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# CLINICAL REVIEW 100

# Evaluation and Treatment of the Infertile Couple*

GIANNI FORTI AND CSILLA KRAUSZ

*Andrology Unit, Department of Clinical Physiopathology, University of Florence, 50139 Florence, Italy*



THE increase in demand for infertility services in Western countries is probably due to different factors: 1) the tendency of women to delay childbearing because of their work so that desired reproduction is condensed into a shorter interval than before and at a more advanced age (*i.e.* >30 or even >35 yr), which by itself is a negative prognostic factor for the woman's fecundability; 2) an increase of effective treatments by assisted reproductive techniques (ARTs); 3) an increased awareness of such treatments. The prevalence of infertile couples differs according to the definition of couple infertility. If we accept the most commonly used definition, *i.e.* the lack of pregnancy after 1 yr of unprotected regular intercourse, infertile couples represent about 10–15% of all couples. According to the definition of the European Society for Human Reproduction and Embryology, *i.e.* the lack of pregnancy within 2 yr by regular coital exposure, the prevalence of infertile couples in Europe and North America is approximately 5–6% (1).

The causes of infertility can be divided into four major categories: 1) the female factor; 2) the male factor; 3) combined factors; 4) unexplained infertility. It is difficult to assign exact percentage to each of these categories; however, it is generally reported that in approximately 35% of cases, infertility is mainly due to a female factor, in 30% to a male factor, in 20% to abnormalities detected in both partners, and in 15% of cases no diagnosis can be made after a complete investigation (Table 1). In some couples there is no possibility of natural conception because of sterility of the male (azoospermia or lack of ejaculation) or of the female (ovarian failure, tubal occlusion, absence of the uterus). Minor degrees of fertility impairment are not necessarily associated with couple infertility when present in only one partner but may reduce the couple's fertility when present in both partners.

The aim of the present review is to give the general endocrinologist a brief overview of a modern diagnostic and therapeutic approach to the infertile couple.

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Address all correspondence and requests for reprints to: G. Forti, Andrology Unit, Viale Pieraccini 6, 50139, Florence, Italy. E-mail: g.forti@dfc.unifi.it.

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## Evaluation of the Infertile Couple

In the diagnostic approach, which should be clearly and thoroughly explained to both partners, the sexual history of the couple is very important. The frequency and timing of intercourse and the use of lubricants should be assessed. It happens frequently that an infertile couple abstains from intercourse and has only 'timed' exposures in the middle of the cycle, but there is no evidence that prolonged abstinence increases the chances of pregnancy: abstinence (7–8 days) should be reasonably recommended only if oligozoospermia is present (2). Use of lubricants should be discouraged because of their detrimental effect on semen quality.

After the sexual history is obtained, a detailed medical history and physical examination, followed by appropriate diagnostic tests, should be performed in both partners in a sequential way, as shown in Fig. 1.

### Female Partner

#### *Medical history and physical examination*

*Medical history.* Ovulatory dysfunction can be suggested by late menarch, presence of premenstrual syndrome, abnormal cycle length, amount of menstrual loss, premenstrual spotting, hot flushes (hypoestrogenism), and excessive physical exercise and/or weight changes (due to eating disorders) greater than 10% in the past year. Systemic diseases such as diabetes mellitus and thyroid dysfunction that are not adequately treated may also have adverse effects on fertility. Medical treatments may cause temporary (sex steroids) or permanent (cytotoxic agents, abdominal irradiation) damage to the ovulatory function. Neuroleptic, antidepressant, and hypotensive drugs and drugs for gastrointestinal symptoms can cause hyperprolactinemia. The use of recreational drugs such as marijuana and cocaine should also be investigated. Spontaneous galactorrhoea must be ascertained and further investigated for suspected hyperprolactinemia. The possible negative effect of environmental and occupational factors in relation to female fertility is under study. Although substances used in shoe, rubber, and textile manufacture have been reported to interfere with menstrual cycle, further controlled, prospective, and clinically detailed research is required (3).

The presence of a vaginal/cervical factor can be suspected by a history of recurrent vaginal infections.

Excessive menstrual losses and dysmenorrhea may suggest not only ovarian dysfunction but also the presence of a uterine fibromyoma.

**TABLE 1.** Approximate percentage distribution of diagnosis in infertile couples (modified from Ref. 1, Table 2)

| | Both partners | Only female | Only male |
|---|---|---|---|
| Sterile (%) | 0.3 | 10 | 6 |
| Subfertile (%) | 20 | 25 | 24 |
| Unexplained (%) | 15 | | |

| Definition | Female partner | Male partner |
|---|---|---|
| Sterile | Bilateral tubal occlusion<br>Amenorrhea with elevated FSH | Aspermia/azoospermia |
| Subfertile | Abnormal ovarian function<br>Unilateral tubal occlusion<br>Endometriosis<br>Cervical/immunological factors | Abnormal semen quality (oligo/astheno/teratozoospermia) |
| Unexplained | Normal endocrine profile<br>Tubal patency | Normal semen quality<br>Normal sexual function |

