Published in final edited form as:
Nat Rev Urol. 2011 February ; 8(2): 86–94. doi:10.1038/nrurol.2010.230.

Contemporary concepts in the evaluation and management of male infertility

Kathleen Hwang, **R. Chanc Walters**, and **Larry I. Lipshultz**
Scott Department of Urology, Baylor College of Medicine, 6624 Fannin Street, Suite 1700, Houston, TX 77030, USA

Abstract

Infertility in men is a common condition. At the core of the medical evaluation of the male partner in a couple who are unable to conceive is the history and physical examination. Special attention should be directed to the patient's developmental history and any use of testosterone products. The physical examination focuses on the genitals, and includes assessments of the size and consistency of the testicles, epididymis, vas deferens, and presence of varicoceles. Although many sophisticated tests are available, semen analysis is still the most important diagnostic tool used to assess fertility, and includes parameters such as sperm count, motility and viability. Treatment of male factor infertility can involve targeted agents, in the case of specific conditions such as hypogonadotropic hypogonadism, or it can be empirical—using medical therapy or assisted conception techniques—for patients in whom no underlying cause has been identified. Although an all-encompassing treatment for male factor infertility has not yet been developed, the field offers many promising avenues of research.

Introduction

Infertility is defined as failure to conceive after 12 months of unprotected intercourse and affects 15% of couples.[1] Male factors account for the difficulties in 50% of couples, thus ~8% of all men of reproductive age may need medical attention for reproductive failure.[2] In addition, underlying medical pathology can be found in 6% of men who present with infertility.[3]

Through the years, the protocols for evaluation and treatment of male infertility have undergone many changes. While there are currently a number of medical and surgical treatment choices available for men who are unable to initiate a pregnancy, the decision to proceed with one treatment over another is dependent upon a thorough evaluation and the results of appropriate testing of the individual. In this Review, we will discuss the evaluation of men who present with difficulty in initiating a pregnancy, describe the various tests

Correspondence to: L.I. Lipshultz, larryl@bcm.edu.

Competing interests
L. I. Lipshultz declares an association with the following company: Auxilium. See the article online for full details of the relationship. The other authors declare no competing interests.

Review criteria
We searched for original articles focusing on male infertility in MEDLINE and PubMed, published between 1980 and 2010. The search terms we used were "male infertility", "genetics", "diagnostics", "sperm function testing" and "male evaluation", alone and in combination. All papers identified full text papers in English. We also searched the reference lists of identified articles for further relevant papers.

Author contributions
K. Hwang and R. C. Walters researched data for the article, and wrote the manuscript. K. Hwang and L. I. Lipshultz reviewed and edited the manuscript before submission and L. I. Lipshultz was also involved in discussions regarding the article content.

available, and note their indications for use. We will then focus on the therapies currently available to treat male factor infertility, and also consider future directions in the management of this condition.

The physiology of infertility

The hypothalamus controls production of reproductive hormones through the pulsatile secretion of gonadotropin-releasing hormone (GnRH). In turn, GnRH stimulates the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH acts on the Leydig cells of the testes to produce testosterone, whereas FSH acts on testicular Sertoli cells to stimulate spermatogenesis. Negative feedback control of reproductive hormone levels is achieved via inhibin (which decreases FSH levels) or via estradiol aromatized from testosterone (which inhibits LH production).[4]

The classic form of testosterone deficiency is found in individuals with hypogonadotropic hypogonadism. This is the form of testosterone deficiency that is best characterized, and which is often the first consideration of the physician, although this potentially treatable[5] form of male factor infertility accounts for <1% of cases. The cause of hypogonadotropic hypogonadism can be congenital, acquired, or idiopathic; the congenital etiologies include Prader–Willi syndrome, Lawrence–Moon–Biedl syndrome, and Kallman syndrome, or hypogonadotropic hypogonadism can be acquired after radiotherapy to the brain, trauma, or secondary to a pituitary tumor. Kallman syndrome is the most common form of primary hypothalamic deficiency. These patients can be identified by their lack of secondary sexual characteristics and anosmia (which is due to failure of GnRH neurons to migrate from the olfactory placode, along with failure of the olfactory bulb to form). The main mechanism of infertility in patients with Kallman syndrome is a failure to initiate spermatogenesis.

Hyperprolactinemia (defined as a serum prolactin concentration ≥20 ng/ml in men) can also be a cause of infertility in both males and females and is usually caused by a pituitary tumor, hypothyroidism, hepatic disease, or secondary to treatment with psychotropic or antihypertensive drugs. Excess prolactin inhibits the hypothalamic secretion of GnRH and impairs binding of LH to Leydig cells in the testis. In addition to hypogonadotropic hypogonadism, patients with hyperprolactinemia have low ejaculate volumes and can experience visual field defects if a tumor is present.

The use of exogenous androgens can have a profound effect on fertility. Androgen excess caused by the use of steroids impairs spermatogenesis by suppressing GnRH, which results in decreased LH and FSH levels and a considerable reduction in intratesticular testosterone levels.[6] Exogenous testosterone replacement therapy can result from self-administration in order to increase lean muscle mass (sometimes seen in athletes), or even as an appropriate treatment in a hypogonadal patient in whom fertility was either not a concern initially or not discussed. Though the anabolic to androgenic ratio in testosterone-derived products can vary, they all have pharmacological effects that can lead to male factor infertility.

Medical history

The work-up of a male patient who reports difficulty in initiating a pregnancy should start with taking a thorough history, the results of which guide the physical examination and diagnostic testing. The physician should ascertain how long the couple has been attempting to conceive, any previous pregnancies in or by either partner, the timing of intercourse, and the use of lubricants. Any other medical problems or interventions should be discussed, especially highlighting scrotal, prostatic, spinal, inguinal, and retroperitoneal surgery. The current or past use of any medications should be identified. Men are now more likely to delay paternity than ever before, and older patients should be directly questioned on the use

of testosterone replacement therapy as many patients will not think to mention the use of a topical gel or cream. Patients should be asked about their developmental history, as well as any history of viral orchitis, childhood cancers, congenital or genetic abnormalities, undescended testes, recent febrile illnesses and genitourinary infections. Finally, any history of occupational or environmental exposures (to chemicals, radiation, pesticides, or chronic heat, for example) recreational drug use, and smoking should be obtained.

