. Fertil Steril. 2000;73(5):955-60. doi: 10.1016/s0015-0282(00)00416-7.

192. Cayan S, Lee D, Conaghan J, Givens CA, Ryan IP, Schriock ED, et al. A comparison of ICSI outcomes with fresh and cryopreserved epididymal spermatozoa from the same couples. Hum Reprod. 2001;16(3):495-9. doi: 10.1093/humrep/16.3.495.
193. Mazzilli R, Cimadomo D, Vaiarelli A, Capalbo A, Dovere L, Alviggi E, et al. Effect of the male factor on the clinical outcome of intracytoplasmic sperm injection combined with preimplantation aneuploidy testing: observational longitudinal cohort study of 1,219 consecutive cycles. Fertil Steril. 2017;108(6):961-72.e3. doi: 10.1016/j.fertnstert.2017.08.033.
194. Lotti F, Corona G, Cocci A, Cipriani S, Baldi E, Degl'Innocenti S, et al. The prevalence of midline prostatic cysts and the relationship between cyst size and semen parameters among infertile and fertile men. Hum Reprod. 2018;33(11):2023-34. doi: 10.1093/humrep/dey298.
195. Boomsma CM, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. Cochrane Database Syst Rev. 2019;10:CD004507. doi: 10.1002/14651858.CD004507.pub4.
196. Van der Zwalmen P, Bertin-Segal G, Geerts L, Debauche C, Schoysman R. Sperm morphology and IVF pregnancy rate: comparison between Percoll gradient centrifugation and swim-up procedures. Hum Reprod. 1991;6(4):581-8. doi: 10.1093/oxfordjournals.humrep.a137383.
197. Englert Y, Van den Bergh M, Rodesch C, Bertrand E, Biramane J, Legreve A. Comparative auto-controlled study between swim-up and Percoll preparation of fresh semen samples for in-vitro fertilization. Hum Reprod. 1992;7(3):399-402. doi: 10.1093/oxfordjournals.humrep.a137657.
198. Sapienza F, Verheyen G, Tournaye H, Janssens R, Pletincx I, Derde M, et al. An auto-controlled study in in-vitro fertilization reveals the benefit of Percoll centrifugation to swim-up in the preparation of poor-quality semen. Hum Reprod. 1993;8(11):1856-62. doi: 10.1093/oxfordjournals.humrep.a137949.
199. Leonetti T, Causio F, Cagnazzo G. Techniques of sperm selection and in vitro fertilization results. Acta Eur Fertil. 1995;26(1):31-4.
200. Hammadeh ME, Zavos PM, Rosenbaum P, Schmidt W. Comparison between the quality and function of sperm after semen processing with two different methods. Asian J Androl. 2001;3(2):125-30.
201. Borges E, Setti AS, Vingris L, Figueira ReC, Braga DP, Iaconelli A. Intracytoplasmic morphologically selected sperm injection outcomes: the role of sperm preparation techniques. J Assist Reprod Genet. 2013;30(6):849-54. doi: 10.1007/s10815-013-9989-x.
202. Kim EK, Kim EH, Kim EA, Lee KA, Shin JE, Kwon H. Comparison of the effect of different media on the clinical outcomes of the density-gradient centrifugation/swim-up and swim-up methods. Clin Exp Reprod Med. 2015;42(1):22-9. doi: 10.5653/cerm.2015.42.1.22.
203. Palini S, Stefani S, Primiterra M, Benedetti S, Barone S, Carli L, et al. Comparison of in vitro fertilization outcomes in ICSI cycles after human sperm preparation by density gradient centrifugation and direct micro swim-up without centrifugation. JBRA Assist Reprod. 2017;21(2):89-93. doi: 10.5935/1518-0557.20170022.
204. coticchio.biogenesi@grupposandonato.it ESIGoEaASiRMEa. The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators. Reprod Biomed Online. 2017;35(5):494-510. doi: 10.1016/j.rbmo.2017.06.015.
205. Teixeira DM, Hadyme Miyague A, Barbosa MA, Navarro PA, Raine-Fenning N, Nastri CO, et al. Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction. Cochrane Database Syst Rev. 2020;2:CD010167. doi: 10.1002/14651858.CD010167.pub3.
206. Lepine S, McDowell S, Searle LM, Kroon B, Glujovsky D, Yazdani A. Advanced sperm selection techniques for assisted reproduction. Cochrane Database Syst Rev. 2019;7:CD010461. doi: 10.1002/14651858.CD010461.pub3.
207. Samuel R, Feng H, Jafek A, Despain D, Jenkins T, Gale B. Microfluidic-based sperm sorting & analysis for treatment of male infertility. Transl Androl Urol. 2018;7(Suppl 3):S336-S47. doi: 10.21037/tau.2018.05.08.
208. Ibrahim E, Brackett NL, Lynne CM. Advances in the management of infertility in men with spinal cord injury. Asian J Androl. 2016;18(3):382-90. doi: 10.4103/1008-682X.178851.
209. Reignier A, Lammers J, Splingart C, Redhead D, Labat JJ, Mirallié S, et al. Sperm cryopreservation and assisted reproductive technology outcome in patients with spinal cord injury. Andrologia. 2018;50(1). doi: 10.1111/and.12833.
210. Koscinski I, Wittemer C, Lefebvre-Khalil V, Marcelli F, Defossez A, Rigot JM. Optimal management of extreme oligozoospermia by an appropriate cryopreservation programme. Hum Reprod. 2007;22(10):2679-84. doi: 10.1093/humrep/dem190.