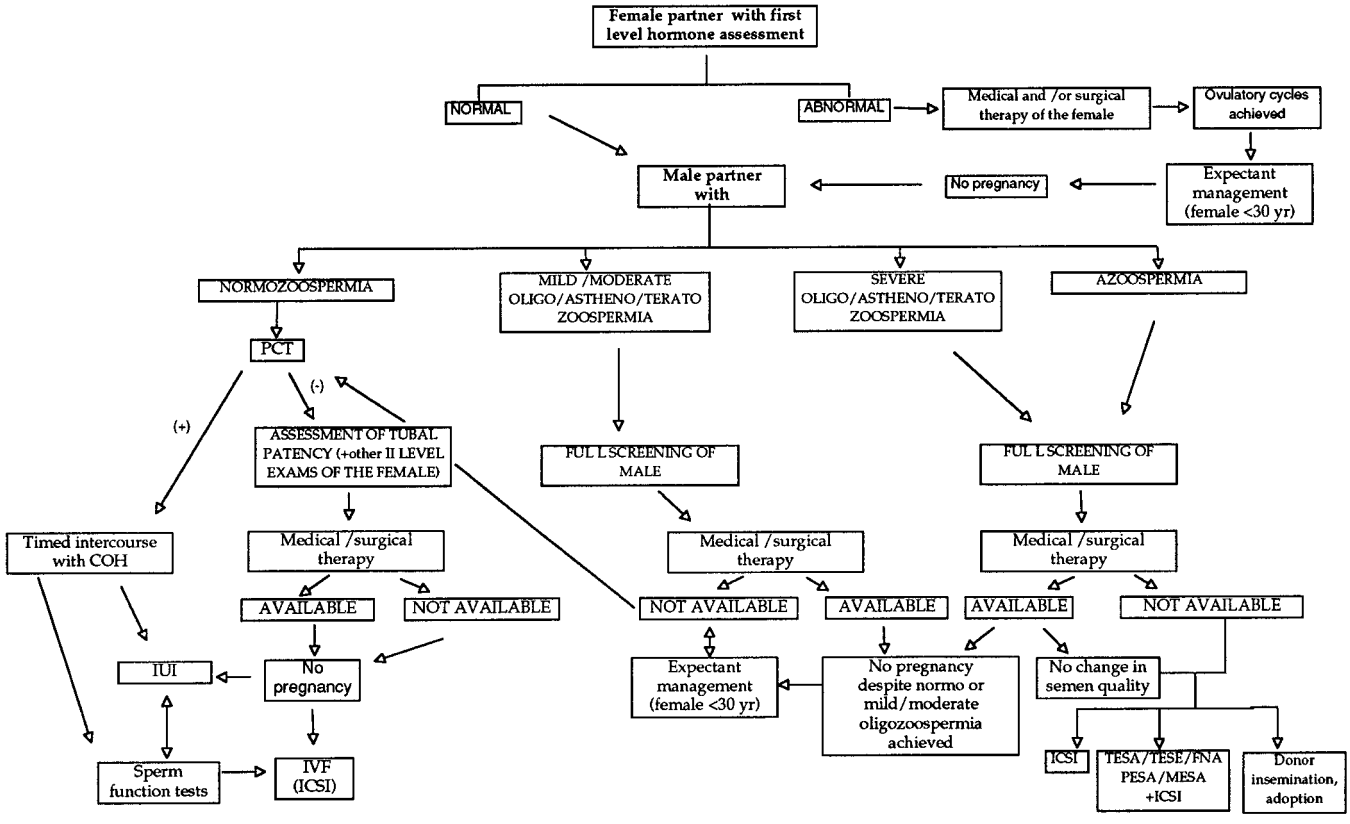


FIG. 1. Flowchart of diagnostic assessment and therapeutic options in couple infertility. PCT, Post-coital test; COH, Controlled ovarian hyperstimulation; IUI, intrauterine insemination; IVF, *in vitro* Fertilization; ICSI, intracytoplasmic sperm injection; TESA, testicular sperm aspiration; TESE, testicular sperm extraction; PESA, percutaneous epididymal sperm aspiration; MESA, microsurgical epididymal sperm aspiration.

Tubal obstruction can be suspected in women using intrauterine devices for contraception who have an increased risk of pelvic inflammatory disease (PID) and therefore of tubal disease. As the number of sexual partners increases the incidence of sexually transmitted diseases, it is also an important associative risk factor for the development of subsequent tubal infertility. Therefore, oral contraceptive users are also at risk of such pathology. Infections by *Chlamydia trachomatis* (4–6 times more frequent than by *Neisseria gonorrhoeae* in Western countries) are the most common causes of infection-related tubal pathology. Previous abdominal surgery, "complicated" appendicectomy, and gynecological operations (ovarian cystectomy, edge resection of the ovaries) all predispose to adhesion formation and increase the likelihood of tubal dysfunction. A history of tuberculosis can be associated with a diagnosis of tubal damage. Severe dysmenorrhea and deep dyspareunia may be related to endometriosis or PID.

In secondary infertility (*i.e.* the woman has previously been pregnant), details of previous pregnancies, including

abortions, miscarriages, and ectopic and molar pregnancies, must be recorded.

*Physical examination.* Physical examination should consider: 1) body shape and stature (short stature with webbed neck suggest Turner's syndrome); 2) calculation of the body mass index (BMI = ratio between weight in kilograms and height in square meters); 3) evaluation of secondary sexual characteristics (hair distribution, breast development, spontaneous and manually induced galactorrhea, inspection of external genitalia); 4) abnormalities such as fibromyomata, ovarian cysts, or fixed retroversion of the uterus can be diagnosed with a bimanual examination of the uterus associated with the palpation of the fornices. The vagina and the cervix must be examined, and any discharge should be further investigated for infection; 5) the cardiovascular, respiratory, and gastrointestinal systems must be carefully examined before pregnancy is planned.

## First-Level Diagnostic Tests

### *Hormonal assessment of ovulatory function*

Anovulatory infertility can be associated with oligo/amenorrhea but also with apparently normal menstrual cycles. Unfortunately, there is no established method to ascertain the completion of a normal ovulatory cycle in a woman. The most cost-effective compromise seems to be the measurement of midluteal progesterone concentrations in plasma (at least two assays) which must be >18 nmol/L (>5.6 ng/mL) and the measurement of basal body temperature for at least 2–3 months (3). In secondary amenorrhea or oligomenorrhea measurement of circulating estradiol or gestagen challenge, measurement of FSH, LH, and PRL (in the early follicular phase, if menses are present) are necessary. When hirsutism and/or acne is present, circulating androgens (testosterone, androstenedione, dehydroepiandrosterone sulfate) and 17-hydroxy-progesterone must be measured. According to the results, if 21-hydroxylase deficiency (usually the late-onset form) is suspected, an ACTH challenge test and/or a dexamethasone suppression test can be indicated. Polycystic ovary syndrome (PCOS) is likely in patients with BMI > 25, hirsutism, a LH/FSH ratio > 2, and high androgen levels. Transvaginal ultrasound scanning of the ovaries can be useful even if there is no general agreement about the ultrasound features of PCOS (4, 5).

In hyperprolactinemia, use of dopamine receptor-blocking agents or central nervous system dopamine-depleting agents must be excluded. TSH should then be measured to exclude primary hypothyroidism. Pituitary imaging with computerized tomography or nuclear magnetic resonance will confirm or exclude sellar (micro- or macroadenoma) or suprasellar organic pathology.

Although luteal phase defect has been clearly described by several authors, the currently available diagnostic tests (endometrial biopsy dating, daily progesterone measurements) are cumbersome and not properly validated.

Premature ovarian failure is suspected in women younger than 40 yr with high FSH levels (>40 IU/L) obtained at least 1 month apart and low estrogen levels. While karyotype is part of the assessment, the necessity of ovarian ultrasound, measurement of ovarian antibodies, and ovarian biopsy is open to question. In the case of suspected autoimmune polyglandular syndrome, screening for other endocrine abnormalities is mandatory.

### *Tubal patency assessment*

Evaluation of tubal patency can be considered a first-level diagnostic test in a normal cycling woman with a normozoospermic partner and a history of dysmenorrhea and/or dyspareunia, previous PID, or abdominal surgery. In order to assess tubal patency, hysterosalpingography (HSG) is usually performed in the early follicular phase. Since both false negative and false positive results have been reported, further investigation of tubal patency may be obtained by the more invasive and expensive laparoscopy. Retrograde tubal cannulation under radiographic guidance during HSG or laparoscopic inspection, completes the diagnosis of proximal tubal occlusion. Laparoscopy, in contrast to HSG, allows the identification of peritubal adhesions either of inflammatory origin or due to endometriosis. A direct visualization of the tubal lumen has been proposed by tubal catheterization via laparoscopy, hysteroscopy, or more recently, transcervically as an outpatient procedure (falloposcopy).

