# Diagnosis of Infertility

# 4

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Whenever a couple has not conceived after 1 year of unprotected intercourse, both partners should undergo a thorough medical examination. An earlier evaluation is suggested in the presence of a known male (i.e., history of cryptorchidism) or female (i.e., age over 35 years) infertility risk factor or if a man wishes to know his fertility potential [1].

The justification for male evaluation by an andrologist relies on the fact that a male factor is solely responsible in about 20 % of infertile couples and is contributory in another 30–40 % [2, 3]. It is presently recommended that, to categorize infertility, both partners should be investigated simultaneously [4]. The goals of a male evaluation for infertility are to identify causes of infertility that after correction could allow natural conception, to identify causes of infertility that after correction could increase the chances of success of assisted reproduction technologies (ARTs), and to explore underlying conditions that, in additionto being related to infertility, could pose a risk for the man's health.

## 4.1 Andrologic Evaluation for Male Infertility: The Initial Office Visit

The minimum andrologic evaluation for male infertility should include a complete medical history, physical examination, and the evaluation of at least two semen analyses [1]. The physical evaluation is usually complemented by the ultrasonographic evaluation of the scrotal content and, if indicated, the prostate. All of these tests can be carried out during a single andrologic office visit. Additional

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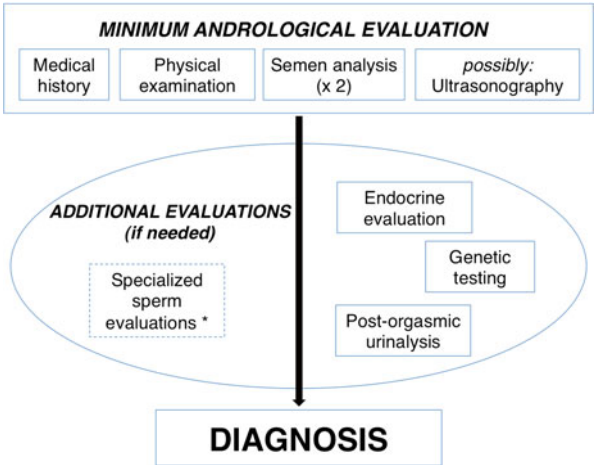


**Fig. 4.1** Andrologic workup flow chart: *specialized sperm evaluations are presently considered experimental (WHO)

investigations may be appropriate, should specific problems emerge at the initial office visit. These tests chiefly comprise endocrine evaluation, genetic testing, post-orgasmic urinalysis, and specialized sperm evaluations (Fig. 4.1). The components of the andrologic office visit are detailed here.

## 4.1.1 Medical History

The medical history should investigate all possible causes that may affect the fertility potential of the man. The following areas should be addressed.

### 4.1.1.1 Reproductive History

Did the man under investigation formerly induce pregnancy or pregnancies with the present or other partners? If so, this would suggest that medical attention be focused more on the present female partner. Should a history of spontaneous abortions be present, this should direct one's attention to sperm DNA evaluations.

### 4.1.1.2 Occupational History

Professions at risk of affecting fertility, such as direct and prolonged exposure to high temperatures (e.g., kitchen work) and exposure to gonadotoxic agents (e.g., pesticides), should be noted. According to the specific agent, fertility may improve after 1–2 spermatogenetic cycles (3–6 months) after discontinuation of exposure.

### 4.1.1.3 Lifestyle Risk Factors

Smoke [5–7], excessive alcohol [8] and coffee [9] intake, recreational drugs [10], elevated body mass index (BMI) [11, 12], and low physical activity [13] also have been linked to impaired male fertility; modifying such risk factors may have a positive impact on male fertility.

**Fig. 4.2** Prader orchidometer

#### 4.1.1.4 Andrologic History

The possible occurrence of the following conditions should be investigated: undescended testis and age of orchiopexy, testis torsion and outcome, former inguinoscrotal surgery such as for inguinal hernia repair, former prostate surgery, pubertal/prepubertal mumps-related orchitis, pubertal development, anosmia, former neoplasia and related treatments, current and recent medications, current and recent genitourinary symptoms or infections, and recent episodes of high fever.

#### 4.1.1.5 Sexual History

The patient should be asked about libido, quality of erection, intercourse frequency, ejaculation, and possible sexual distress related to reproductive difficulties or timed sex.

### 4.1.2 Physical Examination

In the man evaluated for infertility, physical examination is more informative by far than in the woman: male genitalia are external and easily evaluated, even without the aid of ultrasonography. Moreover, the main male sexual accessory gland, the prostate, can be digitally palpated through the anus.