211. Corona G, Pizzocaro A, Lanfranco F, Garolla A, Pelliccione F, Vignozzi L, et al. Sperm recovery and ICSI outcomes in Klinefelter syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2017;23(3):265-75. doi: 10.1093/humupd/dmx008.
212. Tao Y, Sanger E, Saewu A, Leveille MC. Human sperm vitrification: the state of the art. Reprod Biol Endocrinol. 2020;18(1):17. doi: 10.1186/s12958-020-00580-5.
213. Gandini L, Sgrò P, Lombardo F, Paoli D, Culasso F, Toselli L, et al. Effect of chemo- or radiotherapy on sperm parameters of testicular cancer patients. Hum Reprod. 2006;21(11):2882-9. doi: 10.1093/humrep/del167.
214. Paoli D, Rizzo F, Fiore G, Pallotti F, Pulsoni A, Annechini G, et al. Spermatogenesis in Hodgkin's lymphoma patients: a retrospective study of semen quality before and after different chemotherapy regimens. Hum Reprod. 2016;31(2):263-72. doi: 10.1093/humrep/dev310.
215. Dohle GR. Male infertility in cancer patients: Review of the literature. Int J Urol. 2010;17(4):327-31. doi: 10.1111/j.1442-2042.2010.02484.x.
216. Kuczyński W, Dhont M, Grygoruk C, Grochowski D, Wołczyński S, Szamatowicz M. The outcome of intracytoplasmic injection of fresh and cryopreserved ejaculated spermatozoa--a prospective randomized study. Hum Reprod. 2001;16(10):2109-13. doi: 10.1093/humrep/16.10.2109.
217. Yu Z, Wei Z, Yang J, Wang T, Jiang H, Li H, et al. Comparison of intracytoplasmic sperm injection outcome with fresh versus frozen-thawed testicular sperm in men with nonobstructive azoospermia: a systematic review and meta-analysis. J Assist Reprod Genet. 2018;35(7):1247-57. doi: 10.1007/s10815-018-1206-5.
218. Ferrari S, Paffoni A, Filippi F, Busnelli A, Vegetti W, Somigliana E. Sperm cryopreservation and reproductive outcome in male cancer patients: a systematic review. Reprod Biomed Online. 2016;33(1):29-38. doi: 10.1016/j.rbmo.2016.04.002.
219. Trottmann M, Becker AJ, Stadler T, Straub J, Soljanik I, Schlenker B, et al. Semen quality in men with malignant diseases before and after therapy and the role of cryopreservation. Eur Urol. 2007;52(2):355-67. doi: 10.1016/j.eururo.2007.03.085.
220. Di Bisceglie C, Bertagna A, Composto ER, Lanfranco F, Baldi M, Motta G, et al. Effects of oncological treatments on semen quality in patients with testicular neoplasia or lymphoproliferative disorders. Asian J Androl. 2013;15(3):425-9. doi: 10.1038/aja.2012.171.
221. Bujan L, Walschaerts M, Moinard N, Hennebicq S, Saias J, Brugnon F, et al. Impact of chemotherapy and radiotherapy for testicular germ cell tumors on spermatogenesis and sperm DNA: a multicenter prospective study from the CECOS network. Fertil Steril. 2013;100(3):673-80. doi: 10.1016/j.fertnstert.2013.05.018.
222. Ghezzi M, Berretta M, Bottacin A, Palego P, Sartini B, Cosci I, et al. Impact of Bep or Carboplatin Chemotherapy on Testicular Function and Sperm Nucleus of Subjects with Testicular Germ Cell Tumor. Front Pharmacol. 2016;7:122. doi: 10.3389/fphar.2016.00122.
223. Paoli D, Pelloni M, Lenzi A, Lombardo F. Cryopreservation of Sperm: Effects on Chromatin and Strategies to Prevent Them. Adv Exp Med Biol. 2019;1166:149-67. doi: 10.1007/978-3-030-21664-1_9.
224. Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS, et al. Fertility Preservation in Patients With Cancer: ASCO Clinical Practice Guideline Update. J Clin Oncol. 2018;36(19):1994-2001. doi: 10.1200/JCO.2018.78.1914.
225. Tiseo BC, Cocuzza M, Bonfa E, Srougi M, Silva CA. Male fertility potential alteration in rheumatic diseases: a systematic review. Int Braz J Urol. 2016;42(1):11-21. doi: 10.1590/S1677-5538.IBJU.2014.0595.
226. Banerjee A, Scarpa M, Pathak S, Burra P, Sturniolo GC, Russo FP, et al. Inflammatory Bowel Disease Therapies Adversely Affect Fertility in Men- A Systematic Review and Meta-analysis. Endocr Metab Immune Disord Drug Targets. 2019;19(7):959-74. doi: 10.2174/1871530319666190313112110.