## Second-Level Diagnostic Tests

If normal ovulatory function is ascertained, disturbance in the passage of the oocyte and/or the spermatozoa must be considered at different levels of the female genital tract.

*Cervical factors.* All forms of vaginitis may include cervicitis, leading to a change in cervical mucus pH, which is detrimental to the motility and the ascent of sperm. If vaginal discharge and/or low abdominal pain is present, a bacteriological examination is necessary in order to exclude infection by common Gram-positive and Gram-negative bacteria, *Chlamydia trachomatis, Neisseria gonorrheae, Ureaplasma urealyticum,* and *Mycoplasma hominis*. In patients affected by recurrent cervicitis, colposcopy is recommended to rule out cervical metaplasia/dysplasia.

*Abnormal sperm-cervical mucus interaction.* The most commonly used technique for identifying this abnormality is the postcoital test (PCT). Before PCT is performed, vaginal infections must be excluded and optimal timing carefully assessed. A negative (abnormal) result of PCT is accepted as valid only if the mucus is of acceptable quantity and quality (score > 10) (6). The predictive values of normal and abnormal PCT results are rather poor, although a cut-off point of normality at the point of at least one motile spermatozoon per high power (×400) field may be a worthwhile option (7). If a pathological PCT result is found despite favorable cervical mucus score, the cervical secretion should be tested for qualitative and quantitative sperm antibody determination. The significance of female circulating antibodies has yet to be determined.

*Uterus abnormalities.* The diagnosis of infertility secondary to uterine abnormalities is one of exclusion. With the exception of uterine hypoplasia/agenesis, the relationship between congenital or acquired uterine defects (leiomyomata, intra-

uterine adhesions) with an impaired ability to conceive is open to question. On the other hand, uterine anomalies certainly play an important role in recurrent pregnancy losses (12–15%). The integrity of the uterus is usually assessed by pelvic and transvaginal ultrasound. Further information can be obtained by HSG and/or hysteroscopy.

*Tubal factors.* Congenital anomalies are less important than acquired conditions, such as adhesions caused by endometriosis and infections. Since many pelvic infections (especially *Chlamydia* infection) have no or only nonspecific symptoms, tubal patency should be assessed in all women with normal ovulation and normal PCT who do not achieve pregnancy within 6–12 months.

The assessment of tubal patency is not necessary in couples affected by severe male factor in whom *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) and embryo transfer (ET) are the only possible therapeutic options (see Table 2).

*Genetic assessment*

Karyotyping is indicated in women with primary or secondary amenorrhea with elevated serum FSH, in long-term unexplained infertility, and in recurrent spontaneous abortion.

*Recurrent early abortions/previous repeated failures of ARTs*

Autoantibodies against phospholipids such as anticardiolipin have been reported to reduce implantation and pregnancy rates after IVF and to be associated with recurrent early abortions. As the incidence of autoantibodies against phospholipids is much higher in infertile (range, 15–45%) than in fertile women (range, 1–4%), their measurement is advisable in women with previous failure of ARTs. However, standardization of methods of detection, quality control, and careful interpretation of positive results is required (8).

## Treatment of the Infertile Female

*Anovulatory infertility.* There is a large variety of conditions between persistent anovulation and normal ovulation. Irregular ovulation can be a cofactor, whereas chronic anovulation is obviously a definite cause of infertility. Changes in life-style can be simple, but necessary, measures in cases of obesity, malnutrition, overexercise, and drug use. Specific treatments apply if anovulation is due to hormonal abnormalities. The four most frequent conditions are normogonadotropic, hypergonadotropic, hyperprolactinemic, and hypogonadotropic anovulation.

*Normogonadotropic anovulation.* These women (usually with PCOS) have normal FSH levels but LH may be elevated. In overweight patients (BMI $>$ 25) the treatment of choice is an antiestrogen such as clomiphene citrate or tamoxifen combined, when possible, with weight reduction. There is no documented advantage for sequentially combining clomifene with human (h) CG or estrogen. Recently, the insulin-sensitizing agent troglitazone has been reported to improve ovarian function in women with PCOS and therefore proposed as a novel therapy (9). In women unresponsive to clomiphene ($\sim$50%), laparoscopic ovarian electrocautery can be proposed: although the cumulative pregnancy rate reported after this type of treatment has been reported to be around 50%, ensuing risks of postoperative adhesions must be considered (10). A most commonly used alternative therapy is the administration of pulsatile GnRH or human purified or recombinant FSH to induce multiple follicular growth followed by hCG and timed intercourse or an ART.

**TABLE 2.** Definitions of the most used assisted reproductive techniques

| | |
|---|---|
| Intrauterine insemination (IUI): | Spermatozoa previously selected are placed in the uterine cavity using a catheter after ovulation. IUI represents the first step in the treatment of young couples with no evidence of tubal damage or severe male factor. |
| *In vitro* fertilization (IVF) and embryo transfer (ET) | After controlled ovarian stimulation, follicular aspiration is performed under transvaginal ultrasound guidance, and the oocytes are incubated for 3–6 hr and then inseminated with sperm *in vitro*. Two days after oocyte recovery, fertilized oocytes should have cleaved to embryos containing two to eight cells. After grading, two to three embryos are transferred in the uterus. IVF was started to treat infertility due to tubal impairment, but is largely used in many other forms of infertility. |
| Gamete intrafallopian transfer (GIFT) | This technique is an alternative to IVF-ET in women with normal fallopian tubes and involves the direct placement of sperm and oocytes into the ampullary portion of the tube, allowing IVF to occur at the natural site. A laparoscopic procedure under general anesthesia is usually needed. |
| Zygote intrafallopian transfer (ZIFT) | The technique involves IVF and transfer of the zygotes to the fallopian tube. |
| Intracytoplasmic sperm injection (ICSI) | Direct injection of a single sperm into the cytoplasm of an oocyte. This technique is indicated in severe male factor. It is also used in azoospermic patients after retrieval of spermatozoa from the epididymis by microsurgical sperm aspiration (MESA) or from the testis by testicular sperm aspiration (TESA) from a needle biopsy or by testicular sperm extraction (TESE) from an open biopsy. |

Instead of traditional high-dose step-up regimen (which represents a higher risk of ovarian hyperstimulation syndrome), new schedules have been suggested in order to recruit as few follicles as possible and to allow selection and dominance to occur (11).

*Hypergonadotropic anovulation (premature ovarian failure).* In women with persistently high FSH levels (>40 IU/L), no treatment has been prospectively proven to restore ovulation; however, spontaneous, transient remission can occur. Adoption or ovum donation could be an option for these couples. In an early phase of premature ovarian failure, when the woman is still cycling and FSH levels are rather high, but not in the menopausal range (*i.e.* 15–25 IU/L), a controlled ovarian hyperstimulation can be proposed both for an IVF attempt (although with a poor success rate) and/or in order to cryopreserve the oocytes for later ARTs in appropriate laboratories. (Cryopreservation of oocytes, however, has not yet obtained good results.) A follow up for subsequent autoimmune polyglandular syndrome or non-organ-specific autoimmunity is recommended.