The andrologic physical evaluation should comprise: evaluation of secondary sexual characteristics, presence of (pseudo-)gynecomastia, penis inspection with attention to location of the external urethral meatus, and digital rectal examination of the prostate.

A detailed evaluation of the scrotal content is of paramount importance. Testes should be assessed for bilateral presence, location (in place, vs retained, vs ectopic), size (according to Prader orchidometer; Fig. 4.2), consistency, and presence of nodules. Epididymides should be evaluated for their presence, possible dilatations, and associated cysts. Bilateral presence of deferent ducts should be ascertained. The presence of varicocele, and its grading [14], should be sought while in orthostasis (Fig. 4.2).

**Table 4.1** Distribution of values at 5th and 50th centiles for semen parameters from men whose partners became pregnant within 12 months of discontinuing contraceptive use (WHO)

| Parameter | (Units) | Centile | |
|---|---|---|---|
| | | 5th | 50th |
| Semen volume | (mL) | 1.5 | 3.7 |
| Sperm concentration | ($10^6$/mL) | 15 | 73 |
| Total motility (PR + NP) | (%) | 40 | 61 |
| Normal forms | (%) | 4 | 15 |

*PR* progressive motility, *NP* nonprogressive motility

### 4.1.3 Semen Analysis

Semen analysis is the cornerstone of the laboratory evaluation of the infertile male, and helps to define the severity of the male factor [1]. The 2010 World Health Organization (WHO) *Laboratory Manual for the Examination and Processing of Human Semen* details the present standards of semen analysis and the related laboratory protocols [15], and every laboratory performing semen analyses should comply with such standards.

In general, laboratory reports of semen analysis define values within normal as those values that do not fall below the lower reference limit (fifth centile). When critically evaluating sperm analysis reports, it should be remembered that the fifth centile indicates values below which only 5 % of the observations of fertile men will fall, and not the average values of fertile men whose partners had a time to pregnancy of 12 months or less [15]. Table 4.1 lists the latest WHO sperm parameter reference values of the 5th and 50th centiles, with the aim of providing the reader with a more critical interpretation of sperm analysis.

It should always be remembered that semen parameters within the 95 % reference interval do not guarantee fertility; nor do values outside these limits, in isolation from other clinical data, necessarily indicate male infertility or abnormality: a man's semen characteristics need to be interpreted in conjunction with his clinical information [16] (Table 4.2).

### 4.1.4 Ultrasonography Evaluation of Testes and Prostate

Usually the andrologic physical evaluation of the male is complemented by ultrasonographic evaluation of scrotal content and, if indicated, the prostate.

Testis sonography adds useful information regarding the structure of testicular tissue (Fig. 4.3a: microlithiasis), the possible presence of nonpalpable tumors, accurate definition of testes volumes, details of epididymis, and varicocele definition (using Doppler ultrasonography performed while in orthostasis).

If there are clues of obstructive pathology, a transrectal ultrasonogram may unveil an intraprostatic obstructive cyst (Fig. 4.3b). Obstructive intraprostatic cysts should be suspected in the presence of reduced semen volume or even azoospermia, normal-sized testes with distended epididymis, and ejaculatory discomfort/pain.

**Table 4.2** Some examples of medical history, physical examination, semen analysis data, and possible underlying pathology

| Medical history | Physical examination | Semen analysis | Suspected |
|---|---|---|---|
| Former prostate surgery | – | Reduced/absent sperm at orgasm | Retrograde ejaculation |
| Anosmia/ delayed puberty | Cryptorchidism | – | Kallmann syndrome |
| Inguinal hernia repair | Distended epididymis | Oligospermia | Deferent ischemic damage |
| Low libido | Altered sexual characters | OAT | Hypogonadism |
| Low libido | Small firm testes | Azoospermia | Klinefelter syndrome |
| Postpubertal parotitis | Small soft testes | Azoospermia/ cryptozoospermia | Postviral testicular damage |
| – | Varicocele | OAT | Causal role of varicocele |
| Pain at ejaculation | Prostatic tender "nodule" | Low semen volume | Obstructive intraprostatic cyst |
| – | Small testes | Normal volume, azoospermia | Nonobstructive azoospermia |
| – | Distended epididymis | Low volume, azoospermia | Obstructive azoospermia |
| Recent fever | Painful epididymis | Two million WBC/ mL OAT | Infection |

