Why do we need another volume dedicated to infertility? This is the question I posed to the esteemed, late Dr. Martin Resnick when he asked me to edit this issue of the *Urologic Clinics of North America*. The answers are clear. Infertility is important. Reproduction is a basic physiologic function. The individual infertile couple experiences many interpersonal, physiologic, emotional, and financial stresses. Society is also affected as a result of the lost productivity of these individuals and medical expenditures. Urologists need to be prepared to optimally treat the infertile couple. This issue seeks to provide the most current information to those entrusted with the care of infertile couples.

This issue, *Male Infertility: Current Concepts and Controversies*, has enlisted many of the thought leaders in the discipline of male infertility. These authors have been charged with contributing to the most up-to-date issue dedicated to communicating advancements or controversies in the management of the infertile male.

The first section, “Foundations,” reviews the controversial reports of declining sperm counts. If indeed sperm counts are declining, all of the topics that follow gain even greater significance. Urologists have long utilized hormonal evaluation to assess spermatogenesis. The next article in this section addresses the question of whether or not our understanding of the endocrinologic control of spermatogenesis has evolved and, if so, how it has affected our approach to the infertile male. Although integral to the evaluation of the infertile couple, the limitations of semen analysis have long been recognized. Thus, the assessment of sperm structure and function has garnered interest and resulted in the development of the new techniques that are next presented. The last article in this section recognizes the importance of meaningful collaboration between the urologist and the reproductive endocrinologist in the treatment of the infertile couple. This article provides the urologist with the essential elements necessary to understand the evaluation of the female partner and to be an effective collaborator.

The section entitled, “The Vexing Varicocele,” discusses the changing approach to the treatment of the varicocele and provides the reader with an updated analysis of the effectiveness of varicocelectomy with regard to semen parameters and fertility. This information is critically important when counseling the infertile couple.

“Ejaculatory Abnormalities,” present difficult challenges for the urologist. Developments in the treatment and diagnosis of such abnormalities are next highlighted.

The urologist is often faced with treating patients with azoospermia. Those with obstruction...
present a technical challenge. There have been many technical modifications and advancements since these techniques were first introduced. These developments form the basis of the section on obstructive azoospermia. However, even more exciting is the recognition of the entity of non-obstructive azoospermia (NOA) and our ability to acquire sperm from individuals with NOA. These techniques have quickly evolved and are critically reviewed in this issue.

There is probably no more exciting nor more important area in male infertility treatment than the recognition of the importance of genetic factors influencing fertility. The section, “Genetic Foundations,” provides the urologist with a genetic primer as well as the essential information required to understand the role of genetic abnormalities in male factor infertility.

It is widely recognized that “Assisted Reproduction in Male Infertility” has taken an increasingly important role and includes a spectrum of interventions, from intrauterine insemination to in vitro fertilization, with or without intracytoplasmic sperm injection. The effectiveness of intrauterine insemination for male factor has been questioned, and the safety of in vitro fertilization and intracytoplasmic sperm injection is also of ongoing concern. These topics are reviewed and important information is provided permitting urologists to interact with our reproductive endocrinology counterparts and to make recommendations for the utilization of assisted reproductive technologies.

Although areas of controversy are discussed throughout this issue, the section, “Controversies,” highlights two therapeutic debates: Is reconstruction appropriate in men with obstructive azoospermia? Does endocrine manipulation have a role in male infertility?

Lastly, our population is aging and the age of prospective patients has increased. Therefore, there is increasing concern about the notion that aging affects spermatogenesis and fertility outcomes. It is appropriate that the scientific and medical advancements presented in this issue are introduced and concluded by articles that highlight societal issues that may affect fertility.

Infertility affects the individual, the couple, and society; that is why we need this issue dedicated to male infertility.

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Declining Worldwide Sperm Counts:
Disproving a Myth
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Much has been made in the medical and lay literature of an alleged decline in human sperm counts worldwide. More than 100 articles have appeared in the peer-reviewed literature in the past 50 years on this topic. As discussed in more detail later, these articles vary widely in the quality of their methodology and their fundamental results. Most studies have found no decline, an increase, or mixed results in assessing changes in sperm parameters. A few studies have shown an unambiguous decline. It is from the latter studies, however, that the lay media and various advocacy groups have drawn the “findings” supporting theories that purport to explain the “decline” in sperm counts. The primary theory supported by these groups is that minute environmental levels of chemicals acting as “hormone mimics” or “endocrine disruptors” are responsible for this alleged deterioration. The term “endocrine disruptor” refers to chemical substances that exhibit some degree of estrogen-like activity. Although there is no question that estrogenic compounds can be potent modulators of biochemical and physiologic function in high doses, the implication that in utero or adult exposure to low levels of environmental “endocrine disruptors” produces clinically detectable effects in humans is highly uncertain.

Data from the handful of peer-reviewed articles showing a decline in sperm counts and other semen parameters have been quoted often enough in the media and even among researchers that this “fact” has achieved the quality of a paradigm. A dispassionate review of the research to date, however, firmly repudiates not only the alleged connections between “endocrine disruptors” and declines in semen quality but also the declines themselves. Far from being a worldwide and well-proved phenomenon, declines in semen quality are, at best, a highly local phenomenon with an unknown cause and, at worst, a collective artifact arising from the observation of a highly variable physical attribute (sperm counts) with a relatively low-resolution tool (retrospective analysis of non-randomized study populations).

This article explores in detail the issue of the alleged decline in semen quality. The impetus for a comprehensive re-evaluation at this time is threefold: (1) the potential impact of a real decline in semen quality and subsequent human fertility is a priori critical to human welfare; (2) governments have begun to enact “anti-endocrine disruptor” legislation that is based, in part, on selected portions of the published data about semen quality; and (3) confusion and misinformation about semen quality remain widespread in lay and professional circles.

Sources of error

At first blush, it might seem almost trivial to obtain for semen quality the same type of widely accepted physiologic norms that have been determined for other bodily fluids and functions, such as blood or blood pressure. A host of difficulties conspire to make semen the least well understood bodily fluid, however, in terms of the distribution of its normal parameters in the general population. Obtaining human semen for scientific analysis is logistically difficult. As many authors have pointed out, the fact that semen is almost universally obtained by masturbation has
placed profound limits on the ability of researchers to adequately study this issue. If collection of semen samples were as straightforward as obtaining blood samples, the nature of semen quality changes over time (if any) would have been determined decisively decades ago. A prospective, longitudinal study of semen parameters in a large, multicenter, randomized study of community-dwelling men, although time-consuming and expensive, would provide highly reliable data. Of nearly equal quality would be an analysis of a suitably sized population of randomly selected community-dwelling men analyzed by birth cohort. Unfortunately, neither of these high-quality observational tools has been used to investigate the phenomenon of semen quality because of the logistical and emotional obstacles posed by the means of obtaining semen in a timely and well-controlled manner.

Researchers in the past 50 years have studied populations of men who have provided semen samples for reasons such as donation to sperm banks, evaluation for male factor infertility, prevasectomy evaluation, infertility evaluation for a couple, and donation for use with assisted reproductive techniques, such as in vitro fertilization or intracytoplasmic sperm injection. None of these populations represents a random sample of the population at large, and each presents a selection bias, although some of these study populations are more likely to be biased than others. For example, men who provide semen samples as part of a couple’s infertility evaluation in which the female partner is later determined to be the source of the infertility could plausibly be considered nearly representative of the general male population because their inclusion for testing is unrelated to the semen donor’s potential fertility. Other types of male study populations are more likely to be biased, however. Semen donors, for example, may have been screened for problems known to affect fertility or may have been selected precisely because a prior semen analysis showed a robust fertility. Male donors to in vitro fertilization or intracytoplasmic sperm injection programs are more likely than normal to have low fertility, regardless of the fertility status of their partner.

The lack of truly randomized, community-dwelling study populations has posed fundamental limits on our ability to say what is “normal” in terms of semen parameters and renders illegitimate any attempts to generalize from a particular study of semen or semen change over time to the male population at large.

Another source of potential error in studies of semen quality is the highly variable nature of the subject in question. Attributes such as sperm count, semen volume, and sperm morphology not only vary widely between individuals but also vary widely within individuals. Semen quality is sensitive to the following variables:

- Abstinence time (the amount of time since the previous ejaculation). Longer abstinence times lead to higher sperm counts, higher semen volumes, and a higher percentage of sperm displaying abnormal morphology. In turn, abstinence time varies with such things as a man’s age, his current level of sexual activity, and his general health.
- Scrotal temperature. The Sertoli cells of the testicles are temperature sensitive and must be several degrees cooler than normal body temperature to function properly. Anything that either temporarily or chronically raises scrotal temperature can depress semen quality [1]. Such phenomena as fever, hot tubs, exposure to high-temperature working conditions, and occupations that require long periods of sitting have been shown to affect sperm quality.
- Season. Some, but not all, studies of semen quality have shown seasonal fluctuations in mean sperm counts, with averages highest in springtime and lowest in summer [2].
- Smoking. Chronic smokers show a 13% to 17% decline in sperm counts, according to a meta-analysis of 20 studies [3]. Variations in the incidence of smoking between regions or over time may alter mean sperm counts.
- Marijuana use. Various animal, in vitro, and human studies have demonstrated deleterious effects on sperm parameters—including sperm counts—of tetrahydrocannabinol and chronic use of marijuana [4]. Changing regional or temporal trends in the use of marijuana may be a confounding factor in studies of semen quality.