Physical examination

The physical examination should start by noting the overall body habitus, presence or absence of gynecomastia, and distribution of body hair. A genitourinary examination should note the size and location of the meatal opening. The scrotum should be examined to assess testicular size and consistency, presence and possible induration of the epididymis, and presence of the vas deferens on each side. The possible existence of a varicocele should be investigated using the Valsalva maneuver with the patient standing upright in a warm examination room. Finally, a digital rectal examination should note sphincter tone, prostate size and consistency, and look for the presence of a midline cyst or enlarged seminal vesicles, which are suggestive of obstruction.

Endocrine studies

An endocrine work up is required to evaluate the integrity of the hypothalamic–pituitary–gonadal (HPG) axis. Current AUA best practice guidelines[7] recommend the measurements of at least serum FSH levels and serum testosterone levels in patients who have an abnormal semen analysis (especially if the sperm concentration is <10 million/ml) or have impaired sexual function. However, many centers carry out a more extensive laboratory evaluation, which can include measurements of LH, prolactin, thyroid-stimulating hormone (TSH), sex-hormone-binding globulin (SHBG), cortisol and estradiol levels, if the clinical findings indicate an endocrine abnormality. Approximately 3% of subfertile men have an underlying endocrinopathy.[5]

After a thorough history, physical examination in conjunction with appropriate laboratory testing, radiological imaging may be useful in further evaluation of the infertile male. Transrectal or scrotal ultrasonography can be useful in confirming suspicion of genital tract obstruction. Futhermore, cranial imaging with MRI is essential in diagnosing pituitary tumors.

Semen analysis

The pivotal and initial laboratory test when evaluating male factor infertility continues to be the semen analysis, although ‘normal’ semen parameters can vary widely. At least two semen samples between 2 and 4 weeks apart should be obtained; each proceeded by 2–3 days of abstinence from sex. Abstinence periods are important as sperm density will increase by 25% for each day of abstinence up to 4 days.[8] Samples are preferably obtained either by masturbation or using a latex-free, spermicide-free collection condom during intercourse. The sample can be obtained either in the clinic or at home, if kept at body temperature and delivered to the laboratory within 1 h.

The semen sample is first allowed to liquefy before looking for round cells, debris, and bacteria under the microscope. Of note, 20% of infertile men have some leukocytes in their semen, although only 20% of these individuals will have clinically relevant leukocytospermia (defined by the WHO as $>1 \times 10^6$/ml white blood cells).[9,10] If the patient has symptoms—such as pain with ejaculation or urination, scrotal pain, or systemic

symptoms of infection—consistent with genital tract infection or if bacteria are seen on microscopy, semen culture and antibiotic sensitivity testing should be carried out.

Standard semen analysis

In 2010, updated reference values for semen analysis were published by the World Health Organization, using data from men worldwide who had become fathers <12 months previously. The WHO defines semen adequacy as volume ≥1.5 ml, sperm count ≥15 million/ml, total motility ≥40%, total sperm ≥39 million per ejaculate, vitality ≥58% and Kruger strict morphology ≥4%[11,12]—substantial changes from the criteria of the 4th edition in 1999 (Table 1).[13] These guidelines have always been periodically updated every few years as new recommendations arise regarding sperm function testing and other diagnostic and technical advancements. However, this is the first version to take strict morphological criteria into account, and the first edition to report reference ranges that have been derived using strict statistical analysis compared to the previous versions, which were limits of adequacy and derived by consensus. Currently, the guidelines are still so relatively new that most primary physicians have not implemented these new ranges into their patient care. However, in theory they might result in fewer men referred for specialist evaluation, because of the smaller abnormal range in counts, for example.

Sperm counts are performed using specific counting chambers and known concentrations. While there are automated systems available, when evaluating a patient with male factor we believe that a manual analysis by a skilled technician is far more specific and sensitive. However, when these two variables are combined with the ejaculate volume, the total number of motile sperm can be estimated. This is the semen parameter that correlates most closely with pregnancy outcomes.[14] Another commonly used parameter of semen quality is the Kruger strict morphology value, which reflects the percentage of sperm with a ‘perfect’ appearance (Box 1). Although strict morphology can be a useful measure of semen quality in assisted reproduction, as first described by Kruger, its clinical value in other settings remains controversial.[15] Kruger strict morphology is a poor predictor of genetically normal sperm, and has proven unhelpful for selecting sperm without chromosomal abnormalities.[16,17] Sperm motility measurements take into account the function of the sperm, not just quantity. Unlike sperm counts, sperm motility is not an objective value and is, therefore, subject to technician variability (Box 2).

Extended semen analysis

White blood cell assay—In the past, leukocytospermia has been associated with decreased sperm counts, reduced motility, abnormal morphology, and defective fertilization; however, these studies have demonstrated inconsistent results regarding the relationship between leukocytospermia and infertility.[18] Determining the number of leukocytes in an unstained semen sample can be difficult, as immature germ cells can resemble round cells. True leukocytospermia can only be demonstrated using special dyes (papanicolaou) or specific immunofluorescent stains. Stains performed on semen smears were used to distinguish between white blood cells (WBCs) and immature germ cells, however the testing involves a lengthy preparation with proper assessment by a highly trained technician. Flow cytometry has also demonstrated an ability to count seminal WBCs in conjunction with panleukocyte monoclonal antibodies ($CD45^+$),[19] however, flow cytometry is not readily available in every andrology laboratory. The peroxidase test was developed in 1974[20] for enumerating WBCs in semen by assessing the peroxidase enzyme present in granulocytes, which are the predominant WBC in semen. It is a relatively simple, quick and inexpensive test that is now widely used.

Quantification of reactive oxygen species—Reactive oxygen species (ROS) are the harmful byproducts of oxygen metabolism, and include superoxide anions, hydrogen peroxide, and hydroxyl free radicals. An excess of these substances can lead to oxidative damage to proteins, lipids, and DNA.[21] The presence of high levels of ROS can lead to sperm damage and infertility; ROS levels are elevated in up to 40% of subfertile men,[22] and show an inverse relationship with the rate of spontaneous pregnancies.[23] Several methods are currently used to quantify ROS levels in semen; however, most of these assays are unable to determine whether the ROS excess originates from leukocytes in the semen or from the sperm themselves. Thus, if leukocytospermia is also present, treatment to reduce the white blood cell levels must be performed before samples are taken for ROS assay.[24] Prolonged semen processing time can falsely lower ROS quantification, so the test must be performed within 1 h of semen collection.[25] There are various methods to detect seminal ROS, including chemiluminescence, nitroblue tetrazolium test, cytochrome C reduction test, and xylenol orange based assay. The chemiluminescence assay is the most commonly used and utilizes a luminometer to measure chemical reactions between ROS found in human semen and a chemiluminescent probe, such as luminol or lucigenin.