Figure 1

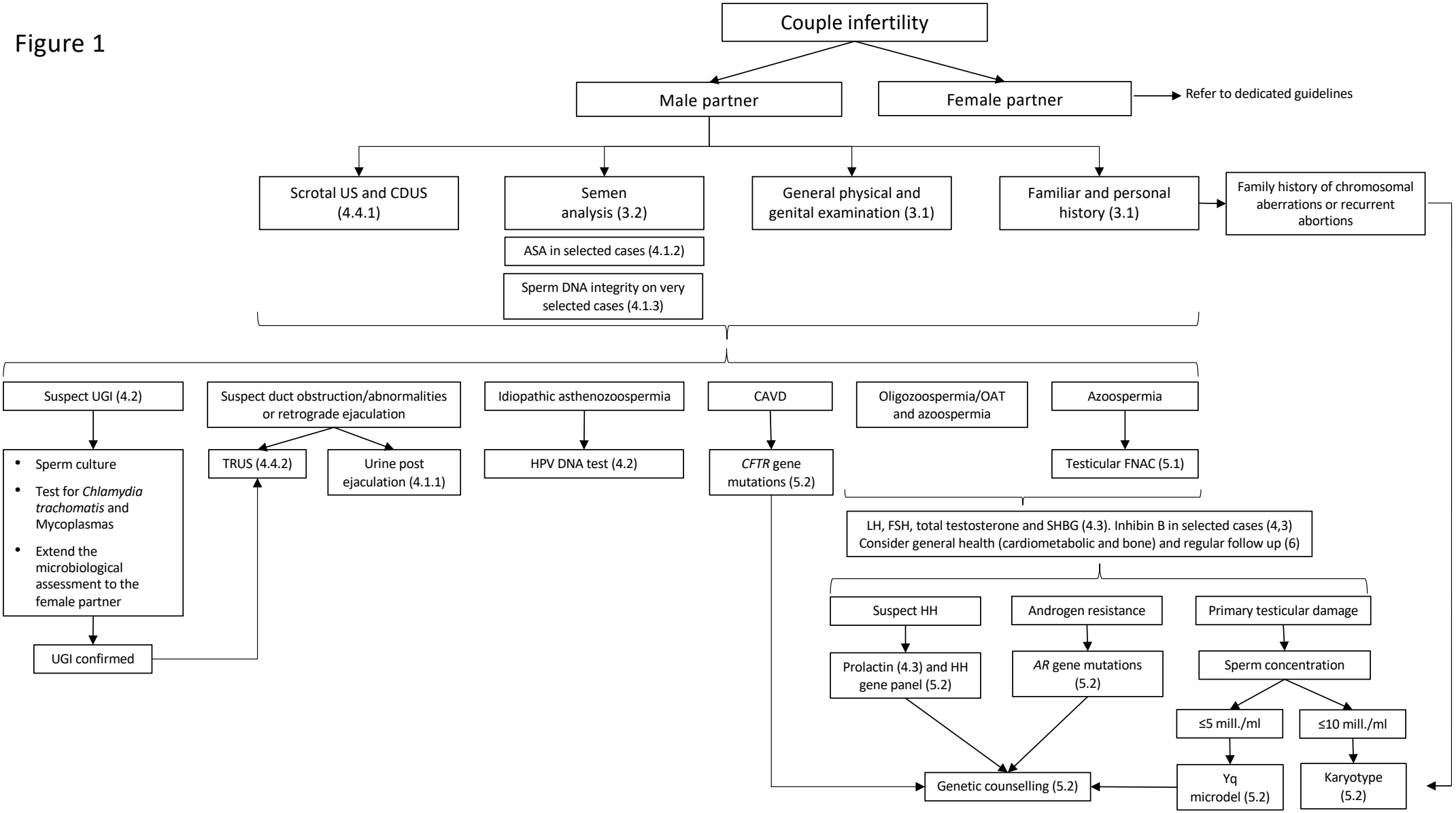