*Hyperprolactinemic anovulation.* The treatment of first choice for pituitary hyperprolactinemia (either functional or due to micro- or macroprolactinoma) is bromocriptine. Transphenoidal surgery can be an alternative option for microprolactinomas or can be necessary if shrinking of a macroadenoma does not occur (12). Chemically releated dopamine agonists such as pergolide and cabergoline may be suggested in cases of intolerance to bromocriptine. If cycling does not return despite normalized PRL concentrations, the pituitary-thyroid and pituitary-adrenal axis function should also be checked. If pituitary function is normal, dopamine agonists can be combined with antiestrogens to induce ovulation.

*Hypogonadotropic anovulation.* Underweight patients (BMI < 19) who are malnourished and/or who have exercised excessively must be counseled and must resume weight before pharmacological treatments. If primary pituitary failure is present, ovulation may be induced with gonadotropins. If the pituitary failure is secondary to hypothalamic dysfunction, pulsatile GnRH therapy is the most appropriate.

At this time no association has been demonstrated between infertility and luteal phase defect detected by histological or hormonal evaluation. In any case, in women with suspected luteal insufficiency progesterone supplementation may be attempted during the luteal phase for three to six cycles.

*Tubal infertility.* If semen analysis is normal, surgical tubal reconstruction (ovariosalpingolysis, salpingoneostomy, fimbrioplasty) can be considered before IVF is attempted. Since the success of surgery depends on the tubal mucosal appearance, in the case of damaged mucosa or impaired semen parameters, IVF-ET is the first treatment choice. On the basis of hormonal characteristics of the woman, several ovarian stimulation protocols are available for IVF.

*Endometriosis.* Women with minimal to mild endometriosis represent the majority of endometriosis-associated infertility. These women are usually treated with ovarian suppression either with GnRH agonists or with danazole, medroxyprogesterone acetate, oral contraceptives, and gestrinone. Some randomized clinical trials have compared ovulation suppression *vs.* placebo therapy and have shown no evidence of a treatment effect. The aggregate pregnancy rates reported after withdrawal of treatment were 41% in women with placebo therapy and 41%, 40%, 36%, 35%, and 28% in women treated with medroxyprogesterone acetate, oral contraceptive, GnRH agonist, danazole, and gestrinone, respectively (1). Severe endometriosis associated with tubal obstruction, ovarian endometriomas, extensive adhesions, and cul-de-sac obliteration are indications for surgical treatment. Compared with ovulation suppression or no therapy, surgical treatment has a significant effect; however, further controlled trials are needed for final conclusions (1).

*Vaginal and cervical factors.* Infection or inflammation of vagina and/or cervix could necessitate medical therapy. In the case of cervical metaplasia/dysplasia, electroresection or laser surgery is recommended. In patients with a negative (abnormal) PCT and the presence of antisperm antibodies, intrauterine insemination (IUI) is recommended.

*Uterus abnormalities.* Intrauterine septa or submucosal fibroids should preferably be treated by hysteroscopic electroresection. Excision of intramural or subserosal myomas must be done by macrosurgery.

*Recurrent early abortion.* If antiphospholipid antibodies are present, heparin and aspirin treatment is the therapy of choice. However, in patients treated by aspirin/heparin and undergoing IVF, the higher implantation and pregnancy rates reported by several authors were obtained in noncontrolled studies. Therefore, prospective, randomized clinical trials are needed to evaluate the role of antiphospholipid antibodies in female infertility and the effects of heparin and aspirin therapy on IVF outcome (8).

### *General cautions for ovulation induction*

Before any ovulation induction, a pelvic ultrasound examination is necessary in order to exclude an ovarian mass and/or uterine abnormalities. In the case of clomiphene treatment the woman should be monitored monthly by ultrasound in order to control persistent ovarian follicular enlargement. There is no need to monitor follicle growth during treatment with antiestrogen and the pulsatile administration of GnRH. Ovarian stimulation with human gonadotropins [human menopausal gonadotropin (hMG)/FSH + hCG] is usually performed in patients undergoing ARTs and can be required also in patients with ovulatory disorders unresponsive to other therapies. Since ovarian hyperstimulation syndrome is a recognized complication of such treatment, a careful ultrasound monitoring every 2 days and/or plasma estradiol measurement are mandatory. However, the number of preovulatory follicles and the estradiol concentrations suggesting the risk of ovarian hyperstimulation at the time of hCG injection are different from center to center (11).

### *Ovulation induction and ovarian cancer risk*

Available data in the literature do not resolve the question concerning a causal relationship between fertility drugs and

ovarian cancer. It seems to be that infertility alone is an independent risk factor. Large prospective studies with carefully selected control groups are needed. Until such data are forthcoming, close clinical surveillance of patients with ultrasound or other imaging techniques before, during, and after treatment of infertility is warranted (13).

## Male Partner

*Medical history and physical examination*

*Testicular dysfunction.* Testicular dysfunction due to acquired gonadotropin defect is associated with few symptoms such as the reduction of the volume of the ejaculate, reduction of beard growth and libido, and asthenia. On the other hand, a eunuchoid habitus with infantile genitalia, sparse or nearly absent body hair, gynecomastia, and low testicular volume (5–10 mL) is typical of congenital gonadotropin deficiency, which can be associated with hypo- or anosmia in Kallmann's syndrome. The assessment of testicular volume by scrotal palpation and comparison with ellipsoids of known volume (Prader's orchidometer) is very important in the infertile male, as the seminiferous tubules represent approximately 80–85% of the testicular mass. Therefore, a small testicular volume (<15 mL) suggests a significant impairment of the seminiferous tubules.

*Nonendocrine testicular dysfunction.* Nonendocrine testicular dysfunction can be suspected in men with varicocele or a history of excess alcohol consumption, drug abuse, human immunodeficiency virus infection, occupational exposure to toxicants (lead, arsenic), ongoing medical treatments (anabolic steroids, cancer chemotherapy, sulfasalazine, nitrofurantoin), high fever in the past 6 months, testicular injury, mumps orchitis, surgery for varicocele, and cryptorchidism. Small testes (<15 mL) can be observed in these men. In men with extremely small (<5 mL), firm testes, with or without eunuchoid habitus and gynecomastia, Klinefelter's syndrome is suspected.

*Impairment of sperm transport and/or accessory gland infections.* Impairment of sperm transport and/or accessory gland infections can be suggested by a history of sexually transmitted disease, epididymitis, prostatitis, urinary infection, or inguinal surgery. These men usually have a normal testicular volume. Signs of epididymal inflammation such as thickening, nodules and/or pain can be also found at scrotal palpation. Absence of the vas deferens can be also suggested, in skilled hands, by scrotal palpation. Examination of the prostate gland by digital rectal examination may be omitted if there is no history, physical signs, or indication from urine or semen analysis that the patient may have any disease of the accessory sex glands.

The presence of penile abnormalities such as hypospadias, surgical or traumatic penile scars, and induration plaques should be assessed in every subject.