Quantification of DNA damage—Damage to the spermatic DNA can occur either during intratesticular spermatogenesis or during semen maturation and transport. Semen samples from subfertile men have considerably more DNA damage than semen from fertile controls.[26] Evidence suggests that DNA fragmentation in spermatozoa is associated with poor pregnancy outcomes, including early embryo death, poor embryo development, and poor implantation.[27] DNA damage increases with elevated ROS levels and with the presence of varicoceles, but subsequent surgical correction of the varicocele can reduce the DNA damage.[28–31] The precise normal value for DNA damage will depend on the particular assay used, but most laboratories set the normal range as <30% DNA fragmentation. Evaluating for a DNA damage level in our clinical practice has been helpful in patients with unexplained recurrent early gestational loss. A variety of different tests are available: the acridine orange–staining test, the sperm chromatin structural assay, terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL), and the comet assay (a single cell gel electrophoresis).

Detection of antisperm antibodies—A multitude of studies have tried to determine the clinical relevance of antisperm antibodies. Currently, the techniques available to detect them are mixed agglutination assay, immunofluorescence assay, and the immunobead test.[32] Antisperm antibodies are present in the male partner's semen in ~13% of subfertile couples and studies show that couples in which either partner is positive for antisperm antibodies have decreased pregnancy rates.[33] However, 2.5% of fertile couples also test positive for antisperm antibodies.[33,34] In practice, testing for these antibodies is useful when sperm clumping is seen on microscopy, in patients with isolated defects in motility, those with an unexplained poor sperm cervical interaction test and in couples whose inability to conceive is unexplained.

The sperm cervical interaction test is the formal assessment of the interaction between sperm and cervical mucus, and is commonly referred to as the postcoital test. An aspirate of cervical mucus is examined 30 min after intercourse during mid-cycle. The aspirate is examined microscopically for the number of motile sperm present and can be a helpful predictor of the success of intrauterine insemination. However, with the advent of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), this test has essentially been made obsolete.

Sperm function

Hypo-osmotic swelling—The hypo-osmotic swelling test checks for sperm viability by determining whether immotile sperm have intact, functional membranes and are, therefore, alive. According to this test, >60% of sperm are viable in a normal ejaculated semen sample.[35] The test is based upon the premise that when placed in a hypo-osmotic condition (150 mOsm/l or less), a normal live sperm maintains an osmotic gradient and absorbs fluid, resulting in a swelling of the plasma membrane. Its main use is to differentiate patients with immotile cilia syndrome (in whom sperm count and morphology are normal, but motility is impaired due to ultrastructural defects in dynein) from those with other sperm motility defects, and to select viable sperm for use in assisted reproduction techniques, such as ICSI.

Acrosome reaction assay—The acrosome is a structure that lies on the head of the sperm and is important for fertilization, as it contains enzymes essential for the breakdown of the zona pellucida. Historically, this test was used to evaluate the sperm before, during and after incubation at 37 °C, after which the sperm are exposed to an agent which stimulates the acrosome reaction, such as progesterone. Approaches to study this reaction include special stains (antibodies), microscopy, acrosin enzyme assay, and electron microscopy. These stains and immunocytochemical approaches are used to measure the basal level and induced level (using agents such as progesterone) of the acrosome reaction. The most commonly used method is the triple stain technique.[36] The value of the test has become more limited with the development of IVF and ICSI and the ability of Kruger strict morphology to detect acrosome abnormalities.

Sperm zona binding assays—Sperm binding to the zona pellucida is an essential step in fertilization. The zona binding assay compares the binding ability of the patient's own sperm to that of a donor.[37] This test compares the ability of fluorescently labeled donor and patient sperm to competitively bind to a fixed zona. The results are then expressed as an index of patient to donor sperm bound. At the moment, use of this test is limited to distinguishing patients who require ICSI from those who are suitable for routine IVF. However, intact ova, which are required to carry out the test, have become extremely rare with the widespread use of ICSI.

Sperm penetration assay—After penetrating the zona pellucida, sperm must undergo capacitation, fuse with the egg cell membrane, and decondense the sperm head to form the pronucleus.[38] All these steps can be assayed using the sperm penetration assay. The patient's sperm is incubated with a "promiscuous" (allowing multiple penetrations) hamster ova and the number of sperm penetrations is calculated over a predetermined amount of time. The results of this test are useful to predict whether IVF will have a successful outcome, but is rarely used, as it is time-consuming and complex, and requires intact hamster ova.[39]

Genetic testing

Genetic testing has become widespread throughout modern medicine, and the field of male factor infertility is no exception. Currently, three major genetic tests are in widespread use in male fertility clinics: karyotype analysis, Y chromosome microdeletion determination, and fluorescence *in situ* hybridization (FISH). Karyotype analysis and Y chromosome microdeletion determination are currently indicated for patients with sperm concentrations $<5 \times 10^6$/ml. Importantly, these tests are often offered in conjunction with genetic counseling, as male offspring of patients with a Y chromosome microdeletion will pass this abnormality to all male offspring.

Karyotype analysis

Approximately 5% of subfertile male patients will have an identifiable chromosomal abnormality on karyotype analysis.[40] In azoospermic patients, the proportion increases to 15%.[41] Karyotype analysis detects large-scale genetic abnormalities, such as deletions of entire chromosomes or substantial portions of a chromosome, as well as translocations. Sequence abnormalities such as frameshift mutations, point mutations and other submicroscopic deletions cannot be identified. The most common chromosomal defect detected by karyotype analysis is Klinefelter syndrome (47,XXY), which occurs in 1:500 male births and is found in up to 10% of azoospermic men and 5% of severe oligospermic patients.[42] The sperm count in these patients can vary from severe oligospermia to azoospermia. For patients with karyotype abnormalities, referral for genetic counseling is essential to discuss the impact of paternity on future generations and options for sperm donation or adoption.

Y chromosome microdeletion

The Y chromosome contains essential genes needed for male development and sperm function, in particular the azoospermia factor region (AZF), which is responsible for sperm development. This region can be subdivided into AZFa, AZFb, and AZFc, according to their location with reference to the centromere and deletions in these locations are responsible for varying degrees of spermatogenic dysfunction, as they encode different gene products which all contribute to the drive of the spermatogenic machinery.[43] Determining the location of the deletion can be beneficial to predict success of future treatments. Entire microdeletions of AZFa or AZFb can portend a poor prognosis for sperm retrieval even with microscopic testicular sperm extraction, as the sperm counts are so low. AZFc microdeletion can result in a spectrum of deficiency from severe oligospermia to azoospermia.[44] These deletions are identified using PCR to analyze sequence tagged sites. Indications for testing for AZF microdeletions are sperm concentrations <5 million/ml.