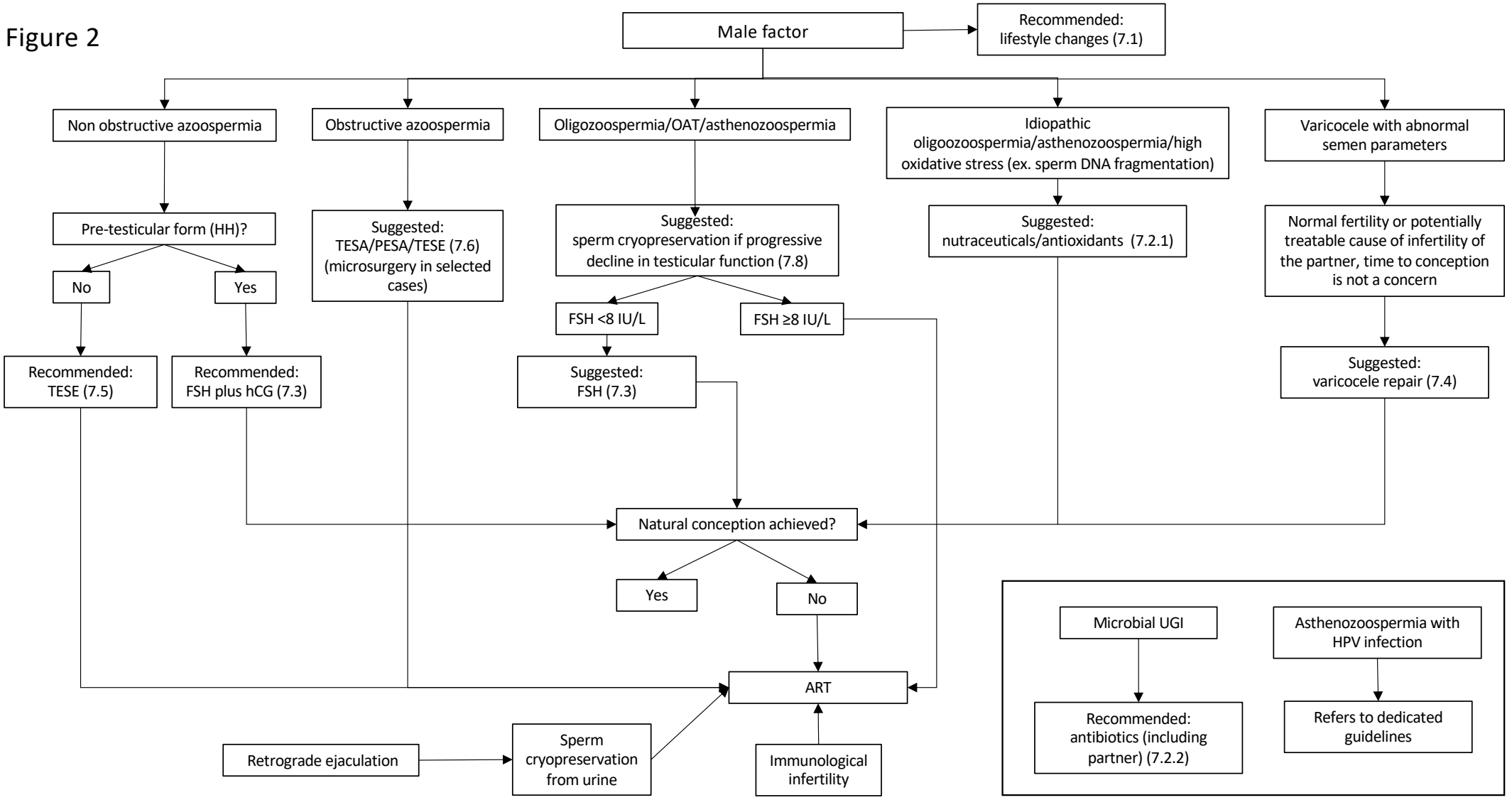