An additional factor that has yet to be explained adequately contributes to the difficulties of scientifically determining population norms for semen and assessing any changes to those norms over time. Semen parameters have been repeatedly shown to vary significantly with geographic region. Even careful studies using identical laboratory methods on similar populations of men recruited for similar reasons have found this effect. For example, a study of 1283 men
from three regions of the United States found a mean sperm concentration in California of \(72.7 \times 10^6\) sperm/mL, whereas the mean concentration in Minnesota was \(100.8 \times 10^6\) sperm/mL, and in New York it was \(131.5 \times 10^6\) sperm/mL [5]. As demonstrated in the following sections, failure to take such variation into account can completely invalidate studies of semen characteristics.

Studies of semen quality have been hampered by three fundamental sources of potential error: inability to study a truly random population of community-dwelling men, wide inherent inter- and intrasubject variation in semen parameters, and wide and unpredictable geographic variations in semen quality. The authors of the best studies of semen quality in the past 50 years are cognizant of some or all of these potential sources of error. Some studies, for example, have attempted to control for variables such as abstinence time or have chosen subjects only from the subpopulations of men that are least likely to be biased in comparison to the population at large. Many studies have not taken these potentials sources of error into account, however. The failure to address such errors in some studies has been compounded by additional methodologic or statistical errors.

A flawed pivotal study

Before 1992, several small-scale or regional studies of men seeking medical help for infertility suggested a decline in sperm counts or other semen parameters in primarily European countries [6–15]. In the same period, however, a large US study found no decline in semen parameters [16]. The divergence in the results of these studies remained a topic of professional discussion and debate during these years but did not reach a wider audience. This thinking changed with the publication in 1992 of a paper by Elisabeth Carlsen and two colleagues from the University of Copenhagen, Denmark [17]. Entitled “Evidence for decreasing quality of semen during past 50 years,” this meta-analysis of 61 previous studies gained worldwide media attention. The attention was caused by the surprising magnitude of the findings (a nearly 50% drop in sperm count from \(113 \times 10^6/mL\) in 1940 to only \(66 \times 10^6\) sperm/mL in 1990) and the fact that the authors suggested a cause for the decline: “compounds with estrogen-like activity or other environmental or endogenous factors.” This study’s findings dovetailed with pre-existing concerns in many quarters about the potential hazards of environmental pollutants, such as herbicides, pesticides, and chemical contamination of all sorts. Although the relevant medical community reacted quickly to the paper with skepticism about its results and criticism of its methodologies, the popular interpretations of the study were unreserved, and subsequent critiques in the medical literature received little, if any, popular notice. As a result, the Carlsen paper has had an impact on the popular imagination and mindset of many environmental advocates that is far out of proportion to its actual scientific value. I argue that the paper has no scientific value. Its primary importance is that it acted as a stimulus for more careful researchers to explore the complex issue of semen quality.

Although the Carlsen paper already has been thoroughly “discredited in a number of professional articles” [18–22], its stature as a pivotal study warrants a summary of its major weaknesses:

- Variability across the 61 studies in the methods and protocols used for sperm collection and measurement
- Inability to control for period of abstinence in study subjects
- Inability to control for lifestyle factors, such as cigarette smoking or recreational drug use
- Failure to include studies that were available and conducted within the time period of the meta-analysis that fail to show a decline or report sperm concentrations higher than other studies included in the meta-analysis from the same time period
- Failure to account for geographic variation among studies (Of the studies from before 1970, all were from the United States, and 80% of these were from New York, where sperm counts (then and now) are the highest. After 1970, only three studies were from the United States, and many were from third-world countries, where sperm counts were low. A reanalysis of the Carlsen meta-analysis that accounts for this geographic variation shows no decline in sperm counts [Fig. 1].)
- Use of an inappropriate statistical analysis. A comprehensive statistical reanalysis of the Carlsen study [23] showed that the linear regression model used was inappropriate because the data distribution was highly non-uniform—most data were collected between 1970 and 1990. When quadratic or spline regression models were used (even when the data were uncorrected for geographic
variation) the data show mean sperm counts increasing since 1940.

Any of these weaknesses alone would serve to cast the results of a scientific study in doubt. Taken together, they justify a deep skepticism regarding the Carlsen study and a removal of the study from consideration in any review of evidence supporting a decline in sperm counts or other semen parameters.

The balance of evidence

Although without use in a scientific sense, the Carlsen paper did stimulate more than a decade’s worth of scientific research, most of it more methodologically sound than the Carlsen paper and written by authors who made far less sweeping extrapolations to the general population and possible causative agents. The 31 major studies published after the Carlsen paper that reported on time trends of semen parameters have been reviewed. Six of these studies showed clear evidence of a decline in either sperm counts or sperm counts and other semen parameters in a given location and period of time (Table 1). Sixteen studies unambiguously showed no decline (either no change or an increase) in semen parameters (Table 2), and 5 studies showed ambiguous results (Table 3). This latter group includes studies such as the 1995 report by Comhaire and colleagues [24], in which motility and normal morphology showed significant decreases but total sperm count was unchanged across the 17-year study period. The other studies in this group reported similarly conflicting results.

The summaries that follow do not include studies that simply reanalyzed existing data from Carlsen or others (although all of them contradict the “declining sperm” hypothesis), nor do they include studies that examined or critiqued in general ways some of the methodologic issues involved in the debate over alleged changes in semen parameters.

As can be seen from the tables, six studies with a combined N of 9215 reported an unambiguous decline in one or more semen parameters (including sperm count). The magnitudes of the reported declines in sperm counts (either in terms of total count or as sperm concentration/density) varied from a 16% decline to a 31.5% decline in the study period. In general, the authors of these studies attempted as best they could to control or account for some of the variables discussed previously.
In comparison, 16 studies with a combined $N$ nearly ten times that of the “pro-decline” studies (103,313) found no decline or a slight increase in the sperm counts of their respective populations. These authors took pains to control for variables such as abstinence time and variation in laboratory techniques and analyzed their data using appropriate statistical techniques. As noted earlier, however, all of these studies, like the “pro-decline” studies, relied on populations of men who do not necessarily represent the general male population and thus share the weakness of all studies to date. With that qualification understood, many of these studies are careful and rigorous and more than sufficiently powered to provide reliable data. If there were an actual decline in sperm counts in these study populations, one can assume that these analyses would have identified it.

An objective observer can draw two firm conclusions from this summary:

1. There is no “worldwide” decline in sperm counts or other semen parameters.
2. No correlations can be drawn from these studies about a causative role for “endocrine disruptors.”

### The question of geographic variation in sperm counts

Both of these conclusions deserve elaboration. Although the evidence firmly refutes the reality of