Fluorescence *in situ* hybridization

FISH detects specific DNA sequences in decondensed sperm nuclei, and can determine the chromosomal constitution of human spermatozoa. After proper incubation, multicolor FISH is performed: triple-color FISH for chromosome 18, X and Y, and dual-color FISH for chromosomes 13 and 21. The slides are analyzed under an epifluorescent microscope and the spermatozoa are scored according to defined criteria. The incidence of numerical chromosomal abnormalities in spermatozoa has been reported in a wide range of individuals including carriers of chromosome anomalies as well as fertile and infertile males. Sperm from oligospermic men with normal karyotypes have substantially increased rates of aneuploidy.[45] In fertile men the percentage of aneuploid sperm is estimated at 6.5%; however, a correlation was found in patients with severe oligospermia who also had increased FSH levels and aneuploidies, reporting a 22.6% incidence of meiotic anomalies.[45,46] Chromosomal aneuploidy rates are inversely correlated with sperm concentration and total motility.[47] Higher aneuploidy rates have been reported in couples with recurrent first trimester miscarriages, as well as in couples who experience difficulty in conceiving.[48] In this instance, FISH is a direct reflection of the chromosomal aneuploidy of the gamete itself and, therefore, a direct reflection of the genetic makeup of the potential future fetus. However, the use of FISH is currently limited by its high cost and variability in results.

Future directions in evaluating male infertility

Several areas of clinical and basic science research could lead to improved care for our patients in the future. One such area is cytogenetics. A standard karyotype test is limited by

the microscope's resolution and, thus, cannot detect subtle chromosomal abnormalities. Array-based comparative genomic hybridization has emerged as a possible solution to this problem, as it can be used to detect submicroscopic genetic abnormalities, including copy-number changes, and carry out chromosomal break point analysis. Other areas of development are in metabolomics, proteomics, and genomics, which can provide molecular data about the underlying biochemical mechanisms of disease and, hopefully, fertility.[49] These tools provide comprehensive molecular data about the underlying biochemical mechanisms of disease, such as addressing the causes of defective sperm–zona interaction with glycomic analyses, assessing the consequence of oxidative stress in the male germline with application of lipidomics to the analysis of sperm quality, and even identifying particular genotypes associated with specific defects in semen quality with advanced diagnostic genomics.

Medical treatments for infertility in men

Hormonal treatments

Enthusiasm for hormonal treatments has waned in recent years but their use is still widespread, as they are the least invasive and the most convenient initial treatment. Hormonal treatments can be targeted to the specific cause of the infertility if the underlying pathology is known, and can also be used to treat idiopathic infertility.

Targeted therapy—Targeted hormonal therapies are available for patients whose infertility is due to altered levels of androgens, prolactin, or TSH. Treatment has two goals. The first goal is to restore virilization and normal sexual function, using testosterone. The second is to initiate spermatogenesis with pulsatile GnRH treatment at 2,500–3,000 U every other day and FSH at 75–150 U every other day. Alternatively, patients can be treated with human chorionic gonadotropin (hCG) (3,000 IU every other day) and adding human menopausal gonadotropin (hMG) after spermatogenesis has been initiated.[50] In patients with hyperprolactinemia, treatment depends on the need for surgical treatment of an underlying pituitary tumor. If a macroadenoma is not present and surgery is not indicated, treatment with the somatostatin analogs bromocriptine (2.5–10 mg daily) or, more commonly, cabergoline (0.5–1.0 mg twice a week)[51] is indicated.

Hormonal treatment of patients using steroids—The first step in treating patients who are using testosterone products is to discontinue their use. Spermatogenesis usually returns, but full recovery can take over a year and may not return to pretreatment levels. In addition, many patients will still need some form of testosterone supplementation, as their endogenous testosterone production has been suppressed. For these patients, multiple hormonal agents have been used, including hCG, clomiphene citrate, or aromatase inhibitors. The choice and dose of potential treatments should be based on each patient's hormone profile. If their testosterone:estradiol ratio is normal (15:1), hCG (3,000 U every other day), clomiphene citrate (25 mg daily), or tamoxifen (10 mg twice daily) can be used. If the ratio is abnormal, then anastrozole (1 mg daily) can be used to block the aromatization of testosterone to estradiol.

Gonadotropins—The use of gonadotropins in the treatment of idiopathic infertility in men is based on the age-old credo that "if a little bit is good, a lot will be better", and thus gonadotropins are often used in extremely high doses. The use of hCG is becoming increasingly prevalent, as hCG administration can increase intratesticular testosterone to normal levels.[52] Since many combinations of gonadotropins and dosages have been used, determining their true efficacy is difficult. In an attempt to clarify these results, a Cochrane analysis has been carried out, focusing only on the randomized, controlled studies.[53] It

concluded that pregnancy rates increased following 3 months of gonadotropin treatment. However, the analysis lacked adequate statistical power, and thus this conclusion must be viewed with some skepticism.

Oxytocin—Though best known for its role in promoting lactation, oxytocin is also produced in the reproductive tract. This hormone promotes sperm progression, increases sperm retrieval (seen in a small single-blinded study with oligospermic men)[54] and also increases the conversion of testosterone to dihydrotestosterone (DHT),[55] a more potent form of testosterone, which cannot be converted to estradiol by aromatase. The only trial of oxytocin to date showed no change in sperm count, ejaculate volume or sperm motility when used once before collection.[56]

Antiestrogens

Clomiphene citrate and tamoxifen are nonsteroidal antiestrogens that function bind to estrogen receptors in the hypothalamus and pituitary, which blocks the effects of estradiol. This action increases GnRH secretion, with a resulting increase in LH, FSH, and testosterone levels. The FSH level should be checked after 4 weeks of this treatment—a twofold or more increase in FSH indicates intrinsic testicular failure. These agents are used in hypogonadal men who wish to remain fertile and who should not be placed on exogenous testosterone as this alone will shut down spermatogenesis.

Nine large studies have investigated clomiphene citrate use and subsequent pregnancy rates. Overall, four studies have shown a positive outcome and four a negative outcome, with one being inconclusive.[57–62] A Cochrane database review showed no difference in pregnancy rates following clomiphene citrate treatment;[63] however, a study on the use of clomiphene to treat nonobstructive azoospermia showed that 64% of treated patients achieved sperm in their ejaculate.[64] Tamoxifen is less potent and less well studied than clomiphene, and a meta-analysis of tamoxifen treatment for infertility failed to show a significant effect on pregnancy outcomes, although an increase in sperm count has been observed.[63] Additionally, a Cochrane meta-analysis of 10 studies showed no significant effect on pregnancy outcomes with antiestrogens.[65] A single study has reported a positive effect on fertility (increases in semen parameters and pregnancy rates) when tamoxifen was administered with low dose testosterone.[66] Enclomiphene, the *trans* isomer of clomiphene, is currently in phase IIb trials for patients with oligospermia and hypogonadism.