## First-Level Diagnostic Tests

*Semen analysis.* In the investigation of a couple at least one semen analysis is always mandatory and should be performed according to the World Health Organization recommended procedure (14). The manual provides normal values (Table 3) as well as nomenclature for normal and pathological findings (Table 4). Normal values help to identify patients who should have no difficulty in inducing a pregnancy; however, unless there is a severe oligozoospermia or azoospermia, the predictive value of subnormal semen variables is limited. The introduction of computer-assisted semen analysis, which requires expensive and sophisticated equipment, has not yet led to any substantial improvement in diagnosis and must be still considered mainly a research tool (1).

If the first semen analysis is normal, there is generally no need for a repeat analysis. In all other circumstances in which an abnormal semen sample is obtained, an additional semen sample should be examined after an interval of 6–12 weeks (spermatogenesis takes ~3 months to be completed) because of the large variability of semen parameters (14). In azoospermia, after centrifugation of the semen sample, a careful analysis of the pellet is also necessary.

When azoospermia is present, obstruction should be distinguished from seminiferous tubular damage (see also Fig. 2B). A reduced bilateral testicular volume, (<10–12 mL), with normal volume ejaculate (>2 mL) and high FSH is indicative of damaged spermatogenesis. Obstructive

**TABLE 3.** Normal values of semen parameters according to WHO (1992)

| | |
|---|---|
| Standard tests | |
| Volume | >2ml |
| pH | 7.2–8.0 |
| Sperm concentration | $>20 \times 10^6$ spermatozoa/mL |
| Total sperm count | $>40 \times 10^6$ spermatozoa/ejaculate |
| Motility | >50% with forward progression (categories a and b) or >25% with rapid progression (category a) within 60 min of ejaculation |
| Morphology | >30% with normal forms |
| Vitality | >75% or more live, *i.e.* excluding dye |
| White blood cells | $<1 \times 10^6$/mL |
| Immunobead test | <20% spermatozoa with adherent particles |
| Mixed agglutination reaction test | <10% spermatozoa with adherent particles |
| Optional tests | |
| $\alpha$-Glucosidase (neutral) | >20 mU per ejaculate |
| Zinc (total) | >2.4 $\mu$mol per ejaculate |
| Citric acid (total) | >52 $\mu$mol per ejaculate |
| Acid phosphatase (total) | >200 U per ejaculate |
| Fructose (total) | >13 $\mu$mol per ejaculate |

**TABLE 4.** Nomenclature for normal and pathological findings in semen analysis according to WHO (1992)

| | |
|---|---|
| Normozoospermia | Normal ejaculate (as defined in Table 2) |
| Oligozoospermia | Sperm concentration $<20 \times 10^6$/ml |
| Asthenozoospermia | <50% spermatozoa with forward progression (categories a and b) or <25% spermatozoa with category a movement |
| Teratozoospermia | <30% spermatozoa with normal morphology |
| Oligo-astheno-terato-zoospermia | Signifies disturbance of all three variables (combination of only two prefixes may also be used) |
| Azoospermia | No spermatozoa in the ejaculate |
| Aspermia | No ejaculate |

azoospermia is usually characterized by a normal testicular volume and normal FSH levels. In these patients, very simple semen parameters such as lack of coagulation, low volume of the ejaculate, and acidic pH can suggest congenital bilateral absence of vas deferens (CBAVD) and seminal vesicles, a condition that is associated with a high incidence of mutations in the cystic fibrosis gene. In CBAVD as well as in the rare cases of acquired ejaculatory duct obstruction, seminal fructose, which is produced by seminal vesicles, is usually very low. Low levels of seminal $\alpha$-glucosidase, the most frequently used epididymal marker, are present in CBAVD, in men with epididymal obstruction, but also in some men with azoospermia due to tubular damage (15).

In astheno/oligoasthenozoospermic patients, immunological infertility and/or infection of sex accessory glands may be suspected (see also Fig. 2A). If the percentage of motile spermatozoa coated by antisperm antibodies is more than 50%, a pure immunological factor is likely and titration of sperm antibodies in serum will add weight and confirm the diagnosis. If semen has high viscosity, pH > 8, and more than $10^6$ leukocytes/mL and/or reduced seminal levels of prostatic markers (acidic phosphatase, citric acid, zinc), accessory gland infection (prostatitis or prostatovesiculitis) is likely. The clinical significance of isolated leukocytospermia as well as the role of subclinical genital infections in male infertility is controversial (16).

*FSH, LH, and testosterone measurement.* The most important hormonal measurement in the infertile male, both from the diagnostic and prognostic point of view, is serum FSH (see also Fig. 2, A and B). High FSH levels and normal LH and testosterone levels are present in the majority of normally virilized infertile men with sperm concentration lower than $5 \times 10^6$/mL and are usually related to the entity of spermatogenetic damage (17). Low levels of FSH, LH, and testosterone suggest acquired hypogonadotropic hypogonadism, which might be due to a prolactinoma or a nonfunctioning pituitary tumor. Serum inhibin B levels have been recently reported to be inversely related to FSH in infertile men, suggesting that inhibin B levels reflect Sertoli cell function (18). However, the diagnostic value of inhibin B measurement in the routine assessment of male infertility has still to be evaluated.

Using this approach (medical history and physical examination with testicular volume assessment, semen analysis, and hormone measurement), a definite diagnosis of the cause of male infertility can be obtained in approximately 70% of cases (19). Further investigation may be warranted, based on the initial findings and the therapeutic approach recommended to the couple (see also Figs. 1 and 2, A and B).

## Second-Level Diagnostic Tests

*Bacteriological examination, transrectal, and scrotal ultrasound.* In men with asymptomatic leukocytospermia and a positive bacteriological examination, some ultrasonographic criteria have been considered indicative of chronic prostatitis (glandular asymmetry, areas of calcification, dilation of the periprostatic venous plexus) or vesiculitis (enlargement and asymmetry, thickening and calcification of the glandular epithelium) (20). Due to its high cost, transrectal ultrasound is a complementary diagnostic tool used mainly in countries with socialized health care (European countries), whereas in the United States, because it does not influence the antimicrobial treatment of the infection, it is a debatable option.

In a prospective study of 1048 consecutive patients attending a fertility clinic, scrotal sonography showed abnormalities of the scrotal content in 50.4% of them. Most abnormalities were benign (varicocele, increased size of the epididymis, epididymal cysts, hydrocele, testicular cysts); however, the 1:200 incidence of testicular tumors in that study was much higher compared with the 1:20.000 incidence in the general male population (21). Therefore, testicular ultrasonography can be suggested as a diagnostic tool especially in patients with an increased risk of malignancy, *e.g.* those with a previous history of cryptorchidism.