Couple infertility
Male partner
Female partner
Refer to dedicated guidelines
Scrotal US and CDUS (4.4.1)
Semen analysis (3.2)
General physical and genital examination (3.1)
Familiar and personal history (3.1)
Family history of chromosomal aberrations or recurrent abortions
ASA in selected cases (4.1.2)
Sperm DNA integrity on very selected cases (4.1.3)
Suspect UGI (4.2)
Suspect duct obstruction/abnormalities or retrograde ejaculation
Idiopathic asthenozoospermia
CAVD
Oligozoospermia/OAT and azoospermia
Azoospermia
Sperm culture
Test for Chlamydia trachomatis and Mycoplasmas
Extend the microbiological assessment to the female partner
TRUS (4.4.2)
Urine post ejaculation (4.1.1)
HPV DNA test (4.2)
CFTR gene mutations (5.2)
Testicular FNAC (5.1)
UGI confirmed
LH, FSH, total testosterone and SHBG (4.3). Inhibin B in selected cases (4,3)
Consider general health (cardiometabolic and bone) and regular follow up (6)
Suspect HH
Androgen resistance
Primary testicular damage
Prolactin (4.3) and HH gene panel (5.2)
AR gene mutations (5.2)
Sperm concentration
≤5 mill./ml
≤10 mill./ml
Yq microdel (5.2)
Karyotype (5.2)
Genetic counselling (5.2)

Figure 2


Male factor
Recommended: lifestyle changes (7.1)
Non obstructive azoospermia
Obstructive azoospermia
Oligozoospermia/OAT/asthenozoospermia
Idiopathic oligoozoospermia/asthenozoospermia/high oxidative stress (ex. sperm DNA fragmentation)
Varicocele with abnormal semen parameters
Pre-testicular form (HH)?
No
Yes
Recommended: TESE (7.5)
Recommended: FSH plus hCG (7.3)
Suggested: TESA/PESA/TESE (7.6) (microsurgery in selected cases)
Suggested: sperm cryopreservation if progressive decline in testicular function (7.8)
FSH <8 IU/L
FSH ≥8 IU/L
Suggested: FSH (7.3)
Suggested: nutraceuticals/antioxidants (7.2.1)
Normal fertility or potentially treatable cause of infertility of the partner, time to conception is not a concern
Suggested: varicocele repair (7.4)
Natural conception achieved?
Yes
No
ART
Retrograde ejaculation
Sperm cryopreservation from urine
Immunological infertility
Microbial UGI
Recommended: antibiotics (including partner) (7.2.2)
Asthenozoospermia with HPV infection
Refers to dedicated guidelines

Supplementary table 1. The most relevant factors causing infertility.