Aromatase inhibitors

Aromatase inhibitors prevent the synthesis of estradiol by blocking aromatization of testosterone. Reduced levels of estradiol result in decreased negative feedback of the HPG axis, leading to increased GnRH, LH, and FSH levels, and raised intratesticular testosterone levels. Noncontrolled studies have reported an increase in semen parameters and pregnancy rates in response to the aromatase inhibitors, anastrozole and testolactone.[67,68] One controlled study showed that hormone parameters increased but pregnancy rates did not change.[69] They are effective in treating men with hypogonadism who are interested in fertility, but are not shown to be effective in patients with Klinefelter syndrome.

Antioxidants

Studies have shown a positive effect of antioxidants on sperm quality.[70] Vitamin C, which is found in high levels in the semen, is reduced in the semen of subfertile men.[71] A randomized study in heavy smokers showed that both 200 mg and 1,000 mg vitamin C improve sperm counts and motility.[72] Vitamin E is also commonly used, though the data are conflicting; some studies suggest a positive effect and some no effect of this agent on semen parameters.[73–75] Other, less well studied antioxidants include selenium, glutathione,

carotenoids, coenzyme Q_{10}, zinc, and copper. Studies of all these compounds have produced conflicting results,[76–80] yielding antioxidant regimens and indications that have not been universally adapted.

One compound of special interest is carnitine. Carnitine is present at increased concentrations in the epididymis, where its levels correlate with spermatozoal energy production and motility.[81] Seminal carnitine levels are lower in subfertile men than in fertile men.[82] Initial reports showed substantial improvements in sperm concentration and motility following carnitine administration, however three subsequently published studies have failed to show a statistically significant effect.[83–86]

Future treatments

Two areas have shown particular promise for future infertility treatment in men: gene therapy and spermatogonial stem cell transplantation. Treatment with spermatogonial stem cells aims to use an adult stem cell population to restore successful spermatogenesis in patients with secondary infertility (the inability to conceive despite having already initiated a successful pregnancy), which is often a consequence of treatment for cancer. Spermatogonial stem cell transplantation was first demonstrated in a mouse model.[87] Testicular tissue or isolated spermatogonial stem cells were obtained and cryo-preserved before administering busulfan (an alkylating agent) that rendered the animal sterile.[88] After thawing the tissue, autotransplantation of spermatogonial stem cells into the efferent ductules or the seminiferous tubules resulted in the production of viable offspring.[89] One major concern associated with autologous transplantation in cancer patients, however, is the reintroduction of malignant cells. The majority of studies addressing this concern involves magnetic or fluorescence-activated cell sorting and capitalizes on the ability to identify specific spermatogonial stem cell markers in rodent models with hematological malignancies.[90] Studies in human models have reported inconsistent results, complicated by the fact that specific spermatogonial stem cell markers in humans have not been completely established.[91,92]

Gene therapy is the other potential area for advanced future study. Gene therapy research is one of the most fascinating areas in contemporary biology, and while it focuses on correcting genetic flaws and curing life-threatening diseases, it raises sharp ethical considerations. Somatic gene therapy can be applied to treating a multitude of disorders including cancer and inheritable diseases, and the ethical issues primarily concern safety and efficacy.[93] However, germline therapy for treating male infertility is particularly controversial as it theoretically manipulates and affects future generations without a precise understanding about the mechanism and control of gene expression;[94] forever changing the genetic make up of progeny.

Today gene therapy is primarily used in animal models, although some human trials have been carried out. Some of the first human trials were used to target patients with adenosine deaminase deficiency and severe combined immune deficiency (SCID). Yomogida *et al.*[95] examined the conditions for the introduction and stable expression of transgenes in Sertoli cells using electroporation in mutant infertile mice. The transgene demonstrated stable expression in mature Sertoli cells for a limited period of time, although no pups were obtained under normal mating conditions. Although this method is still fraught with challenges, the potential for future application to patients with Sertoli cell dysfunction is promising.

Conclusions

The options for treatment of infertility in men have expanded over the past few decades. Similarly, tools to evaluate and diagnose infertility have also progressed. The information gleaned from these diagnostic tests enables targeted treatment of male factor infertility, and can potentially improve the overall health of the patient, as it has fewer adverse effects and treats underlying conditions. Furthermore, understanding of the molecular and biochemical mechanisms of infertility continues to evolve, providing physicians with the knowledge to improve evaluation techniques and provide better targeted and more successful treatment for the subfertile couple.

Acknowledgments

This study was supported in part by the 1K12DK083014 Multidisciplinary K12 Urology Research Career Development Program at Baylor to K. Hwang from the National Institute of Kidney and Digestive Diseases.

References

1. Thonneau P, et al. Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988–1989). Hum. Reprod. 1991; 6:811–816. [PubMed: 1757519]
2. Centers for Disease Control. 2010
3. Kolettis PN, Sabanegh ES. Significant medical pathology discovered during a male infertility evaluation. J. Urol. 2001; 166:178–180. [PubMed: 11435851]
4. Brugh VM 3rd, Matschke HM, Lipshultz LI. Male factor infertility. Endocrinol. Metab. Clin. North Am. 2003; 32:689–707. [PubMed: 14560894]
5. Sigman M, Jarow JP. Endocrine evaluation of infertile men. Urology. 1997; 50:659–664. [PubMed: 9372871]
6. McLachlan RI, et al. Hormonal regulation of spermatogenesis in primates and man: insights for development of the male hormonal contraceptive. J. Androl. 2002; 23:149–162. [PubMed: 11868805]
7. AUA. 2010
8. Carlsen E, Petersen JH, Andersson AM, Skakkebaek NE. Effects of ejaculatory frequency and season on variations in semen quality. Fertil. Steril. 2004; 82:358–366. [PubMed: 15302284]
9. Wolff H. The biologic significance of white blood cells in semen. Fertil. Steril. 1995; 63:1143–1157. [PubMed: 7750580]
10. Keck C, Gerber-Schafer C, Clad A, Wilhelm C, Breckwoldt M. Seminal tract infections: impact on male fertility and treatment options. Hum. Reprod. Update. 1998; 4:891–903. [PubMed: 10098479]
11. Cooper TG, et al. World Health Organization reference values for human semen characteristics. Hum. Reprod. Update. 2010; 16:231–245. [PubMed: 19934213]
12. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 5th edn.. Cambridge University Press; 2010.
13. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th edn.. Cambridge University Press; 1999.
14. Bostofte E, Bagger P, Michael A, Stakemann G. Fertility prognosis for infertile men: results of follow-up study of semen analysis in infertile men from two different populations evaluated by the Cox regression model. Fertil. Steril. 1990; 54:1100–1106. [PubMed: 2245836]
15. Menkveld R, Rhemrev JP, Franken DR, Vermeiden JP, Kruger TF. Acrosomal morphology as a novel criterion for male fertility diagnosis: relation with acrosin activity, morphology (strict criteria), and fertilization *in vitro*. Fertil. Steril. 1996; 65:637–644. [PubMed: 8774300]
16. Celik-Ozenci C, et al. Sperm selection for ICSI: shape properties do not predict the absence or presence of numerical chromosomal aberrations. Hum. Reprod. 2004; 19:2052–2059. [PubMed: 15205404]