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<th>Date</th>
<th>Author(s)</th>
<th>Sample size (N)</th>
<th>Study period</th>
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<th>Major findings</th>
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<tr>
<td></td>
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<td>2. Mean sperm concentration declined 2.1% per year from 89 million/mL to 60 million/mL</td>
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<td>3. Percent motile sperm decreased 0.6% per year</td>
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<td>4. Percent normal sperm decreased by 0.5% per year</td>
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<td>2. Total motile sperm fell from 169 million to 129 million</td>
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<td>3. Concentration declined 2.1%/y</td>
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<td>4. Motility increased 0.18%/y</td>
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<td>1996</td>
<td>Adamopoulos</td>
<td>2385</td>
<td>1977–1993</td>
<td>Greece</td>
<td>1. Total sperm count declined from 154.3 million to 130.1 million</td>
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<td>2. No significant drop in semen volume</td>
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<td>2. Median total sperm count dropped from 206 million and 117 million, respectively</td>
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<td>1999</td>
<td>Bilotta [35]</td>
<td>1068</td>
<td>1981–1995</td>
<td>Italy</td>
<td>1. 31% decline in sperm concentration over the study period</td>
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<td>2. 8% decline in motility</td>
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<td>3. 9% decline in sperm with “typical morphology”</td>
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<td>2. Motility declined by 0.5% per year</td>
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<td>Total</td>
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<td>9215</td>
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<td>Date</td>
<td>Author(s)</td>
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<td>1996</td>
<td>Paulsen</td>
<td>510</td>
<td>1972–1993</td>
<td>United States</td>
<td>1. No decrease in sperm count, volume, sperm concentration, or normal morphology</td>
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<tr>
<td>1996</td>
<td>Vierula</td>
<td>238</td>
<td>NA</td>
<td>Finland</td>
<td>1. Mean sperm concentration was high (133.9 million/mL) and unchanged across the study period</td>
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<td>2. Total sperm count and sperm density were unchanged</td>
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<td>3. No trends up or down when data were analyzed by birth cohort</td>
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<td>1996</td>
<td>Fisch [5,22]</td>
<td>1283</td>
<td>1970–1994</td>
<td>United States</td>
<td>1. Statistically significant increase in sperm concentration over study period from mean of 77 million/mL to 89 million/mL (0.65% increase/year)</td>
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<td>2. Motility also constant, although mean volume decreased slightly</td>
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<td>2. Sperm with normal morphology rose from 58% to 66.4%</td>
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<td>3. Volume declined</td>
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<td>2. Total motile sperm count rose 7.7%/y</td>
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<td>3. Motility increased 0.27%/y</td>
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<td>1997</td>
<td>Handelsman</td>
<td>689</td>
<td>1980–1995</td>
<td>Australia</td>
<td>1. Overall mean for period was 69 million/mL</td>
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<td>2. No significant change in semen volume, total sperm count, or sperm concentration over study period</td>
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<tr>
<td>1997</td>
<td>Rasmussen [32]</td>
<td>1055</td>
<td>1950–1970</td>
<td>Denmark</td>
<td>1. Variation found in semen parameters year to year but no decline observed over the period studied</td>
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<td>2. Comparison of four birth cohorts revealed no association with changes in sperm quality</td>
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<td>1998</td>
<td>Emanuel [21]</td>
<td>374</td>
<td>1971–1994</td>
<td>United States</td>
<td>1. No significant differences between mean or median sperm counts between subjects in modern group compared with 1000 subjects in MacLeod and Gold's 1951 study</td>
</tr>
<tr>
<td>1998</td>
<td>Younglai</td>
<td>48,968</td>
<td>1984–1996</td>
<td>Canada</td>
<td>1. Linear regression analysis of the means of each of 11 centers studied over study period showed no significant trend</td>
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<tr>
<td>1999</td>
<td>Andolz</td>
<td>20,411</td>
<td>1960–1996</td>
<td>Spain</td>
<td>1. 0.2% decline in volume/year</td>
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<td>2. 0.04% increase in sperm count/year</td>
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<td>3. 0.4% increase in motility</td>
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<td>4. 3.6% decline in normal sperm/year</td>
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a worldwide decline in sperm counts, these studies clearly demonstrate that semen parameters vary dramatically geographically and temporally. In addition to the previously mentioned data from the United States, a study completed in Europe in 2001 found significant differences in mean sperm count among fertile men in Denmark, France, Scotland, and Finland [25]. For example, sperm concentration in Copenhagen, Denmark was only 74% that of Turku, Finland.

It is important to recognize that even large interregional differences in sperm count, sperm concentration, or semen volume may not have high clinical relevance because these parameters are only weakly associated with male fertility [26]. On the other hand, sperm motility and morphology are more strongly associated with fertility, and many of the studies reviewed herein demonstrate that these parameters also vary widely.

Currently, no data exist to explain the observed geographic variations in semen parameters. The range of possible causative agents is large and includes the following candidates:

- As-yet undetected differences in laboratory techniques, methods of analysis and interpretation, subject recruitment, or subject health and lifestyle differences between regions
- Differences in sexual behavior that would alter mean abstinence times between regions
- Differences in emotional/psychologic stress between selected populations of men
- Genetic differences among populations
- Variation in lifestyle factors, such as smoking or recreational drug use
- In utero exposure of male subjects to compounds with mutagenic or teratogenic potential
- Exposure of adult men to differences in environmental pollutants, such as lead or industrial chemicals found in herbicides, pesticides, or other materials

The data assembled to date fail to support any particular causative agent. No useful correlations have been found that would point to differences in exposure to industrial pollutants [27]. The lack of association between rural and urban areas or between areas with known high levels of air pollution and those with less pollution suggests that these factors are unlikely to explain the differences observed. The regional differences described to

Table 2 (continued)

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<tr>
<th>Date</th>
<th>Author(s)</th>
<th>Sample size (N)</th>
<th>Study period</th>
<th>Location</th>
<th>Major findings</th>
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</thead>
</table>
| 1999 | Gyllenborg [2] | 1927 | 1977–1995 | Denmark | 1. Increase in mean sperm concentration from 53 million/mL to 72.7 million/mL  
2. Increase in total sperm count from 166 million to 227 million  
| 1999 | Zorn | 2343 | 1983–1996 | Slovenia | 1. Volume, concentration, and total sperm count did not change in study period  
2. Sperm concentration analyzed by birth cohort showed a decline from 1950–1960, then an increase after 1960 |
| 2000 | Acacio | 1347 | 1951–1997 | United States | 1. No decline in sperm concentration found when compared with MacLeod data from 1951 and 1979 |
| 2000 | Tae Seo | 22,249 | 1989–1998 | Korea | 1. Mean sperm concentration was 60.5 million/mL  
2. No change in concentration, volume, or motility in study period |
| 2001 | Itoh | 711 | 1975–1998 | Japan | 1. Volume was unchanged  
2. Sperm concentration was 70.9 million/mL in early study compared with 79.6 million/mL in later Study |
| Total | | 103,313 | | | |

### DECLINING WORLDWIDE SPERM COUNTS

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date warrant further research, some of which is ongoing [28]. It is similarly impossible to draw any meaningful conclusions from the conflicting studies exploring temporal changes in semen parameters. Although the preponderance of data strongly suggests that there has been no decline in sperm counts or concentration in the past few decades, the handful of well-conducted studies reviewed herein that came to opposite conclusions cannot be dismissed. These data may be real for the specific region and time period examined. If so, then the observed declines are highly localized. For example, a study of men in the vicinity of Paris showed a decline [29], whereas a study of men in the Toulouse region of southern France showed no decline [30]. Likewise, one study of Danish men showed a decline [31], whereas another study that drew men from the same small country showed no decline over the 20-year study period [32].

Explanations for a decline in the regions studied include all of the potential causative factors mentioned and another that has not received much attention to date. It is well known that weight gain in men, particularly the deposition of adipose tissue around the waist, can depress serum total testosterone levels and increase serum estradiol levels [33]. Given the epidemic of obesity in the developed world in the past few decades, a gain in mean male weight coupled with type 2 diabetes and metabolic syndrome, which are related to weight gain, could explain some of the observed declines of sperm in specific populations of men studied. Weight gain has not been included in the data analysis of any of the studies of changing semen parameters conducted to date. It is worth noting that

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<th>Date</th>
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<td></td>
<td>2. Total sperm count did not decrease</td>
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<td>3. 40% of donors after 1990 exhibited “subnormal” sperm compared with only 5% of group investigated before 1980</td>
</tr>
<tr>
<td>1996</td>
<td>Van Waeleghem</td>
<td>416</td>
<td>NA</td>
<td>Belgium</td>
<td>1. Volume increased slightly</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Mean concentration declined by 12.4 million/mL in study period</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>3. Sperm count was unchanged</td>
</tr>
<tr>
<td>1996</td>
<td>DeMouzon</td>
<td>7714</td>
<td>1989–1995</td>
<td>France</td>
<td>1. No decline in sperm counts when data were analyzed by year of collection</td>
</tr>
<tr>
<td></td>
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<td>2. Sperm counts declined “regularly” for men born from 1950 to 1975</td>
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<td>2. From 1950 onward there was a gradual decline in sperm count and normal sperm</td>
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<td>forms but not in semen volume</td>
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<td>3. Decline in total sperm count was 1.9 million/mL per year of advancing year of</td>
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<td>birth</td>
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<tr>
<td>1999</td>
<td>Ulstein</td>
<td>5180</td>
<td>1975–1994</td>
<td>Norway</td>
<td>1. Two subgroups of study subjects showed declines in semen parameters</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Subgroup of men with previous children did not show decline in semen parameters</td>
</tr>
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</table>
contrary to the data on an alleged decline in semen parameters, good data from randomized, adequately sized populations of community-dwelling men show a clear and consistent decline in mean testosterone levels [34]. Such declines also may contribute to a decline in semen parameters in the few locations in which this phenomenon has been observed, although this has not yet been explored scientifically. It is certainly another area ripe for further investigation.

Summary

This article critically examines allegations for a worldwide decline in sperm counts and other semen parameters, as most visibly presented by Carlsen and colleagues in their 1992 paper. Despite the lack of scientific support for this hypothesis and for related claims that a “decline” is related to “endocrine disruptors,” these constructs remain firmly entrenched in the popular literature and are being used, in part, as a justification for legislation banning certain suspected “disruptors,” such as phthalates. A review of the data amassed to date on this issue clearly demonstrates that the bulk of the evidence refutes claims for a widespread decline in semen parameters. The initial study by Carlsen and colleagues has been criticized widely and thoroughly, and the number of methodologic flaws contained in the study warrants its exclusion from any review of data supporting a decline. The total population of subjects in which no decline in semen parameters was found is ten times larger than the population of men in whom a decline was found.