*Genetic assessment.* Chromosome abnormalities are much more frequent in infertile males (5.3%) than in the general population (0.6%) (22); therefore, karyotype must be performed in men with azoospermia and severe oligozoospermia if FSH levels are increased and testicular volume is markedly reduced. The most frequent abnormalities are sex chromosome aneuploidies such as the 47,XXY and the 47, XYY karyotype (1:500 and 1:750 newborns, respectively), autosomal Robertsonian translocations, and other types of translocations. Of the various genes considered critical for the regulation of male fertility, genes on the long arm of the Y chromosome (Yq), especially within deletion interval 6, also known as Yq 11.23, are the most promising. Most macroscopic deletions in Yq11 are associated with impaired spermatogenesis, but microdeletions of three regions of the Y chromosome were found recently in a large sample of oligozoospermic and azoospermic men, and the regions were named azoospermia factor AZFa, AZFb, and AZFc, respectively; within the AZFc region a gene family termed DAZ (deleted in azoospermia) has been identified. AZFb deletion intervals include members of a related gene family (RBM, for RNA binding motif) which, like DAZ, are predicted to encode testis-specific RNA binding proteins (23).

The frequency of microdeletions of these regions of the Y

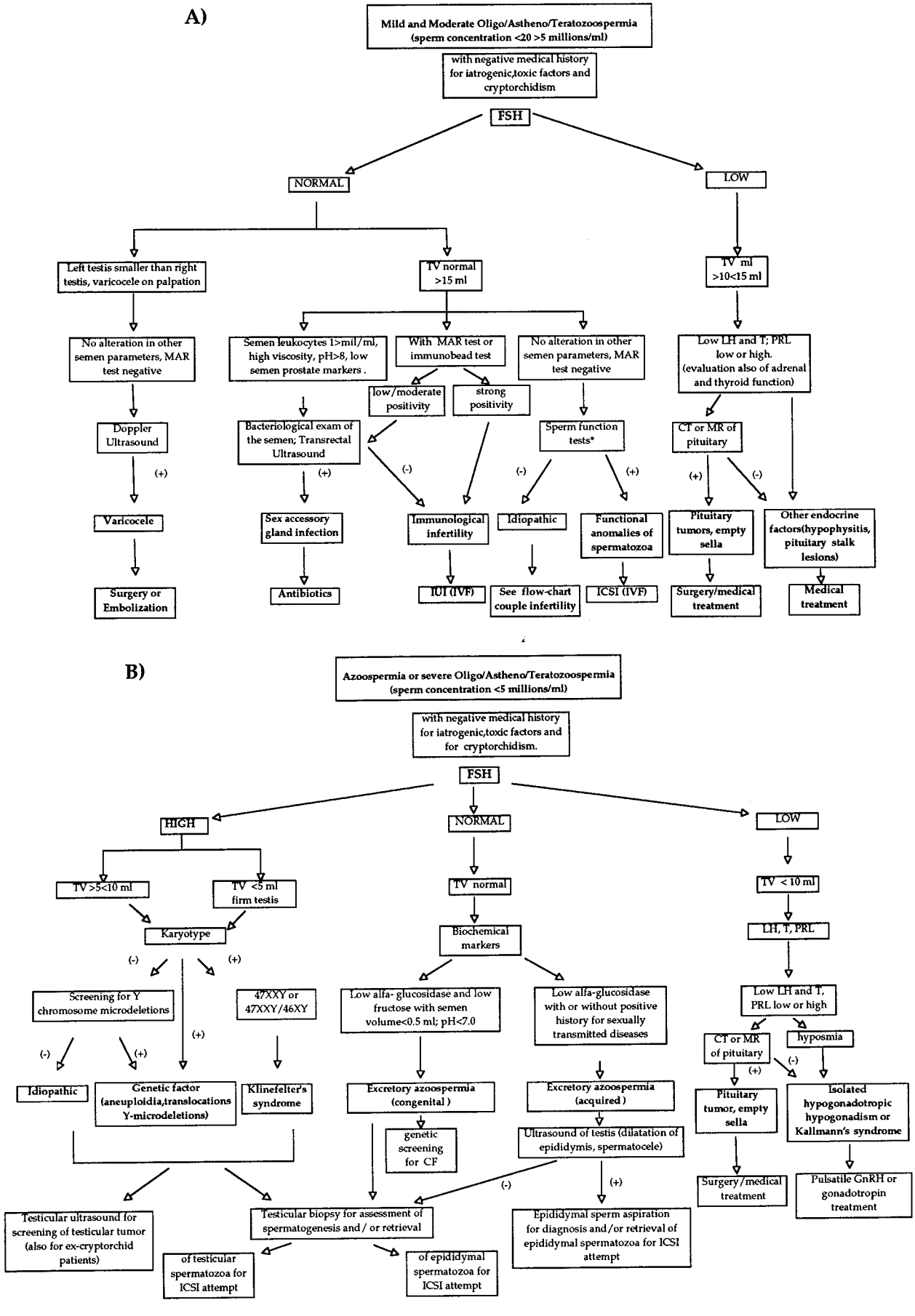

**TABLE 5.** Main characteristics of some sperm function tests: correlation (*r* value) with *in vitro* % fertilization rate of oocytes, sensitivity, specificity, positive and negative predictive values (PPV and NPV)

| Test | Correlation with % fertilization rate (*r* value) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|
| Morphology by strict criteria | 0.31 | 94 | 40 | 22 | 98 |
| Hemizona assay | 0.75 | 100 | 94 | 85 | 100 |
| AR induction by A23187 | 0.30 | 85 | 58 | 87 | 53 |
| AR induction by follicular fluid | 0.39 | 55 | 71 | 75 | 49 |
| AR induction by progesterone | 0.49 | 89 | 60 | 91 | 64 |
| $[Ca^{2+}]_i$ induction by progesterone | 0.44 | 96 | 53 | 89 | 77 |

AR, Acrosome reaction; $[Ca^{2+}]_i$, intracellular free calcium concentration.
(Adapted from Ref. 28).

chromosome reported by several authors in azoospermic and severely oligozoospermic males ranges between 3 and 18% (24). Men with deletions in peripheral blood lymphocytes also have the deletions in ejaculated sperm.

In patients with CBAVD, which is now considered a mild form of cystic fibrosis, screening for cystic fibrosis gene mutations should be performed since mutations of this gene have been reported in approximately 70–80% of such patients, the most frequent being the ΔF508 mutation (25). Due to the high carrier frequency in Europe and North America (1:25) genetic screening should also be done in the female partner especially if an ART attempt (usually epididymal sperm aspiration coupled to ICSI) is planned.

*Third-level diagnostic tests*

*Testicular biopsy.* Until recently testicular biopsy was a procedure designed to confirm a normal spermatogenesis in patients with suspected obstructive azoospermia (with normal FSH and normal testicular volume) before reconstructive microsurgery. Today, testicular biopsy can be considered more a therapeutic than a diagnostic procedure. Testicular sperm can be obtained from testicular biopsies of men with azoospermia caused by obstruction, maturation arrest, or Sertoli cell-only Syndrome and can be successfully used for ICSI treatment (26). There is no general agreement concerning the most successful way for testicular sperm retrieval. Some authors found open testicular biopsy to be the best way (27), but fine-needle testicular aspiration or testicular sperm extraction, in expert hands, seems to be a most effective alternative. Cryopreservation of a fraction of retrieved testicular spermatozoa is suggested for further ICSI cycles.