17. Ryu HM, et al. Increased chromosome X, Y, and 18 nondisjunction in sperm from infertile patients that were identified as normal by strict morphology: implication for intracytoplasmic sperm injection. Fertil. Steril. 2001; 76:879–883. [PubMed: 11704105]
18. Saleh RA, et al. Leukocytospermia is associated with increased reactive oxygen species production by human spermatozoa. Fertil. Steril. 2002; 78:1215–1224. [PubMed: 12477515]
19. Rasanen ML, Hovatta OL, Penttila IM, Agrawal YP. Detection and quantitation of sperm-bound antibodies by flow cytometry of human semen. J. Androl. 1992; 13:55–64. [PubMed: 1372598]
20. Endtz AW. A rapid staining method for differentiating granulocytes from “germinal cells” in Papanicolaou-stained semen. Acta Cytol. 1974; 18:2–7. [PubMed: 4129934]
21. Tremellen K. Oxidative stress and male infertility—a clinical perspective. Hum. Reprod. Update. 2008; 14:243–258. [PubMed: 18281241]
22. Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil. Steril. 1992; 57:409–416. [PubMed: 1735495]
23. Aitken RJ, Irvine DS, Wu FC. Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. Am. J. Obstet. Gynecol. 1991; 164:542–551. [PubMed: 1992700]
24. Aitken RJ, Buckingham DW, West K, Brindle J. On the use of paramagnetic beads and ferrofluids to assess and eliminate the leukocytic contribution to oxygen radical generation by human sperm suspensions. Am. J. Reprod. Immunol. 1996; 35:541–551. [PubMed: 8792938]
25. Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J. Reprod. Fertil. 1987; 81:459–469. [PubMed: 2828610]
26. Spano M, et al. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil. Steril. 2000; 73:43–50. [PubMed: 10632410]
27. Tesarik J, Mendoza C, Greco E. Paternal effects acting during the first cell cycle of human preimplantation development after ICSI. Hum. Reprod. 2002; 17:184–189. [PubMed: 11756385]
28. Aitken RJ, Baker MA, Sawyer D. Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. Reprod. Biomed. Online. 2003; 7:65–70. [PubMed: 12930576]
29. Smit M, et al. Decreased sperm DNA fragmentation after surgical varicocelectomy is associated with increased pregnancy rate. J. Urol. 2010; 183:270–274. [PubMed: 19913801]
30. Smith R, et al. Increased sperm DNA damage in patients with varicocele: relationship with seminal oxidative stress. Hum. Reprod. 2006; 21:986–993. [PubMed: 16361286]
31. Greco E, et al. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J. Androl. 2005; 26:349–353. [PubMed: 15867002]
32. Ackerman S, McGuire G, Fulgham DL, Alexander NJ. An evaluation of a commercially available assay for the detection of antisperm antibodies. Fertil. Steril. 1988; 49:732–734. [PubMed: 3350171]
33. Heidenreich A, Bonfig R, Wilbert DM, Strohmaier WL, Engelmann UH. Risk factors for antisperm antibodies in infertile men. Am. J. Reprod. Immunol. 1994; 31:69–76. [PubMed: 8049027]
34. Collins JA, Burrows EA, Yeo J, YoungLai EV. Frequency and predictive value of antisperm antibodies among infertile couples. Hum. Reprod. 1993; 8:592–598. [PubMed: 8501191]
35. Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJ. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil. 1984; 70:219–228. [PubMed: 6694140]
36. Kallajoki M, Virtanen I, Suominen J. The fate of acrosomal staining during the acrosome reaction of human spermatozoa as revealed by a monoclonal antibody and PNA-lectin. Int. J. Androl. 1986; 9:181–194. [PubMed: 3100456]
37. Burkman LJ, et al. The hemizona assay (HZA): development of a diagnostic test for the binding of human spermatozoa to the human hemizona pellucida to predict fertilization potential. Fertil. Steril. 1988; 49:688–697. [PubMed: 3350165]