Even granting the reality of the declines reported for a handful of localized regions, no conclusions can be drawn from any of the existing studies about the role of putative causative agents. The range of such agents is wide, and no associations have yet been found between any of the reported declines and exposure of the men involved (either in utero or as adults) to “endocrine disrupting” compounds. The cause (or causes) of the well-documented geographic variations in semen parameters deserves further investigation; however, the evidence accumulated to date showing no decline in sperm counts or sperm concentration in populations throughout the developed world should be accepted. Advocates of “endocrine disruptor” theories are unjustified in using an alleged “decline” in semen parameters to support their cause, and public officials should be advised that this “leg” of supposed evidence for proposed legislation is weak to the point of breaking.

Acknowledgment

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References

Hormonal Evaluation of the Infertile Male: Has It Evolved?

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In addition to the surgical correction of a varicocele, the evaluation and management of the various endocrinopathies encountered in urologic practices remains invaluable in the management of the infertile male. The question posed at the time of this publication is “has it evolved?” In their retrospective review of 1035 patients in 1997, Sigman and Jarow [1] found that 9.6% of their patients had abnormal endocrine studies. The most common finding was an isolated serum elevation of follicle-secreting hormone (FSH) in 7.9% of the patients. FSH may be elevated in patients who have abnormal spermatogenesis caused by Sertoli cell dysfunction and inadequate negative feedback inhibition by inhibin. Elevation of FSH above 7.6 mIU/mL along with a testis axis of less than 4.6 cm has a probability of nonobstructive azoospermia (NOA) of 89% [2].

The present authors believe, however, that the presence of a normal semen analysis does not always guarantee a eugonadal state. Furthermore, it is widely recognized that a normal FSH level does not always guarantee the presence of intact spermatogenesis.

Steroidogenesis

Testosterone is the primary circulating androgen in the human male. Testosterone release from the Leydig cells accounts for approximately 95% of the total testosterone production, with the remaining 5% secreted by the adrenal cortex. Androgen biosynthesis in the testes starts with the formation of cholesterol. Cholesterol is produced from de novo synthesis from acetate, via low-density lipoprotein delivery and preformed cholesterol esters present in the Leydig cells. Cholesterol then is converted to pregnenolone under the influence of luteinizing hormone (LH). This conversion is the rate-limiting step in the testicular synthesis of testosterone. Testosterone then can be converted further to either dihydrotestosterone (DHT) via 5-alpha-reductase or estradiol via the P-450 aromatase pathways. Aromatase is present in the testes, brain, skin, liver, and adipose tissues [3,4]. Testosterone and DHT bind to the same androgen receptor. Estradiol binds to its own receptor.

Once one of these androgens binds to the receptor complex, DNA transcription ensues, and several key physiologic and developmental effects are produced. The effect is specific to the target tissue. There are a variety of androgen target tissues. Some respond directly to testosterone and some to DHT (Box 1). Developmentally, testosterone stimulates the Wolffian duct system (epididymis, vas deferens, and seminal vesicles). Testosterone also is converted in the genital folds to DHT, which is very important for genital fold development. This conversion facilitates the closure of the labio-scrotal folds and results in the urethral meatus assuming its distal-most position.

Both FSH and testosterone are essential for normal functioning of the seminiferous tubules in vitro and in vivo. Testosterone is required for completion of the meiotic division and spermatid development and thus plays an important role in...
the initiation and maintenance of spermatogenesis [5]. After the third and final rise of testosterone that occurs at puberty, the serum level is maintained near 6 ng/mL (600 ng/dL) during adult life until it begins to decline gradually with aging [6].

Hypogonadism

By traditional epidemiologic methods, an estimated 1 in 200 men have abnormally low levels of testosterone [7]. Hypogonadism is defined as a low total testosterone level (Food and Drug Administration [FDA] normal range, 300–1000 ng/dL) that can be associated with clinical findings such as decreased libido, infertility, anemia, mood changes, alterations in body hair distribution, and decreases in lean muscle mass and bone mineral density. Although men initially have 700 million Leydig cells, they lose approximately 6 million per year after age 20 years [8]. Nieschlag and colleagues [9], however, did not find any significant differences in sperm counts or fertilizing capacity between younger men and men beyond their seventh decade. Hypogonadism can be separated further into primary and secondary forms. Primary hypogonadism is defined as a low serum testosterone level with normal or elevated LH. Serum FSH levels may be normal or elevated, as seen in patients who have Klinefelter’s syndrome, anorchia, or other iatrogenic causes.

Hypothalamic-pituitary axis abnormalities

Secondary hypogonadism can be categorized further into congenital forms such as idiopathic hypogonadotropic hypogonadism (IHH) (Box 2) [10] or acquired forms. IHH is defined as a low serum testosterone level associated with a low LH level with or without a low FSH level. An important caveat is that low LH levels do not always correlate with low FSH levels, and visa versa. Acquired forms of inappropriately low LH levels include hypopituitarism, hyperprolactinemia, estradiol excess, and noncongenital idiopathic causes. The clinical presentation and the diagnostic implications are based in part on whether this form of androgen deficiency is pre- or postpubertal.

Kallmann syndrome is the anosmic form of IHH. Associated clinical features may include neurologic abnormalities (synkinesia, oculomotor deficits, deafness, mental retardation, and cerebellar defects), unilateral renal agenesis, midline facial defects, and pes cavus. Kallmann syndrome is a common X-linked recessive form of human hypogonadotropic hypogonadism [11,12]. In a recent report from Bhagavath and colleagues [13], KAL1 mutations were an uncommon cause of Kallmann syndrome in male patients, occurring in about 3.7% of all IHH males and in 6.3% of

**Box 1. Androgen target tissues**

*Testosterone mediated*
- Brain
- Breast
- External genitalia enlargement
- Internal genitalia differentiation
- Liver
- Muscle
- Testis

*Dihydrotestosterone mediated*
- External genitalia development
- Hair follicle
- Prostate
- Sebaceous gland
- Seminal vesicles

**Box 2. Etiology of congenital hypogonadotropic hypogonadism**

Congenital idiopathic hypogonadism
- Anosmic (Kallman syndrome)
- Nonanosmic; normosmic idiopathic hypogonadotropic hypogonadism

Fertile eunuch syndrome
Adrenal hypoplasia congenital

Genetic defects of the gonadotropin subunits
- Follicle-stimulating hormone-beta mutations
- Luteinizing hormone-beta mutations
- Mutations in leptin and leptin receptor genes

Hypogonadotropic hypogonadism associated with other pituitary hormone deficiencies
- PROP-1 mutations
- HERS1 mutations

Complex syndromes that include hypogonadotropic hypogonadism
- Prader-Willi syndrome
- Congenital spherocytosis
- Moebius syndrome
- Cerebellar ataxia
- Retinitis pigmentosa
anosmic/hyposmic IHH males in their study population. IHH thus is probably far more common that previously expected, with patients who have Kallmann syndrome representing merely the “tip of the iceberg.”

Pituitary

Hypopituitarism is the partial or complete insufficiency of anterior pituitary hormone secretion and may result from pituitary or hypothalamic disease. The reported incidence (12–42 new cases per million population per year) and prevalence (300–455 per million population) probably are underestimated. Clinical manifestations depend on the extent of hormone deficiency and may be nonspecific (eg, fatigue, hypotension, cold intolerance) or more specific (eg, growth retardation secondary to insufficiency of growth hormone or impotence and infertility as a result of gonadotropin deficiency). A number of inflammatory, granulomatous, or neoplastic diseases as well as traumatic or radiation injuries involving the hypothalamic-pituitary axis can lead to hypopituitarism [14].

Recent observations

At the University of Illinois in Chicago, the authors recorded the incidence of hypogonadism in various diagnostic categories of patients presenting to an infertility clinic by retrospectively reviewing the charts of 120 consecutive patients. Inclusion criteria were diagnoses of NOA, obstructive azoospermia (OA), oligospermia defined by World Health Organization (fourth edition) criteria, and men who had normal semen analyses. Serum testosterone was measured by electrochemiluminescent immunoassay or turbulent flow liquid chromatography tandem mass spectrometry; FDA criteria defined hypogonadism as a morning serum testosterone level less than 300 ng/dL. Interestingly, the incidence of hypogonadism in men who had NOA was 45.0%. The incidence of hypogonadism was 42.9%, 35.3%, and 16.7%, respectively in men who had oligospermia, normal semen analyses, and OA (Fig. 1). This subset of men who have OA may be considered a negative control because of the relatively exclusive nature of this anatomic condition. The fact that the incidence of hypogonadism in the OA group parallels that of the general population serves to validate the expectation that the OA group serves as a control. The lack of compensatory elevation of LH above 12.0 IU/L in the patient population suggested a form of hypogonadotropic hypogonadism. The finding that one third to nearly one half of the men who had NOA had an incidence of hypogonadism by FDA criteria supports the thesis that hypogonadotropic hypogonadism may be considerably more prevalent in the infertile male population than previously understood.