Infertility category	Disease
Alterations of the hypothalamus-pituitary-testicular axis (pre-testicular causes)	• Congenital: - Congenital hypogonadotrophic hypogonadism, syndromic (e.g. Kallmann syndrome) or non-syndromic (normosmic) • Acquired: - Pituitary adenoma - CNS malignancy - Infiltrative diseases (e.g. haemochromatosis, sarcoidosis) - Iatrogenic: transphenoidal resection or radiation - Exogenous androgen or testosterone use
Primary testicular dysfunction (testicular causes)	• Congenital - Karyotype anomalies (e.g. Klinefelter syndrome, translocations, inversions) - Y chromosome microdeletions (*AZF* regions) - 46,XX male syndrome or isodicentric Y chromosome - Partial androgen insensitivity syndrome (mild form) - Monogenic mutations - Cryptorchidism • Acquired - Orchitis - Orchiepididymitis - Varicocele - Testis malignancy - Orchiectomy - Cytotoxic chemotherapy or radiotherapy - Post-hernioplastic testis atrophy - Systemic illnesses (liver or kidney insufficiency)
Ductal obstruction/dysfunction (post-testicular causes)	• Congenital: - Congenital absence of vas deferens with or without unilateral renal agenesis - Young syndrome • Acquired: - Vasectomy, - Idiopathic epididymal occlusion - Bilateral inguinal hernia repair - Ejaculatory duct obstruction - Infections - Diabetes mellitus with vasal peristaltic deficiency - Spinal cord injury - Multiple sclerosis - Neural tube defects - Retroperitoneal lymph node dissection and pelvic surgery

Supplementary table 2. Pathophysiology of sperm DNA integrity.

Sperm DNA damage	Cause
Physiological DSBs (double strand breaks)	Sperm DNA nicks to promote the remodelling of sperm chromatin
Abortive apoptosis	Lifestyle (smoking, obesity, alcohol) Diseases and genito-urinary infections/inflammation Ageing Iatrogenic (drugs, antineoplastic treatments, etc.)
Defect of chromatin compaction	
Oxidative stress	

Supplementary table 3. Scrotal ultrasound features in male infertility.

	Feature	Level of evidence	References
Testis	Volume/symmetry	ØØØØ	[1-7]
	Echo-texture	ØØØO	[8, 9]
	Normal echogenicity		[8, 10]
	Low echogenicity		[11, 12]
	High echogenicity		[8, 10]
	Nodules	ØØØØ	[13-15]
	Micriolithiasis	ØØOO	[16, 17]
Pampiniform plexus	Vessels > 2 mm – varicocele	ØØOO	[10, 13]

Supplementary table 4. Colour Doppler ultrasound features of urogenital infection/inflammation.

	Feature	Level of evidence	References
Prostate	Asymmetry	ØØOO	[18-20]
	Non-homogeneity	ØØOO	[18, 19, 21, 22]
	Areas of low echogenicity	ØØOO	[19, 23-25]
	Areas of high echogenicity	ØØOO	[19, 23-25]
	Calcifications	ØØOO	[19, 23, 24, 26, 27]
	Hyperaemia	ØOOO	[28]
Seminal vesicles	Increased antero-posterior diameter (>14 mm), uni- or bilateral	ØØOO	[19, 29, 30]
	Reduced antero-posterior diameter (<7 mm), uni- or bilateral	ØOOO	[19]
	Asymmetry >2.5 mm compared to the contralateral vesicle	ØOOO	[19, 30]
	Fundus-to-body ratio <1 or >2.5	ØOOO	[25]
	Polycyclic areas separated by hyperechoic septa in one or both vesicle	ØØOO	[19, 31, 32]
	Calcifications	ØØOO	[19, 33]
Epididymis	Low echogenicity	ØOOO	[34]
	High echogenicity	ØOOO	[34]
	Hyperaemia	ØØOO	[19, 33, 35]
	Calcifications	ØØOO	[19, 33, 35]
	Increased head craniocaudal diameter (>12 mm) and/or increased tail craniocaudal diameter (>6 mm)	ØOOO	[36]