38. Yanagimachi R, Yanagimachi H, Rogers BJ. The use of zona-free animal ova as a test-system for the assessment of the fertilizing capacity of human spermatozoa. Biol. Reprod. 1976; 15:471–476. [PubMed: 974200]
39. Johnson A, Bassham B, Lipshultz LI, Lamb DJ. A quality control system for the optimized sperm penetration assay. Fertil. Steril. 1995; 64:832–837. [PubMed: 7672158]
40. O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil. Steril. 2010; 93:1–12. [PubMed: 20103481]
41. Ferlin A, et al. Male infertility: role of genetic background. Reprod. Biomed. Online. 2007; 14:734–745. [PubMed: 17579990]
42. Foresta C, Ferlin A, Gianaroli L, Dallapiccola B. Guidelines for the appropriate use of genetic tests in infertile couples. Eur. J. Hum. Genet. 2002; 10:303–312. [PubMed: 12082505]
43. Kuroda-Kawaguchi T, et al. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nat. Genet. 2001; 29:279–286. [PubMed: 11687796]
44. Hopps CV, et al. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum. Reprod. 2003; 18:1660–1665. [PubMed: 12871878]
45. Egozcue S, et al. Human male infertility: chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion. Hum. Reprod. Update. 2000; 6:93–105. [PubMed: 10711834]
46. Egozcue J, Blanco J, Vidal F. Chromosome studies in human sperm nuclei using fluorescence *in situ* hybridization (FISH). Hum. Reprod. Update. 1997; 3:441–452. [PubMed: 9528909]
47. Vegetti W, et al. Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence *in-situ* hybridization in infertile men. Hum. Reprod. 2000; 15:351–365. [PubMed: 10655307]
48. Huang WJ, et al. Germ-cell nondisjunction in testes biopsies of men with idiopathic infertility. Am. J. Hum. Genet. 1999; 64:1638–1645. [PubMed: 10330350]
49. Aitken RJ. Whither must spermatozoa wander? The future of laboratory seminology. Asian J. Androl. 2010; 12:99–103. [PubMed: 20111089]
50. Haidl G. Management strategies for male factor infertility. Drugs. 2002; 62:1741–1753. [PubMed: 12149044]
51. De Rosa M, et al. Cabergoline treatment rapidly improves gonadal function in hyperprolactinemic males: a comparison with bromocriptine. Eur. J. Endocrinol. 1998; 138:286–293. [PubMed: 9539303]
52. Coviello AD, et al. Low-dose human chorionic gonadotropin maintains intratesticular testosterone in normal men with testosterone-induced gonadotropin suppression. J. Clin. Endocrinol. Metab. 2005; 90:2595–2602. [PubMed: 15713727]
53. Attia AM, Al-Inany HG, Proctor ML. Gonadotrophins for idiopathic male factor subfertility. Cochrane Database of Systematic Reviews. 2006; (Issue 4) Art. No.: CD005071.
54. Filippi S, et al. Identification, localization and functional activity of oxytocin receptors in epididymis. Mol. Cell. Endocrinol. 2002; 193:89–100. [PubMed: 12161007]
55. Nicholson HD, Guldenaar SE, Boer GJ, Pickering BT. Testicular oxytocin: effects of intratesticular oxytocin in the rat. J. Endocrinol. 1991; 130:231–238. [PubMed: 1919394]
56. Byrne MM, Rolf C, Depenbusch M, Cooper TG, Nieschlag E. Lack of effect of a single i.v. dose of oxytocin on sperm output in severely oligozoospermic men. Hum. Reprod. 2003; 18:2098–2102. [PubMed: 14507828]
57. Abel BJ, et al. Randomised trial of clomiphene citrate treatment and vitamin C for male infertility. Br. J. Urol. 1982; 54:780–784. [PubMed: 6817845]
58. A double-blind trial of clomiphene citrate for the treatment of idiopathic male infertility. World Health Organization. Int. J. Androl. 1992; 15:299–307. [No authors listed]. [PubMed: 1516979]
59. Sokol RZ, Steiner BS, Bustillo M, Petersen G, Swerdloff RS. A controlled comparison of the efficacy of clomiphene citrate in male infertility. Fertil. Steril. 1988; 49:865–870. [PubMed: 3129318]

60. Wang C, Chan CW, Wong KK, Yeung KK. Comparison of the effectiveness of placebo, clomiphene citrate, mesterolone, pentoxifylline, and testosterone rebound therapy for the treatment of idiopathic oligospermia. Fertil. Steril. 1983; 40:358–365. [PubMed: 6411497]
61. Ronnberg L. The effect of clomiphene citrate on different sperm parameters and serum hormone levels in preselected infertile men: a controlled double-blind cross-over study. Int. J. Androl. 1980; 3:479–486. [PubMed: 6777310]
62. Micic S, Dotlic R. Evaluation of sperm parameters in clinical trial with clomiphene citrate of oligospermic men. J. Urol. 1985; 133:221–222. [PubMed: 3918178]
63. Liu PY, Handelsman DJ. The present and future state of hormonal treatment for male infertility. Hum. Reprod. Update. 2003; 9:9–23. [PubMed: 12638778]
64. Hussein A, Ozgok Y, Ross L, Niederberger C. Clomiphene administration for cases of nonobstructive azoospermia: a multicenter study. J. Androl. 2005; 26:787–793. [PubMed: 16291975]
65. Vandekerckhove P, Lilford R, Vail A, Hughes E. Clomiphene or tamoxifen for idiopathic oligo/asthenospermia. Cochrane Database of Systematic Reviews. 1996; (Issue 4) Art. No.: CD000151. CD000151.
66. Adamopoulos DA, et al. Effectiveness of combined tamoxifen citrate and testosterone undecanoate treatment in men with idiopathic oligozoospermia. Fertil. Steril. 2003; 80:914–920. [PubMed: 14556812]
67. Raman JD, Schlegel PN. Aromatase inhibitors for male infertility. J. Urol. 2002; 167:624–629. [PubMed: 11792932]
68. Pavlovich CP, King P, Goldstein M, Schlegel PN. Evidence of a treatable endocrinopathy in infertile men. J. Urol. 2001; 165:837–841. [PubMed: 11176482]
69. Clark RV, Sherins RJ. Treatment of men with idiopathic oligozoospermic infertility using the aromatase inhibitor, testolactone. Results of a double-blinded, randomized, placebo-controlled trial with crossover. J. Androl. 1989; 10:240–247. [PubMed: 2663800]
70. Eskenazi B, et al. Antioxidant intake is associated with semen quality in healthy men. Hum. Reprod. 2005; 20:1006–1012. [PubMed: 15665024]
71. Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch. Biochem. Biophys. 1993; 300:535–543. [PubMed: 8434935]
72. Dawson EB, Harris WA, Teter MC, Powell LC. Effect of ascorbic acid supplementation on the sperm quality of smokers. Fertil. Steril. 1992; 58:1034–1039. [PubMed: 1426355]
73. Kessopoulou E, et al. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertil. Steril. 1995; 64:825–831. [PubMed: 7672157]
74. Keskes-Ammar L, et al. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Arch. Androl. 2003; 49:83–94. [PubMed: 12623744]
75. Rolf C, Cooper TG, Yeung CH, Nieschlag E. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. Hum. Reprod. 1999; 14:1028–1033. [PubMed: 10221237]
76. Lewin A, Lavon H. The effect of coenzyme Q10 on sperm motility and function. Mol. Aspects Med. 1997; 18(Suppl):S213–S219. [PubMed: 9266524]
77. Lenzi A, Gandini L, Picardo M. A rationale for glutathione therapy. Hum. Reprod. 1998; 13:1419–1422. [PubMed: 9688363]
78. Lloyd DR, Carmichael PL, Phillips DH. Comparison of the formation of 8-hydroxy-2'-deoxyguanosine and single- and double-strand breaks in DNA mediated by fenton reactions. Chem. Res. Toxicol. 1998; 11:420–427. [PubMed: 9585472]
79. Iwanier K, Zachara BA. Selenium supplementation enhances the element concentration in blood and seminal fluid but does not change the spermatozoal quality characteristics in subfertile men. J. Androl. 1995; 16:441–447. [PubMed: 8575984]