*Sperm function tests.* As routine semen analysis provides little information on sperm-fertilizing ability and the percentage of fertilization failure in ARTs is rather high in cases where the male partner is subfertile, the possibility of predicting the sperm-fertilizing ability would be of great help in choosing the most appropriate ART. Several sperm function tests have been reported to highly predictive of the sperm's ability to fertilize human oocytes *in vitro* (28). As shown in Table 5, the best results are obtained with the hemizona assay, which measures the ability of spermatozoa to bind to the zona pellucida of a human oocyte, but the scarcity of human oocytes makes this test unlikely to be used for routine purposes. A good alternative option seems to be the sperm responsiveness to progesterone. Progesterone is able to induce, through a nongenomic mechanism, both intracellular calcium increase and acrosome reaction in human spermatozoa. We have recently reported, in a large group of unselected couples, that increases both in acrosome reaction and intracellular free calcium concentration in response to progesterone were good predictors of IVF rate (28).

## Treatment of the Infertile Male

In contrast to infertile females, only a small percentage of infertile or subfertile males can undergo a rationale, effective treatment. Before considering specific treatments, however, we must remember that expectant management can be an option if the duration of infertility is less than 3 yr and the woman's age is less than 30 yr, because the fecundity of the woman can often compensate for the presence of low sperm concentration (even $< 5 \times 10^6$/mL) and motility (<20%) (29, 30).

*Rationale treatment (medical or surgical)*

In the few patients in whom infertility is due to hypogonadism secondary to pituitary or hypothalamic failure, treatment with hMG + hCG or pulsatile GnRH, respectively, is highly effective in achieving sperm quality sufficient to induce a pregnancy. If infection of the sex accessory glands is present, appropriate treatment with antibiotics must be performed in both partners. Disorders of ejaculation may be treated in various ways, *e.g.* by electroejaculation or by recovery of spermatozoa from urine coupled to an ART. Obstructive azoospermia may be further investigated with vasography, and in some cases microsurgical reconstruction or anastomosis can give good results. Better results, however, are usually obtained with microsurgical epididymal sperm aspiration and ICSI (see below). Concerning varicocele there is still controversy about whether treatment improves male fertility, and of the four published controlled, prospective randomized trials recently reviewed, treatment improved fertility in two and had no effect in the other two (31). However, there is some consensus that, in view of the apparent progressive nature of this condition, intervention should be suggested 1) in adolescents as a preventive measure if the testicular volume of the affected size is reduced 3 mL or more

FIG. 2. Flowchart of diagnostic assessment and therapeutic options in the male partner of an infertile couple affected with (A) mild oligoasthenozoospermia or (B) azoospermia (or severe oligozoospermia). TV, Testicular volume; MAR test, mixed agglutination reaction test (see also Table 2); T, testosterone; CT, computerized tomography; MRI, magnetic resonance imaging; CF, cystic fibrosis. *, See also flowchart for couple infertility (Fig. 1): sperm function tests are required mainly for appropriate selection of an ART.

compared with the contralateral testis, and 2) in young infertile couples (both partners < 30 yr), with a duration of infertility lower than 3 yr if the male partner has subnormal semen analysis (31).

*Empirical treatment*

In patients with idiopathic semen abnormalities, different kinds of empirical pharmacological treatments have been tried: androgens, hMG/hCG, clomiphene, bromocriptine, aromatase inhibitors, mesterolone, tamoxifene, kallicrein. None of these treatments, however, has been demonstrated to be effective in controlled, double-blind randomized studies (32).

*ARTs*

It is obvious that when few sperm are available, the chances of sperm-egg interaction *in vivo* are reduced. To overcome this difficulty, initial attempts have been made to bring selected sperm closer to the oocyte by IUI. The clinical effectiveness of IUI with sperm selected by appropriate techniques in couples with male factor is not significantly different from no treatment; however if IUI is coupled to induction of multiple ovulation, a significant increase of pregnancy rates occurs (1). The results of IUI are also related with the number and quality of inseminated sperm. Usually a minimal number of selected motile sperm ($1–2 \times 10^6$) is required for an IUI attempt. If pregnancy does not occur within the first three cycles, further IUI cycles should not be performed. IVF for male factor is a further possible option, with a success rate depending mainly on the woman's age (see Table 6). Gamete intrafallopian transfer and zygote intrafallopian transfer have been reported to be more effective than IVF, but are less used techniques because tubal transfer of the gametes (or the embryo) requires a laparoscopic procedure. In couples in which IVF of oocytes does not occur and in patients with severe oligozoospermia and/or severe teratozoospermia or asthenozoopermia, ICSI can be an effective approach. ICSI has been successfully used in obstructive azoospermia with spermatozoa obtained by epididymal microsurgery (microsurgical epididymal sperm aspiration) or percutaneous aspiration (percutaneous epididymal sperm aspiration). ICSI can be also performed with success with testicular spermatozoa obtained by multiple (fine) needle aspiration (testicular sperm aspiration) or extensive open testicular biopsies (testicular sperm extraction) in 50–70% of men with azoospermia due to testicular damage, reduced testicular volume, and high FSH levels. The implantation rates obtained with ICSI performed with ejaculated, epididymal, and testicular spermatozoa are substantially comparable to the results of standard IVF treatments in infertile couples with pure female factor and unexplained infertility (33). The multiple, very complex aspects of ICSI for male factor infertility have been recently reviewed (33).

Flow charts of step-by-step diagnostic assessment and therapeutic options in the oligoasthenozoospermic and azoospermic (or severely oligozoospermic) male are reported in Fig. 2, A and B, respectively.

*Unexplained infertility*

When routine infertility workup yields normal results in both partners, the couple is defined as suffering from unexplained infertility. However, the extent of the routine infertility workup has never been established thoroughly. A basic protocol must include history and physical examination of both partners, hormonal profile including midluteal progesterone estimation in the woman and semen analysis in the man. If no abnormalities are found, PCT, HSG, and/or laparoscopy can be considered second-level tests. Nevertheless, as reported above, additional techniques are available to further the infertility investigation: bacteriological, immunological screening, genetic assessment, and sperm function tests. A deep understanding of sperm biology/biochemistry and the molecular mechanisms of sperm-egg interaction can lead to a more accurate identification of oocyte/sperm anomalies responsible for impaired fertilizing capacity in couples presenting unexplained infertility (34, 35).

More than 30% of couples with unexplained infertility will become pregnant within 3 yr of expectant management (30). However, in case of long-standing, unexplained infertility, with a female partner more than 30 yr of age it is difficult to apply this approach. The most effective treatment seems to be superovulation combined with one ART (IUI, IVF) giving nearly 2 times higher pregnancy rate compared with that obtained by superovulation alone (36).