1. Jørgensen N, Carlsen E, Nermoen I, Punab M, Suominen J, Andersen AG, et al. East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. Hum Reprod. 2002;17(8):2199-208. doi: 10.1093/humrep/17.8.2199.
2. Dogra VS, Gottlieb RH, Oka M, Rubens DJ. Sonography of the scrotum. Radiology. 2003;227(1):18-36. doi: 10.1148/radiol.2271001744.
3. Goede J, van der Voort-Doedens LM, Sijstermans K, Hack WW. The volume of retractile testes. J Urol. 2011;186(5):2050-4. doi: 10.1016/j.juro.2011.07.020.
4. Fisher AD, Rastrelli G, Bandini E, Corona G, Balzi D, Melani C, et al. Metabolic and cardiovascular outcomes of fatherhood: results from a cohort of study in subjects with sexual dysfunction. J Sex Med. 2012;9(11):2785-94. doi: 10.1111/j.1743-6109.2012.02865.x.
5. Condorelli R, Calogero AE, La Vignera S. Relationship between Testicular Volume and Conventional or Nonconventional Sperm Parameters. Int J Endocrinol. 2013;2013:145792. doi: 10.1155/2013/145792.
6. Ventimiglia E, Capogrosso P, Colicchia M, Boeri L, Serino A, Castagna G, et al. Metabolic syndrome in white European men presenting for primary couple's infertility: investigation of the clinical and reproductive burden. Andrology. 2016;4(5):944-51. doi: 10.1111/andr.12232.
7. Pozza C, Kanakis G, Carlomagno F, Lemma A, Pofi R, Tenuta M, et al. Testicular ultrasound score: A new proposal for a scoring system to predict testicular function. Andrology. 2020;8(5):1051-63. doi: 10.1111/andr.12822.

8. Lenz S, Giwercman A, Elsborg A, Cohr KH, Jelnes JE, Carlsen E, et al. Ultrasonic testicular texture and size in 444 men from the general population: correlation to semen quality. Eur Urol. 1993;24(2):231-8. doi: 10.1159/000474300.
9. Westlander G, Ekerhovd E, Granberg S, Hanson L, Hanson C, Bergh C. Testicular ultrasonography and extended chromosome analysis in men with nonmosaic Klinefelter syndrome: a prospective study of possible predictive factors for successful sperm recovery. Fertil Steril. 2001;75(6):1102-5. doi: 10.1016/s0015-0282(01)01793-9.
10. Lotti F, Maggi M. Ultrasound of the male genital tract in relation to male reproductive health. Hum Reprod Update. 2015;21(1):56-83. doi: 10.1093/humupd/dmu042.
11. Béres J, Papp G, Pazonyi I, Czeizel E. Testicular volume variations from 0 to 28 years of age. Int Urol Nephrol. 1989;21(2):159-67. doi: 10.1007/BF02550804.
12. Loberant N, Bhatt S, McLennan GT, Dogra VS. Striated appearance of the testes. Ultrasound Q. 2010;26(1):37-44. doi: 10.1097/RUQ.0b013e3181c6b284.
13. Isidori AM, Giannetta E, Lenzi A. Male hypogonadism. Pituitary. 2008;11(2):171-80. doi: 10.1007/s11102-008-0111-9.
14. Isidori AM, Pozza C, Gianfrilli D, Giannetta E, Lemma A, Pofi R, et al. Differential diagnosis of nonpalpable testicular lesions: qualitative and quantitative contrast-enhanced US of benign and malignant testicular tumors. Radiology. 2014;273(2):606-18. doi: 10.1148/radiol.14132718.
15. Pozza C, Gianfrilli D, Fattorini G, Giannetta E, Barbagallo F, Nicolai E, et al. Diagnostic value of qualitative and strain ratio elastography in the differential diagnosis of non-palpable testicular lesions. Andrology. 2016;4(6):1193-203. doi: 10.1111/andr.12260.
16. Barbonetti A, Martorella A, Minaldi E, D'Andrea S, Bardhi D, Castellini C, et al. Testicular Cancer in Infertile Men With and Without Testicular Microlithiasis: A Systematic Review and Meta-Analysis of Case-Control Studies. Front Endocrinol (Lausanne). 2019;10:164. doi: 10.3389/fendo.2019.00164.
17. D'Andrea S, Martorella A, Castellini C, Cordeschi G, Totaro M, Parisi A, et al. Clinical and seminal parameters associated with testicular microlithiasis and its severity in males from infertile couples. Hum Reprod. 2021;36(4):891-8. doi: 10.1093/humrep/deaa354.
18. Christiansen E, Purvis K. Diagnosis of chronic abacterial prostato-vesiculitis by rectal ultrasonography in relation to symptoms and findings. Br J Urol. 1991;67(2):173-6. doi: 10.1111/j.1464-410x.1991.tb15103.x.
19. Vicari E. Seminal leukocyte concentration and related specific reactive oxygen species production in patients with male accessory gland infections. Hum Reprod. 1999;14(8):2025-30. doi: 10.1093/humrep/14.8.2025.
20. La Vignera S, Vicari E, Condorelli R, D'Agata R, Calogero AE. Hypertrophic-congestive and fibro-sclerotic ultrasound variants of male accessory gland infection have different sperm output. J Endocrinol Invest. 2011;34(10):e330-5. doi: 10.1007/BF03346729.
21. Di Trapani D, Pavone C, Serretta V, Cavallo N, Costa G, Pavone-Macaluso M. Chronic prostatitis and prostatodynia: ultrasonographic alterations of the prostate, bladder neck, seminal vesicles and periprostatic venous plexus. Eur Urol. 1988;15(3-4):230-4. doi: 10.1159/000473440.
22. Behre HM, Kliesch S, Schädel F, Nieschlag E. Clinical relevance of scrotal and transrectal ultrasonography in andrological patients. Int J Androl. 1995;18 Suppl 2:27-31.
23. Doble A, Carter SS. Ultrasonographic findings in prostatitis. Urol Clin North Am. 1989;16(4):763-72.
24. Purvis K, Christiansen E. Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. Int J Androl. 1993;16(1):1-13. doi: 10.1111/j.1365-2605.1993.tb01146.x.
25. La Vignera S, Vicari E, Condorelli RA, D'Agata R, Calogero AE. Male accessory gland infection and sperm parameters (review). Int J Androl. 2011;34(5 Pt 2):e330-47. doi: 10.1111/j.1365-2605.2011.01200.x.
26. Meares EM. Infection stones of prostate gland. Laboratory diagnosis and clinical management. Urology. 1974;4(5):560-6. doi: 10.1016/0090-4295(74)90490-7.
27. Shoskes DA, Lee CT, Murphy D, Kefer J, Wood HM. Incidence and significance of prostatic stones in men with chronic prostatitis/chronic pelvic pain syndrome. Urology. 2007;70(2):235-8. doi: 10.1016/j.urology.2007.04.008.
28. Cho IR, Keener TS, Nghiem HV, Winter T, Krieger JN. Prostate blood flow characteristics in the chronic prostatitis/pelvic pain syndrome. J Urol. 2000;163(4):1130-3.