80. Comhaire FH, El Garem Y, Mahmoud A, Eertmans F, Schoonjans F. Combined conventional/antioxidant “Astaxanthin” treatment for male infertility: a double blind, randomized trial. Asian J. Androl. 2005; 7:257–262. [PubMed: 16110353]
81. Hinton BT, Snoswell AM, Setchell BP. The concentration of carnitine in the luminal fluid of the testis and epididymis of the rat and some other mammals. J. Reprod. Fertil. 1979; 56:105–111. [PubMed: 469829]
82. Matalliotakis I, et al. l-Carnitine levels in the seminal plasma of fertile and infertile men: correlation with sperm quality. Int. J. Fertil. Womens Med. 2000; 45:236–240. [PubMed: 10929687]
83. Lenzi A, et al. Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertil. Steril. 2003; 79:292–300. [PubMed: 12568837]
84. Lenzi A, et al. A placebo-controlled double-blind randomized trial of the use of combined l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia. Fertil. Steril. 2004; 81:1578–1584. [PubMed: 15193480]
85. Costa M, Canale D, Filicori M, D’Lddio S, Lenzi A. l-Carnitine in idiopathic asthenozoospermia: a multicenter study. Italian Study Group on Carnitine and Male Infertility. Andrologia. 1994; 26:155–159. [PubMed: 8085668]
86. Sigman M, Glass S, Campagnone J, Pryor JL. Carnitine for the treatment of idiopathic asthenospermia: a randomized, double-blind, placebo-controlled trial. Fertil. Steril. 2006; 85:1409–1414. [PubMed: 16600222]
87. Brinster RL, Avarbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. Proc. Natl Acad. Sci. USA. 1994; 91:11303–11307. [PubMed: 7972054]
88. Sadri-Ardekani H, et al. Propagation of human spermatogonial stem cells *in vitro*. JAMA. 2009; 302:2127–2134. [PubMed: 19920237]
89. Goossens E, Frederickx V, De Block G, Van Steirteghem AC, Tournaye H. Reproductive capacity of sperm obtained after germ cell transplantation in a mouse model. Hum. Reprod. 2003; 18:1874–1880. [PubMed: 12923142]
90. Fujita K, et al. Transplantation of spermatogonial stem cells isolated from leukemic mice restores fertility without inducing leukemia. J. Clin. Invest. 2005; 115:1855–1861. [PubMed: 15965502]
91. Fujita K, et al. Isolation of germ cells from leukemia and lymphoma cells in a human *in vitro* model: potential clinical application for restoring human fertility after anticancer therapy. Cancer Res. 2006; 66:11166–11171. [PubMed: 17145860]
92. Geens M, et al. The efficiency of magnetic-activated cell sorting and fluorescence-activated cell sorting in the decontamination of testicular cell suspensions in cancer patients. Hum. Reprod. 2007; 22:733–742. [PubMed: 17082221]
93. Smith KR. Gene therapy: theoretical and bioethical concepts. Arch. Med. Res. 2003; 34:247–268. [PubMed: 12957520]
94. Kojima, YKK. Springer: Heiderberg; 2006. p. 592-598.
95. Yomogida K, Yagura Y, Nishimune Y. Electroporated transgene-rescued spermatogenesis in infertile mutant mice with a sertoli cell defect. Biol. Reprod. 2002; 67:712–717. [PubMed: 12193376]
96. Kruger TF, et al. Sperm morphologic features as a prognostic factor in *in vitro* fertilization. Fertil. Steril. 1986; 46:1118–1123. [PubMed: 2946611]
97. Amelar RD, Dubin L, Schoenfeld C. Semen analysis. An office technique. Urology. 1973; 2:605–611. [PubMed: 4766016]

Key points

- Male infertility is often underdiagnosed and even if identified, frequently approached in a disorganized manner
- Male infertility should be addressed in the same way as any medical problem, with a history constructed to identify risk factors, a complete physical examination and appropriate laboratory tests
- Male factors account for up to 50% of reproductive difficulties in couples of reproductive age and men reporting difficulty in initiating a pregnancy warrant a thorough evaluation
- The most important aspect of the initial laboratory evaluation is the semen analysis; it is recommended that ≥2 semen analyses are obtained with similar abstinence periods of 2–3 days
- Our understanding of genetic causes underlying defects in sperm function, as well as the process of spermatogenesis and fertilization has drastically improved, improving our ability to treat these patients

Box 1 | Kruger strict morphology

Although there is a common classification scheme, which designates sperm as normal (oval), amorphous, tapered, duplicated, and immature, more strict criteria to identify "normal" spermatozoa have been developed by Kruger *et al.*[96] Kruger reported that, using his strict criteria, patients with fewer than 4% normal sperm had a fertilization rate of 7.6% of oocytes in comparison to over 50% in patients with 4–14% normal forms. Using these strict morphological criteria, a normal spermatozoon is characterized by a smooth oval head, 4–6 μm in length and 2.4–3.5 μm in width. The acrosome must be well defined, covering 40–70% of the sperm head. There cannot be any mid piece or tail defects. Finally, there should be no cytoplasmic droplets larger than 50% of the size of the sperm head.

Box 2 | Sperm motility measurement

There are two commonly used scoring systems for sperm motility. The first utilizes a 5-point scale, with a rating of 0 indicating no motility, 1 indicating sluggish or nonprogressive movement, 2 referring to sperm moving with a slow, meandering, forward progression, 3 indicating movement in a reasonably straight line with moderate speed, and 4 indicating sperm movement with high speed in a straight line.[97] An alternative system categorizes all of the moving sperm into one of four categories: category A indicates rapid progressive motility, B represents sluggish or slowly progressive motility, C is motility that is nonprogressive, and D indicates lack of motility.[13]

Table 1

World Health Organization semen parameter reference values

Semen parameters	4th edition*[13]	5th edition†[12]
Volume of ejaculate (ml)	≥2	≥1.5
Sperm concentration (10^6/ml)	≥20	≥15
Total sperm count ($\times 10^6$)	≥40	≥39
Motility (%)	≥50 progressive (a + b) ≥25 a only	≥40 total motility ≥28 progressive (a + b)
Morphology by Kruger strict criteria (%)	≥15	≥4
Vitality (% viable)	≥75	≥58
White blood cells (10^6/ml)	<1	<1

*Limits of adequacy, determined by consensus.

†Well-defined reference ranges derived using strict statistical analysis.