*OAT* oligoasthenoteratospermia, *WBC* white blood cells

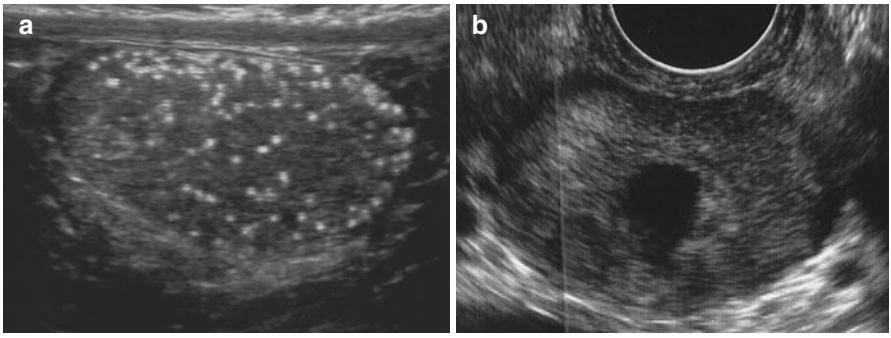


**Fig. 4.3** Possible ultrasonographic findings (**a**): testicular microlithiasis; (**b**): intraprostatic obstructive cyst

## 4.2 Andrologic Evaluation for Male Infertility: Additional Investigations

Some elements gathered during the initial andrologic office visit may prompt further diagnostic workup. The more frequent aspects of additional investigation are endocrine status, genetic testing, postorgasmic urinalysis, and specialized sperm evaluations.

**Table 4.3** Simplified endocrinologic differential diagnoses in the presence of altered sperm analysis

| FSH | LH | TT | Interpretation |
|---|---|---|---|
| > | > | < | Primary hypogonadism: the problem is the testis |
| < | < | < | Secondary hypogonadism: the problem is hypothalamus/hypophysis |
| > | N | N | Possibly: maturation arrest, germinal aplasia, genetic causes |
| N | N | N | Nonendocrine causes vs functional hypogonadotropic hypogonadism |

*FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *TT* total testosterone, *N* normal

### 4.2.1 Endocrine Evaluation

It is appropriate to perform an endocrine evaluation whenever medical history and physical findings suggest an endocrinopathy, in the presence of a low sperm count, and if there is concomitant sexual dysfunction. Gonadal activity relies on the pituitary input provided by luteinizing hormone (LH) and follicle-stimulating-hormone (FSH). Although a minimum initial hormonal evaluation consists of FSH and total serum testosterone, the concomitant assessment of LH, prolactin, and estradiol permits one to obtain a more comprehensive picture of the endocrine status of the patient (Table 4.3).

### 4.2.2 Genetic Testing

When nonobstructive azoospermia or severe oligozoospermia (sperm count $<10\times10^6$/mL) is present, karyotype assessment and a search for microdeletions of the long arm of the Y chromosome are recommended [17]. When bilateral or unilateral congenital absence of the vas is detected, and in the presence of obstructive azoospermia or severe oligozoospermia (sperm count $<10\times10^6$/mL), screening for CFTR mutations is strongly advised [17]. If anosmia has been identified during medical history taking, and even more so if associated with azoospermia, KAL1 gene screening is recommended, with the aim of detecting the X-linked variety of Kallmann syndrome [18].

### 4.2.3 Postorgasmic Urinalysis

This test is indicated in men with low-volume or absent ejaculation at orgasm. The presence of any sperm in a postejaculatory urinalysis in these cases is suggestive of retrograde ejaculation.

### 4.2.4 Specialized Sperm Evaluations

#### 4.2.4.1 Reactive Oxygen Species

Reactive oxygen species (ROS) are generated by both seminal leukocytes and sperm cells; although they have a normal physiological role in capacitation and

acrosome reaction, if in excess they can interfere with sperm function by peroxidation of sperm lipid membranes, and induce DNA damage in both the nuclear and mitochondrial genomes [19]. Chemiluminescent procedures may be used to measure ROS production and the redox activity of human spermatozoa.

#### 4.2.4.2 Sperm Chromatin Assessments

Several methods have been used to test the normality of sperm chromatin and DNA. At present the most used tests are the TUNEL test (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling], the COMET assay (single-cell gel electrophoresis), and the SCD (sperm chromatin dispersion) test. The results of these tests are correlated with each other [20] and with sperm morphology, motility, and viability [2].

Although sperm chromatin assessments are often advocated in cases of inability to conceive by intercourse, intrauterine insemination, in vitro fertilization (IVF), and IVF using intracytoplasmic sperm injection, it is still controversial as to whether there is any relationship between the results of these tests and the specific reproductive problems [2].

Of note, currently both ROS determinations and sperm chromatin assessments are considered research procedures [2].

## 4.3 Thinking of the Man, Not Only of the Sperm: Potentials of Infertility Male Workup to Detect Underlying Abnormalities and Risk Factors for Male Health

It has been recently reported that infertile men are overall less healthy than fertile men [21, 22] and that poor semen quality may be a biomarker of general health, associated with worse survival [22].