Hyperprolactinemia

Hyperprolactinemia suppresses both FSH and LH and may be caused by medications, concurrent medical illnesses, stress (both physiologic and psychologic), or pituitary tumors or may be idiopathic. Prolactin is produced in the anterior pituitary; it is responsible for lactation in women but has negligible physiologic effects in men. Normal prolactin levels in men should be less than 18 ng/dL, and testing should be repeated if the level is elevated, because biologic variability is particularly high with this assay. The most common medications that induce hyperprolactinemia are phenothiazines, imipramine, methyldopa, and reserpine. The clinician also must consider the possibility of prolactinomas, both micro- and

Fig. 1. Incidence of hypogonadism in infertility patients.
macroadenomas. Symptoms of prolactinomas include infertility, depressed libido, galactorrhea, headache, fatigue, and erectile dysfunction.

A small percentage of hyperprolactinemic men who have adenomas may have borderline-normal serum testosterone levels [15,16]. Brain MRI with gadolinium enhancement is diagnostic of anatomic pituitary pathology. Most cases of prolactin-secreting microadenomas respond to medical treatment with either bromocriptine or cabergoline. Both function in a similar way as dopamine agonists. Cabergoline has a longer half-life and therefore offers the advantages of fewer side effects and less frequent dosing. Patients who have idiopathic hyperprolactinemia also may be treated with medication, which may be withdrawn yearly to determine whether hyperprolactinemia persists [17,18].

Thyroid status

Thyroid diseases, both hyper- and hypofunction, can have an adverse impact on male reproduction. A lack of consensus exists, mainly because of the paucity of well-controlled studies, on the exact effect of thyroid abnormalities on the male reproductive system. Hyperthyroidism seems to affect both steroid hormone metabolism and sperm quality. Evidence of consistent elevations of sex hormone–binding globulin (SHBG) and total testosterone is presented in the literature; however, serum levels of free testosterone usually are not affected [19,20].

More recently, several groups of investigators examined the effects of hyperthyroidism on semen quality. Abalovich and colleagues [21] observed the following alterations in semen analysis in a series of 21 patients who had hyperthyroidism: asthenospermia in 85.7%, hypospermia in 61.9%, oligospermia in 42.9%, necrospermia in 42.9%, and teratospermia in 19.0%. These alterations seem to be reversible, because 85% were normalized when a euthyroid state was re-established. In 2002, Krassas and colleagues [22], in a prospective, controlled study compared semen parameters in 23 thyrotoxic males and 15 healthy controls. These investigators reported that mean semen volume was normal for both groups, but mean sperm density and motility were lower in the hyperthyroid group, although this number did not reach statistical significance. The deficient parameters improved following treatment.

Little is known and there are scant data about the effects of hypothyroidism on human spermatogenesis and fertility. Investigators Dubin and Amelar [23] in the early 1970s cited hypothyroidism as one of the syndromes of hormonal deficiency that rarely cause male infertility. Serum free testosterone and SHBG levels are normal or low [24–26]. Also, in hypothyroid males the LH response to gonadotropin-releasing hormone seems to be blunted [27]. Subclinical hypothyroidism does not seem to impact semen density, motility, or morphology [28].

Determining androgen status

The circulating testosterone concentration depends on the rate of production, steroid interconversion, metabolic clearance, and binding protein concentration [29]. Testosterone measurements are separated into the bioavailable fraction and the fraction that is SHBG bound. Bioavailable testosterone is composed of free testosterone and albumin-bound testosterone. Albumin has a lower affinity but a higher capacity for testosterone, whereas SHBG-bound testosterone has a higher affinity but lower capacity than albumin for this hormone fraction. Even a minor amount of SHBG-bound testosterone is available to certain cells with specific receptor binding sites [30,31].

Currently available tests

Measuring free or bioavailable testosterone continues to be problematic [32]. With currently available commercial laboratory assays, free testosterone measurements are notoriously inaccurate and require equilibrium dialysis or ultrafiltration techniques. Measurement of bioavailable testosterone requires ammonium sulfate precipitation.

Free testosterone concentrations may be estimated by using a mass action formulation that includes the theoretic equilibrium of steroids and their binding proteins in serum. An inverse relationship between SHBG concentration and percentage of free testosterone has been demonstrated by computer modeling [33].

Free androgen index

The free androgen index (FAI) is the ratio of total testosterone (TT) to SHBG. It provides a simple assessment of physiologically active testosterone:

$$\text{FAI} (\text{cFT}) = \frac{100 \times \text{TT} (\text{in nmol/L})}{\text{SHBG} (\text{in nmol/L})}$$

Calculated bioavailable or non–SHBG-bound testosterone is equivalent to the sum of free and
albumin-bound testosterone, which also is physiologically bioactive. Like calculated free testosterone (cFT), estimated concentrations of bioavailable testosterone are determined by a mass action formulation model that uses measured total testosterone, SHBG, and optional serum albumin values (http://issam.ch/freetesto.htm). Calculated bioavailable testosterone may be particularly useful for individuals suspected of having plasma protein abnormalities, such as in nephrotic syndrome, cirrhosis of the liver, or glucocorticoid administration.

**Sex hormone–binding globulin**

SHBG is synthesized by liver cells and has a 7-day half-life in circulation. Table 1 shows average SHBG levels for healthy adults. SHBG, also known as “testosterone-estrogen–binding globulin,” “sex steroid–binding globulin,” or “sex steroid–binding protein,” specifically binds 17β-hydroxysteroids in a 1:1 ratio. The glycosylated heterodimer (80–100 kD) binds 5α-DHT most tightly, followed by testosterone and estradiol. As previously mentioned, circulating testosterone is bound tightly with high affinity to SHBG and is bound loosely to albumin with only 0.5% to 3% in the unbound form.

Hormone carriers exist predominantly in the unbound form, providing a large excess of unfilled steroid binding sites. More than 80% of the circulating SHBG is unbound in females, and more than 40% is unbound in males. The other principle testosterone and estrogen carrier, albumin, is 99% unbound [34]. Changes in the total hormone concentration produce relatively minor changes in the size of the free fraction because excess carrier-protein binding sites modulate extreme variations in hormone concentrations.

In contrast, changes in the SHBG concentration greatly affect the amount of steroid available to tissues (Box 3). SHBG binds testosterone with high affinity, and changes in the SHBG concentration result in large shifts in the free and albumin-bound testosterone fractions. Because albumin’s steroid affinity is low (on the order of 10⁴ mol/L), the albumin-bound testosterone fraction may be considered bioavailable or able to diffuse into cells [35].

The circulating androgen concentration affects SHBG synthesis. Elevated testosterone levels cause SHBG synthesis to decrease, whereas high estrogen levels stimulate SHBG production. The regulation of SHBG synthesis, combined with the high affinity of SHBG for testosterone compared with estrogen, results in SHBG effectively amplifying the estrogen level.

**Inhibin B**

Since its discovery in the mid-1990s, inhibin B has been found to be significantly reduced in men who have infertility problems, independent of cause, compared with fertile men [34]. Kumanov and colleagues [36] found that correlations between inhibin B levels and sperm parameters were more significant than the correlations of FSH levels and sperm parameters. Inhibin B levels may be correlated to specific causes of male-factor infertility. Mormandi and colleagues [37] and Fujisawa and colleagues [38] reported that inhibin B had a significant positive correlation with sperm parameters in men who had varicoceles. Studies reported that infertile men who had a previous

<table>
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<th>Population</th>
<th>N</th>
<th>Concentration (nmol/L)</th>
<th>Control (nmol/L)</th>
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<tr>
<td>Males</td>
<td>122</td>
<td>32</td>
<td>13–71</td>
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<tr>
<td>Nonpregnant females</td>
<td>111</td>
<td>51</td>
<td>18–114</td>
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</table>

**Box 3. Sex hormone–binding globulin levels**

**Decrease**
- Androgens
- Growth hormone
- Progestins
- Glucocorticoids
- Insulin
- Hypothyroidism
- Acromegaly
- Nephrotic syndrome
- Obesity

**Increase**
- Estrogens
- Thyroid hormones
- Anticonvulsants
- Hyperthyroidism
- Pregnancy
- Cirrhosis
- Aging
History of cryptorchidism have lower inhibin B levels than normal controls [39]. deGouveia Brazao and colleagues [40] reported that inhibin B levels also were lower in infertile men who had cryptorchidism than in men who had idiopathic subfertility or in normal controls.

This decrease in inhibin B levels probably resulted from irreversible spermatogenic damage that had occurred in these cryptorchid patients.