## Role of the Endocrinologist in the Management of the Infertile Couple

According to these guidelines, it is rather evident that the general endocrinologist can easily perform a first-line diagnostic evaluation of the infertile couple as the hormonal assessment has an important role in the initial workup of both partners, and semen analysis can be easily interpreted. It is also rather obvious that if the infertility has an endocrine cause (*e.g.* hypogonadotropic hypogonadism, PCOS, or hyperprolactinaemia) the endocrinologist has an important role in the treatment. On the other hand, further diagnostic workup of the couple requires appropriate knowledge of the physiology and pathology of the male and female reproductive tract as well as of second- and third-level diagnostic procedures. Such experience can be acquired only by endocrinologists who want to focus their activity in the area of reproduction.

**TABLE 6.** The table summarizes the outcome of all IVF procedures performed in the United States and Canada in 1994

| Category | No. of IVF cycles with retrieval | No. of deliveries | % Deliveries for retrieval |
|---|---|---|---|
| Women <40 yr with no male factor | 14,990 | 3671 | 24.5% |
| Women ≥40 yr with no male factor | 2709 | 243 | 9.0% |
| Women <40 yr with male factor | 4485 | 908 | 20.2% |
| Women ≥40 yr with male factor | 866 | 119 | 8.5% |

The negative effect of female age and male factor diagnosis (when the woman's age is <40 yr) is evident (Adapated from Ref. 40).

A flowchart showing the sequential steps of the diagnostic assessment and the therapeutic options in couple infertility is shown in Fig. 1.

### Summary and Conclusion

Infertility by itself does not threaten physical health but has a strong impact on the psychological and social well-being of couples. In the last two decades, progress in caring for the infertile couple, in particular progress in the field of assisted reproduction and micromanipulation, has provided significant hope for many couples for whom hope could not have been offered in the past. This is especially true for bilateral tubal disease and for male factor infertility, as nearly all couples with male factor infertility can now undergo either one (or more) IVF or ICSI attempt(s). For couples with other causes of infertility, however, the differences in pregnancy rates often do not reach statistical significance (37). We must also remember that the total cost incurred for successful delivery for couples with a better chance of successful IVF (*i.e.* those with tubal disease) increases from approximately $55,000 in American dollars for the first cycle to $73,000 by the sixth cycle (38). Because of these high costs, many insurers in the United States and many public health systems in Europe do not cover or only partially cover these procedures. Consequently, the availability of IVF and related therapies frequently depends on the couple's ability to pay. Therefore, after having established the correct diagnosis, appropriate treatment should be counseled to the infertile couple keeping in mind the following points: 1) in subfertile couples expectant management should be reasonably counselled if the age of the woman is less than 30 yr and the duration of infertility is less than 36 months, even if oligozoospermia is present (30) (Fig. 3); 2) superovulation and timed intercourse seems also to be a reasonable approach in couples with anovulatory, mild/moderate endometriosis, and unexplained infertility (30, 39); 3) in unexplained infertility, ovarian stimulation (with clomiphene or gonadotropin) and IUI seem to offer some advantage over ovarian stimulation and timed intercourse (1); 4) IVF can be a first-line approach in tubal sterility and when IUI or IPI cannot be performed because the number of motile sperm is insufficient, but is usually also the final treatment attempt when other methods have failed. The outcome of IVF is negatively influenced mainly by the woman's age; however, the number of deliveries is also generally lower in couples with male factor (40) (Table 6); 5) ICSI is a further option, which should be limited to couples: a) with very poor semen parameters; b) previous failed fertilization; c) presence of obstructive or nonobstructive azoospermia in which ICSI is combined with sperm extraction from the epididymis or the testis; 6) international register studies demonstrate that the risk of malformation after conventional IVF is not increased (41); 7) some reports suggest that incidence of congenital major and minor malformations is not increased in children born after ICSI (42). However, the rate of sex chromosome anomalies in ICSI fetuses has been reported to be approximately 1% in 585 prenatal diagnoses (43), a frequency increased by a factor of 4 if compared with naturally conceived live-born babies. ICSI bypasses the physiological selection of spermatozoa that occurs at the level of the testis and epididymis, and in the female reproductive tract as well as at the sperm-oocyte interface. As genetic abnormalities are present in a significant percentage of infertile males with impaired spermatogenesis, karyotyping and analysis of the Y chromosome for microdeletions should be carried out in all potential ICSI fathers. Screening for cystic fibrosis gene mutations should also be performed in azoospermia caused by congenital absence of the vas deferens and seminal vesicles. Appropriate genetic counseling should be made available to all ICSI couples whenever a gene or chromosomal anomaly has been identified.

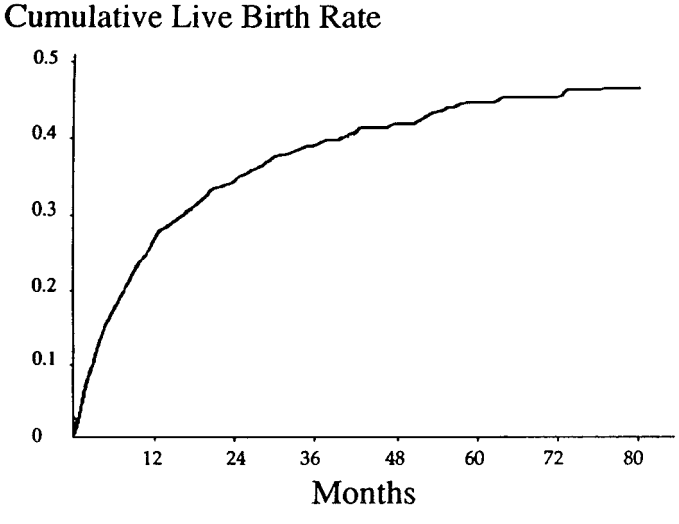


FIG. 3. Cumulative rate of conceptions leading to live birth in 873 untreated couples with infertility of more than 1 yr in duration. Cumulative rate at 36 months was 38.2%. The observed conception rates at 36 months were 33.3, 29.2, 22.6, 20.5, and 20.0% for unexplained infertility, oligozoospermia, ovulation defects, minor tubal defects, and mild endometriosis, respectively [Adapted from Ref. 30, Fig. 1].

With most ARTs the average delivery rate per cycle is approximately 15% and the cumulative delivery rate after several cycles is about 50%. In other words, ARTs, up to now, can reach approximately 50% of positive results in terms of live births in couples who decide to assume the direct and indirect costs of these procedures and their emotional and social burden. Further research is therefore needed in the next years to improve the effectiveness of ARTs, on one hand, and to reduce the need of ARTs on the other. In particular, as embryo implantation rate after ARTs is considerably lower than that of natural conceptions, and effective medical treatment is possible only in a small fraction of infertile males, research should be focused on: 1) elucidation of factors influencing embryonic implantation; 2) identification of regulatory mechanisms of spermatogenesis, spermiation, and epididymal function; 3) identification of sperm receptors that react with oocyte ligands and of the signaling cascades that lead to sperm activation (acrosome reaction, hyperactivated motility); 4) examination of sperm factors involved in oocyte activation and molecules that mediate sperm-egg interaction after penetration and their relationship with early embryo development.

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