While the above studies refer chiefly to comorbidities are not typically related to male infertility, two specific conditions more prevalent in infertile men are directly linked to male fertility: testicular germ cell tumors and elevated BMI. Testicular cancer has a 20-fold greater incidence in infertile men than in men with normal fertility [23], and semen parameters of men affected with testis tumor are altered in comparison with healthy controls [24]. Elevated BMI is in turn known to negatively correlate with sperm density [25], sperm motility, and sperm chromatin integrity [26].

Two other risk factors for poor male fertility, namely cigarette smoke [5] and low physical activity [25], though not more prevalent in the infertile male population, are worth mentioning because, along with elevated BMI, they are also well-known cardiovascular risk factors.

The andrologic evaluation of the infertile man has the extra benefit of opening a window on the general health of the man besides his fertility, with the potential to discover life-threatening conditions such as testis cancer, and to identify cardiovascular risk factors which, if corrected, may positively affect the health quality and survival of affected men (Table 4.4).

**Table 4.4** Life expectancy, principal conditions, and risk factors more prevalent in infertile men/men with poor semen parameters, versus fertile men

| Conditions | Reference |
|---|---|
| Increased mortality[a] | [22] |
| Testicular germ cell tumors | [23, 27–29] |
| Colorectal cancer | [30] |
| Melanoma | [30] |
| Prostate cancer | [30] |
| Cardiovascular disorders | [21] |
| Pulmonary diseases | [21] |
| Connective tissue disorders | [21] |
| Liver diseases | [21] |
| Diabetes mellitus | [21] |
| Body mass index | [21] |

[a]Increased mortality was due to a wide range of diseases and not particularly diseases related to lifestyle or socioeconomic status

## 4.4 When to Refer, How to Refer: Key Elements of the Referral Letter

At the conclusion of the andrologic diagnostic workup, the management of the male patient will depend on both the outcome of the workup itself and the possible presence of female infertility risk factors, including advanced female age (>35 years). If a female factor is present, priority should be given to a male treatment strategy that does not delay access to assisted conception programs.

The possible scenarios are summarized in Table 4.5.

As outlined in Table 4.5, a frequent outcome of the andrologic workup is a referral for assisted conception. When this occurs, it is important to write an appropriate referral letter to the assisted reproduction colleagues to adequately summarize the situation regarding the male partner.

Recently, an Italian panel of andrologists and gynecologists proposed a schematic referral letter, aimed to synthetically provide the ART physicians with all the key clinical information concerning the male partner [31]. These five sections of the proposed referral letter are outlined here.

1. *Heading*: This section should report the name and age of both partners
2. *Reason for referral*. This section should summarize the male reproductive history: for how long the man has tried to conceive with the present partner, and possible formerly induced pregnancies (and related outcomes) with the present and, if pertinent, previous partners. Furthermore, results of the male diagnostic evaluation should be summarized: nontreatable male factor infertility versus potentially treatable male factor infertility but suspect/presence of female factor infertility, versus unexplained infertility
3. *Summary of male workup*: Diagnostic conclusions (i.e., varicocele, hypergonadotropic hypogonadism, etc.)

**Table 4.5** Management priorities of the infertile couple, according to workup outcomes

| MFI | FFI | Management |
|---|---|---|
| +, treatable | – | Male treatment + reassessment |
| +, treatable | + | Male treatment + parallel referral to ART |
| +, not treatable | +/– | Referral to ART |
| – (unexplained infertility) | +/– | Referral to ART |

*MFI* male factor infertility, *FFI* female factor infertility, *ART* assisted reproduction treatment

4. *Indications on how to improve sperm quality*: For example, removal of identified risk factors (cigarette smoke, elevated BMI, etc.), specific treatments that may parallel ART (e.g., varicocele correction, genital inflammation treatment)
5. *Special notes*, *in case of azoospermia*: Type of azoospermia (obstructive vs nonobstructive), results of performed genetic testing, and suggestion on the most appropriate sperm retrieval procedure, in light of the specificity of the case

**Conclusions**

Male andrologic workup is mandatory when addressing a couple's reproductive difficulties. The presence of ejaculated sperm, even if sperm values are not below the lower recommended WHO thresholds, should not prevent the male from being evaluated by means of medical history, physical examination, and, possibly, ultrasonography.

Male investigation can allow identification of conditions and risk factors which, when corrected, may improve the chances of both spontaneous conception and success with ART. Furthermore, male andrologic workup may unveil underlying conditions that pose a previously unknown risk for male health.

The extent of male diagnostic investigations must be always appraised in light of the possible presence of female factor infertility.

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