Inhibin B levels may be a better marker than FSH for evaluating male-factor fertility. In patients who have infertility, measuring inhibin B levels may provide useful information on spermatogenesis and possibly may serve as a more direct marker of spermatogenesis than FSH. Currently, however, clinically available assays of inhibin cost approximately three times as much as assays of FSH, and it is not clear that the available evidence justifies the additional cost of an inhibin assay rather than FSH assay in the common evaluation of the infertile man.

Estradiol

The combination of a low serum testosterone level and an increased estradiol level may affect spermatogenesis adversely, because the former steroid enhances spermatogenesis and the latter inhibits it [41–43]. Raman and Schlegel [44] studied subfertile men who had high estradiol levels and depressed testosterone-to-estradiol (T/E2) ratios (<10:1) using two aromatase inhibitors, testolactone and anastrozole. Both produced increases in T/E2 ratios and an improvement in seminal parameters. Oligospermic men experienced an improvement in semen quality. The azoospermic patients continued to be azoospermic after treatment. Anastrozole (Arimidex), 1 mg/d, seemed to have a greater effect in increasing the T/E2 ratio and in reducing estradiol. Although both testolactone and anastrozole may elevate liver function tests, anastrozole, unlike its steroid-based counterpart, avoids the risk of adrenal steroid inhibition. In the cohort of patients who had Klinefelter’s syndrome, testolactone, 50 to 100 mg twice daily, was observed to be more effective in increasing the T/E2 ratio and reducing estradiol.

Evaluation

Initial evaluation

The initial endocrine evaluation of the infertile male after a careful history and physical examination should include serum testosterone and FSH levels. Although the authors prefer a morning testosterone, this consideration may not be as important in older men, because the normal diurnal variation decreases as men age. If testosterone is abnormal, the authors’ recommendation is to obtain testosterone and LH every 30 minutes three times between 8 and 10 o’clock in the morning. These samples are averaged by pooling samples into equal aliquots to address the variability of these hormones. The authors also include a single initial draw for SHBG and albumin (to calculate bioavailable testosterone), along with a single determination of estradiol. In general, the authors would consider an LH that is not elevated above the upper threshold of normal to suggest an element of a hypogonadotropic state that would respond to medical therapy. Imaging is reserved for LH levels that are below the limit of normal. Although the authors complete this evaluation in all such patients, they recognize that this evaluation schema may not be feasible for all clinicians or patients.

If pooled testosterone is determined to be very low (<150 ng/dL) or other signs of pituitary insufficiency are noted along with decreased libido, a complete hypothalamic-pituitary-gonadal axis investigation is indicated. In these cases the authors recommend adding a prolactin level, and, although the yield of finding significant pathology is low, the clinician also might consider adding a thyroid-stimulating hormone and free thyroxine assay. If hyperprolactinemia (especially in the presence of diminished libido) is discovered, or other laboratory evidence of panhypopituitarism is uncovered, a gadolinium-enhanced MRI with special attention to the sella turcica is indicated.

Treatment

It is important to provide the clinician with evidence supporting the importance of a well-considered approach to evaluating the endocrinologic aspects of male-factor infertility.

Hypogonadism: normal or low gonadotropins

The authors’ initial intervention of choice in hypogonadal men who have low or borderline-normal testosterone levels and unexplained male-factor infertility is clomiphene citrate (Clomid). They begin this relatively inexpensive medication at 50 mg every other day. This is an off-label use of clomiphene citrate, a generic agent that has
not been submitted to the FDA for approval in the medical treatment of male infertility but is in widespread clinical use. Doses can be titrated up to a maximum of 100 mg every day monitored with a serum testosterone evaluation within several weeks after dose adjustment. In the authors’ experience, estradiol levels do not seem to rise concurrently with testosterone increases in patients who show a positive response to clomiphene citrate treatment.

In 2005, in a multi-institutional nonplacebo controlled study in which the patients served as their own controls, Hussein and colleagues [45] demonstrated that the administration of clomiphene citrate could produce sperm in the ejaculate in 64.3% of men who had NOA associated with either hypospermatogenesis or maturation arrest. Patients who had Sertoli cell–only syndrome and patients who had malignancy were excluded from the study population. The statistically significant variable affecting the results was not the dose but the duration of clomiphene citrate therapy, with most patients requiring 4 months of treatment.

Of the patients who remained azoospermic after 3 to 9 months of clomiphene citrate therapy (with a target total testosterone level of 600–800 ng/dL), all had successful sperm retrievals sufficient for intracytoplasmic sperm injection after surgical intervention. Additionally, clomiphene citrate resulted in a statistically significant increase in testis biopsy patterns associated with a greater likelihood of obtaining sperm by surgical extraction. Of the 42 patients in this study, fewer than half (42.9%) had an initial testosterone level lower than 300 ng/dL. This study must be considered a pilot, because it was neither placebo controlled nor blinded. The authors expect future controlled studies will verify these results.

In contrast to classic Kallman syndrome, some acquired forms of IHH have functional, not anatomic, defects that present after puberty. These patients represent a select population that may benefit from pharmacologic manipulation of the hypothalamic-pituitary-gonadal axis. Clomiphene citrate may accomplish this task by competitively blocking the E2 receptor at both the hypothalamic and pituitary level. This blocking increases gonadotropin-releasing hormone, FSH, and LH, and thus testosterone levels.

In a small retrospective review, Whitten and colleagues [47] identified patients who had IHH that presented after puberty. Because these men had an otherwise normal evaluation, testicular volumes greater than 10 mL, and some evidence of gonadotropin secretion, treatment with clomiphene citrate, 50 mg three times per week, was selected for a 3-month trial. Three of the four men in this subgroup demonstrated improvement in testosterone levels and semen parameters by their 3-month follow-up visit.

**Hypergonadotropic hypogonadism**

Patients who have Klinefelter’s syndrome already have high FSH and LH levels, so Leydig cell reserves already are low. They may benefit from testosterone replacement therapy if fertility is not an issue. If fertility is an issue, this special patient population may benefit, as mentioned earlier, from an aromatase inhibitor that blocks the conversion of what little testosterone is in circulation. Testolactone, 50 to 100 mg twice daily, may be of benefit.

**Summary**

An endocrinologic evaluation of patients who have male-factor infertility has clearly evolved and leads to specific diagnoses and treatment strategies in a large population of infertile men. A well-considered endocrine evaluation is especially essential with the ever-growing popularity of assisted reproductive techniques and
continued refinements with intracytoplasmic sperm injection. It is also imperative that urologists work intimately with reproductive specialists, because timing and coordination of care may help achieve the ultimate goals in addition to the proper hormonal milieu sought to maximize sperm quality in these patients.

References


Assessing Sperm Function
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Every male infertility work-up should start with the basics: a good history, physical examination, and at least two semen analyses. Throughout the past 50 years or so that it has been in existence, the semen analysis largely has remained unchanged. This basic test is inexpensive, noninvasive, and remains the cornerstone of the infertility evaluation. As advances are made, however, other tests are introduced—not to supplant or replace this test—but rather to delve further into the specific causes of male infertility. Just like any other aspect in the dynamic field of medicine, this role of the semen analysis has been challenged, its validity questioned, and its techniques scrutinized.

This article reviews basic semen tests and new fertility tests that are providing great insights to the rapidly developing understanding of male infertility. Finally, promising new tests under development are mentioned with their potential clinical applications.

The basic test: the semen analysis

Collection and timing

Suboptimal sperm collection remains a frequent cause of error in the semen analysis. It should be emphasized to patients that there should be 2 to 7 days of sexual abstinence before collection. Two separate samples should be analyzed. These samples should be not less than 7 days apart [1,2]. The duration of the abstinence should be constant if possible, because each additional day can add as much as 25% in sperm concentration [3]. Lubricants should be avoided, as they may interfere with motility results. Coitus interruptus often leads to inaccurate results, as the first part of the ejaculate that contains most of the sperm may be lost. A clean, sterile container should be used as a receptacle. A complete list of the guideline is provided in the World Health Organization (WHO) laboratory manual for examination of human semen and sperm–cervical mucus interaction [4].

Semen collection

Semen specimens can be produced in various ways. At times patients will require assistance.

Masturbation in a clinical setting. This is the recommended procedure where the collection is done in a private room in the same facility where the semen will be analyzed. The glans and the penis should be cleaned with a wet paper towel (soap should be avoided). Lubricant use is discouraged but if needed should not be applied to the glans. The container should be provided by the laboratory to avoid contamination or spermicidal effects. The main advantage of this collection method is its simplicity, noninvasiveness, and inexpensiveness. Optimal specimens, however, may be difficult to procure for some men who are uncomfortable providing a sperm specimen in this environment [1].

Masturbation with assistance. Some men may not be able to achieve adequate erection and
ejaculation. Assistance can be provided for by oral medications such as PDE5 inhibitors given 30 to 60 minutes before collection. Cavernosal and subcutaneous injections are less popular but possible options to administer to patients who have erectile dysfunction. Seminal pouches that do not contain any spermicides also can be used and allow the patient to engage in sexual activity should he be incapable or uncomfortable producing specimens by masturbation.