29. Jarow JP. Transrectal ultrasonography of infertile men. Fertil Steril. 1993;60(6):1035-9. doi: 10.1016/s0015-0282(16)56406-1.
30. La Vignera S, Calogero AE, Arancio A, Castiglione R, De Grande G, Vicari E. Transrectal ultrasonography in infertile patients with persistently elevated bacteriospermia. Asian J Androl. 2008;10(5):731-40. doi: 10.1111/j.1745-7262.2008.00425.x.
31. Kim ED, Lipshultz LI. Role of ultrasound in the assessment of male infertility. J Clin Ultrasound. 1996;24(8):437-53. doi: 10.1002/(SICI)1097-0096(199610)24:8<437::AID-JCU4>3.0.CO;2-L.
32. Kim B, Kawashima A, Ryu JA, Takahashi N, Hartman RP, King BF. Imaging of the seminal vesicle and vas deferens. Radiographics. 2009;29(4):1105-21. doi: 10.1148/rg.294085235.
33. Lotti F, Corona G, Mancini M, Filimberti E, Degli Innocenti S, Colpi GM, et al. Ultrasonographic and clinical correlates of seminal plasma interleukin-8 levels in patients attending an andrology clinic for infertility. Int J Androl. 2011;34(6 Pt 1):600-13. doi: 10.1111/j.1365-2605.2010.01121.x.
34. Woodward PJ, Schwab CM, Sesterhenn IA. From the archives of the AFIP: extratesticular scrotal masses: radiologic-pathologic correlation. Radiographics. 2003;23(1):215-40. doi: 10.1148/rg.231025133.
35. Lotti F, Maggi M. Interleukin 8 and the male genital tract. J Reprod Immunol. 2013;100(1):54-65. doi: 10.1016/j.jri.2013.02.004.
36. La Vignera S, Calogero AE, Condorelli RA, Vicari LO, Catanuso M, D'Agata R, et al. Ultrasonographic evaluation of patients with male accessory gland infection. Andrologia. 2012;44 Suppl 1:26-31. doi: 10.1111/j.1439-0272.2010.01132.x.