**Vacuum erection devices.** These can be used to obtain erection by creating a vacuum around the penis generating a pressure differential that fills the corpora with blood. A constrictive band is placed at the base, however, to maintain erection, and this can inhibit the flow of semen with ejaculation.

**Vibratory stimulation and electroejaculation.** Mechanical/vibratory stimulation may be used for patients who have suffered spinal cord injury (if the spinal cord lesion is T8 and above) [5]. Rectal probe electrostimulation (RPE) may induce ejaculation by stimulation of the efferent fibers of the hypogastric plexus. Precautions for autonomic dysreflexia should be performed while doing these procedures, as some patients with high spinal cord lesions can have life-threatening hypertension [1].

**Technical aspects of the semen analysis**

It should be emphasized that nonspecialized laboratories often will have inadequate equipment and inexperienced personnel to perform the semen analysis. Semen analysis is one of the few manually performed examinations remaining in medical laboratories, and ideally it should be performed in an experienced laboratory [6]. There is no reliable and cost-effective automation. Experienced laboratories often will use the Neubauer chamber (Zeiss, Jena, Germany) as recommended by WHO for sperm counting. This requires careful dilution and frequent cleaning. Incorrect use can increase chamber depth, producing erroneous results [6]. Counting chambers, such as the Makler (Sefi Medical Instruments, Haifa, Israel), that do not require dilution are also subject to the same variation [7]. Disposable counting chambers (Cell Vu (Millennium Sciences, New York), Microcell (Conception Technologies, San Diego, California)) are fairly inexpensive and offer less exposure of the clinician to bodily fluids by eliminating the cleaning process. The availability of an appropriate centrifuge also can be crucial. Semen samples without spermatozoa in an initial assessment should be centrifuged at 2000 g for 10 minutes and reexamined for the presence of sperm. If no sperm is visible at this point, further centrifugation and microscopic examination at 3000 g for 15 minutes are advised. There should be repeated centrifugation and sperm counting performed before azoospermia can be reported in a single semen analysis [8]. The assessment of motility and morphology is an acquired skill for the medical technologist requiring both didactic lectures and practical experience. Quality control testing is a critical component of an accurate semen analysis and often is underemphasized in nonspecialized laboratories [6,9]. It is therefore crucial that patients are referred to a laboratory that can provide reliable results. This may eliminate the need for repeated tests and in the end allow the clinician to make an accurate and cost-effective diagnosis [9]. Clinical laboratories engaged in diagnostic work in the reproductive field in the United States are accredited by agencies such as the College of American Pathologist (CAP). They follow rigorous procedures and protocols and provide superior test results over a laboratory without any external inspection of its records and protocols.

**Standard procedures**

The semen sample should be examined within 1 hour of production and receipt in the laboratory. Some of the semen parameters can be affected by a delay in assessment. Motility decreases significantly after 2 hours and progressively diminishes afterwards while reactive oxygen species (ROS) level increases. Ideally, semen is placed in a 37°C gently shaking incubator for 30 minutes to allow liquefaction and mixing. The semen analysis characteristics can be classified into three groups: macroscopic, microscopic and physiologic.

**Macrosopic**

Table 1 lists the five macroscopic measurements in a standard sperm analysis. These parameters have remained fairly constant, with the normal values remaining relatively unchanged since the inception of the semen analysis. Some variation in macroscopic parameters (ie, liquefaction) is relatively common and has little clinical significance, although it also can be found in accessory gland dysfunction [4,10]. The specifics of how the tests are conducted (for all variables) are found in the WHO manual [2].
Microscopic examination of the semen in essence assesses spermatogenesis. This part of the semen analysis is subject to technical error, and even reliable laboratories can display variable results. The normal values are also subject to some patient variation, with variability from one ejaculate to the next. The following are the parameters of the microscopic analysis.

Sperm agglutination. The microscopic examination starts with the creation of a wet smear (a drop of semen on a slide covered with a cover slip) visualized under 1000 × magnification. Sperm agglutination, sperm presence, and subjective motility can be assessed under this method. When sperm adheres to nonsperm elements (nonspecific agglutination), it may be indicative of accessory gland infection. Sperm-to-sperm agglutination (site-specific agglutination) can be secondary to antisperm antibodies; however, it should be remembered that a small degree of agglutination is normal [4]. When agglutination is observed, semen cultures and antibody assessment should be preformed.

Sperm count and concentration. Assessment of sperm concentration (number of sperm per milliliter) and sperm count (number of sperm/ejaculate) is conducted after liquefaction. Dilution of the semen is required if a Neubauer counting chamber is used, while Makler and other disposable sperm counting chambers (Micro cell and Cell-Vu) do not require dilution [11]. These disposable counting chambers are accurate and fairly inexpensive and minimize the clinician’s exposure to body fluids as cleaning process is not required. The Neubauer chamber remains the gold standard of sperm-counting chambers [8], but it is not without its flaws. The normal sperm concentration is reported at greater than 20 million sperm/mL. Oligospermia (less than 20 million sperm/mL) may be indicative of incomplete collection or a short abstinence period. When collection problems are eliminated, further evaluation as outlined elsewhere in this issue should be undertaken. Azospermia (absence of sperm) may be the result of abnormal spermatogenesis, ejaculatory dysfunction, or obstruction. Polyspermia (abnormally elevated sperm concentration) rarely is reported but may be caused by long sexual abstinence and often is associated with sperm of poor quality.

When oligospermia is reported, the levels of motility and morphology become especially important, as total motile sperm counts guide
decisions on appropriate therapies, including the use of assisted reproductive techniques (ART). In cases of azoospermia and severe oligospermia, hormonal evaluation (follicle-stimulating hormone [FSH] and testosterone) should be requested. Karyotyping and Y microdeletion may provide valuable information regarding the etiology of the patient’s abnormal semen parameters and important information if in vitro fertilization (IVF) is being entertained as a treatment option (see article on genetic causes of male infertility). Foci of microdeletions in the Y chromosome are associated with impaired spermatogenesis, and depending on their location, may predict poor sperm retrieval even with testicular biopsy [12]. Karyotyping may detect autosomal or x-linked genetic aberrations causing infertility. Knowledge of the chromosome status is important, as male offspring conceived with intracytoplasmic sperm insemination (ICSI) or even natural conception most likely will inherit the same microdeletion [12,13].

Sperm morphology. Table 2 compares the two widely used criteria in morphology (WHO and Tygerberg Strict criteria). Among the semen parameters, this is the most subjective and the most difficult to standardize [6,8]. Accurate assessment of morphology is critical in the evaluation of the infertile male. This also can be a significant predictor of pregnancy. When correctable causes of male infertility are not identified, couples with teratozoospermia (less than 15% normal morphology) by WHO method may be directed to proceed with IVF with ICSI versus intrauterine insemination (IUI) [8,14]. Studies on sperm selection for ICSI reports of lower pregnancy outcomes and higher abortion rates [15,16] when morphologically abnormal sperm were used. There is no evidence, however, that abnormal morphology is associated with an increase spontaneous miscarriage rate in natural conception. Abnormal morphology, however, is associated with decreased fertilization or pregnancy rates [14,15].

Nonsperm cells. Immature germ cells, epithelial cell, and leukocytes are some of the nonsperm elements noted on seminal microscopic examination [17,18]. Epithelial cells are indicative of poor collection when present in high numbers. Leukocytes are the most significant nonsperm cellular elements in the semen and are a frequent finding in patients who have unexplained infertility [18]. In the initial microscopic analysis, the immature spermatozoa may be confused with leukocytes. To confirm findings, additional tests may be requested when there are greater than 5 round cells/hpf. Immunocytochemistry is the procedure of choice, but given its expense, most laboratories do not have this test. The Endtz test is a reliable alternative, as it allows accurate identification of leukocytes that contain enzymes that will react with the peroxide and be visualized with the

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<td>Sperm morphology classification</td>
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<tr>
<td>Normal reference range</td>
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<tr>
<td>Head Shape</td>
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<tr>
<td>Acrosome 40% to 70% of head surface</td>
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<tr>
<td>Size 2.5–3.5 μm width</td>
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<tr>
<td>Vacuoles &lt;20% head area</td>
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<td>Midpiece Shape</td>
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orthotoluidine dye [19]. Initially considered solely as a marker for genital tract infection, contemporary research has shown that leukocytes can be present with no other signs of infection or immune response [20] and have intimate links to reactive oxygen species (ROS) [19,21–23]. The WHO has defined leukocytospermia as levels above $1 \times 10^6$ white blood cells (WBC)/mL. Studies have shown, however, that ROS levels are elevated even at WBC counts of less than $20 \times 10^6$, suggesting that much lower levels of leukocytes are pathologic [22,24]. In a 12-month follow-up, men who had a negative Endtz test (zero) had a 23.7% chance of initiating pregnancy, while levels less than $1 \times 10^6$ lowered the chances to 15.5% [25]. In IUI, high seminal leukocyte levels (greater than $2 \times 10^6$/mL) result in lower pregnancy rates. Leukocytospermia determination still has to be requested separately in many andrology laboratories. Its significance and the ease of determination that is reproducible in most laboratories should place this test among the standard testing that accompanies a basic semen analysis. When leukocytospermia is identified, semen cultures should be performed.

Red blood cells (RBC) are often present in semen. Although small amounts are usually a normal finding, they can be indicative of infection, inflammation, ductal obstruction, or rarely vascular abnormalities.

**Physiologic variables**

**Sperm motility**

Sperm motility is a reflection of the normal development of the axoneme and the maturation the sperm undergoes within the epididymis. This is a parameter that is subject to significant potential for technical mistakes in the laboratory. The most common method employed by laboratories is to simply estimate the motility of sperm on several fields. This is a subjective method and prone to inaccuracy. Some argue that in vitro measurement of motility is not reflective of the true motility within the female reproductive tract [26,27]. The progressive motility grading system recommended by the WHO is ideal for technicians trained in andrology but can be daunting for those who do not perform the tests regularly [6]. Some authors recommend that temperature should be reported and the time from submission to examination, as slight temperature increases and delay of examination can decrease the number of motile sperm counted dramatically [8]. Asthenospermia (sperm motility less than the WHO cutoff levels) also can be artifactual when spermicides, lubricants, or rubber condoms are used. Jouannet and colleagues and Nallella and colleagues compared semen parameters and found that sperm motility and concentration combined provided accurate prediction of fertility [28,29] with a high sensitivity (.74) and specificity (.90). Total motile sperm count is thought by many to be the best independent predictor [26,30] of pregnancy outcome with IUI (less than $1 \times 10^6$ motile sperm with pregnancy rate of 2% and 5–8 $\times 10^6$ motile sperm with pregnancy rate of 19%) [30]. High cumulative pregnancy rates (motility greater than 30% with pregnancy rate of 74%) also were reported in females undergoing ovarian stimulation with clomiphene citrate and human menopausal gonadotropin in conjunction with IUI [31]. Postwash total motile sperm count with a cut-off rate of 40% also was found to be a better predictor of IUI success [27].

**Viability**

When the motility is reported as less than 5% to 10%, viability testing is recommended, as profoundly low motility can be from dead sperm or necrospermia [6,8]. The most common viability assessment involves staining with Eosin Y (Sigma-Aldrich, St. Louis, Missouri) followed by the blue-black counter stain of Nigrosin (Sigma-Aldrich, St. Louis, Missouri). The viable sperm with its intact cell membrane will not take up the dye and remain unstained. This test will differentiate necrospermia from immotile sperm secondary to structural flagellar defects such as in Kartagener’s syndrome and primary cilia dyskinesia.

Hypo-osmotic swelling test (HOST) is an alternative method to assess sperm viability. It is based on the principle that viable sperm have intact cell membranes. Exposure of the sperm to hypo-osmotic fluid will cause water to flow into the viable cells seen as swelling of the cytoplasmic space and curling of the sperm tail. Nonviable sperm with nonfunctional cell membranes will not exhibit this effect, as they cannot maintain an osmotic gradient. One application of this test is to aid selection of viable sperm for use in IVF or ICSI, especially when there are no motile sperm seen in the testicular sperm or cryopreserved specimens. As this test is reproducible and relatively inexpensive, it also is recommended by some to be done routinely [32]. Nevertheless, it has not been employed widely in the routine management of infertile males.
**Computer-assisted sperm analysis**

Computer-assisted sperm analysis (CASA) has two advantages: high precision and quantitative assessment of sperm kinematics. It is a semiautomated technique that provides data on sperm density, motility, straight-line and curvilinear velocity, linearity, and average path velocity, amplitude of lateral head displacement, flagellar beat frequency, and hyperactivation. Sperm concentration, sample preparation and frame rate can affect accuracy of the CASA [33]. Stains used also have affected the accuracy of determining morphology. Although this technology had theoretic advantages, it does not realize these advantages in clinical practice. This test requires expensive equipment and still requires the active participation of a technician. At present, these machines are found more commonly in andrology laboratories, and not in general pathology laboratories, where most of the initial semen analysis is analyzed [6,34]. The most important role of CASA at this time seems to be in standardizing aids in quality control and quality assurance in andrology laboratories, as the emerging use of IVF and ICSI diminished the role of motility assessment in sperm selection in assisted reproduction [34]. Future applications are being explored in reproductive toxicology [35].

**Sperm home testing kits**

A decade or so ago, a few male fertility home kits were introduced to the market. Some kits even have microscope sets, and others are not true home kits but merely transport systems. These tests were developed to decrease the patient’s embarrassment of having a semen analysis in a clinic or laboratory. The first notable home tests (Fertilmarq and Babystart, Lake Consumer Products Inc, Wisconsin) were based on sperm staining, and the color intensity will test positive when the sperm count is equal or above 20 million/mL. The kits have two separate tests per pack and claim 97% accuracy. The latest test to be introduced on the market is a combined male and female test kit (Fertell, Genosis Inc, Surrey, UK). The female kit tests for FSH on day 3 of the menstrual cycle from the early morning urine sample as an ovarian reserve test. The male kit is comprised of a container with a lid and is based on the principle of using hyaluronic acid as a cervical mucus substitute that will allow sperm to swim up. These sperm will be coded red in an antigen–antibody reaction. The test will show positive (indicated by a second line in the result box) when the motile sperm has a concentration of greater than 10 million/mL [36]. This test claims 95% accuracy [37]. The disadvantage of this test is that it deals only with motility parameter, which is only one of the aspects of the male infertility spectrum.

Proponents of these home tests feel that these home kits may increase awareness of couples that the male factor needs to be considered in infertility evaluations. Its detractors are skeptical that it will achieve that purpose. Proper treatment is often as multifaceted as the causes of infertility, and these home tests may result in wrong focus and unnecessary delay. Males who test positive may be lulled into thinking that they are normal and may not seek urologic assessment. Only time will tell if these tests truly can promote male factor infertility awareness.

**Limitations of the basic sperm analysis**

The true litmus test for male fertility remains the ability to cause pregnancy in vivo. Although the semen analysis is used as a surrogate measure of a man’s fertility potential, it is not a direct measure of this. Clinical research has shown that a normal semen analysis may not reflect defects in sperm function (idiopathic infertility), and men with poor sperm parameters still may cause spontaneous pregnancy. Only fifty percent of infertile men have recognizable causes detectable by the basic semen analysis [8]. One out of every seven couples are subfertile [38] when based on WHO standards. The presence of several criteria further reinforces the emerging opinion that the current standards (WHO and Tygerberg) do not reflect the true fertility potential of subjects. The current normal values fail to satisfy clinical and statistical standards [8,28] and pose the risk of misclassifying a subject’s true fertility status. In fact, using the WHO cut-off of $20 \times 10^6$, 20% of 18-year olds would be classified as subfertile [39]. Studies on sperm donors with known fertility status reveal a significant overlap in the sperm characteristics between fertile and subfertile men [28,40].

Guzick and colleagues [40] in 2001 conducted a study of 1461 fertile and infertile men with no female infertility factor and found different cut-off levels in:

- Sperm concentration (less than $13.5 \times 10^6$ in subfertile and $48 \times 10^6$ in fertile men)
- Percent motility (less than 32% subfertile and greater than 63% fertile men)
- Normal morphology (less than 9% subfertile and greater than 12% fertile men)
Nallella and colleagues [28] in 2006 did a similar study (n = 572) and used the WHO and Tygerberg criteria on these subjects with known fertility. They noted that there is low sensitivity (0.48) in detecting subfertile subjects using WHO reference values for sperm concentration and low sensitivity (0.83) using Tygerberg criteria for % normal morphology. Among the variables, motility had the least overlap range and gave the best prediction of the subject’s fertility potential. This is in contrast with the earlier study by Guzick and colleagues, where morphology was reported to provide the highest discriminating power in detecting subfertility among all the semen variables. Clearly, each variable alone is neither a powerful sole discriminator nor predictor of fertility status, and they must be considered in the context of other parameters and the clinical setting.

As the need for new reference values emerges, it is inevitable that the definition of normal semen parameters continues to be revisited. Nallella and colleagues [28], in analyzing receiver operating characteristic curves (ROC) for concentration, motility, and morphology, suggested the following values based on the equal sensitivity and specificity of each:

- Concentration greater than 31.2 \( \times 10^6 \)/mL
- Motility greater than 57.8%
- Normal morphology greater than 33% (WHO) and greater than 11% (Tygerberg strict criteria)

These values are also close to earlier studies by Zinaman and colleagues [14] who noted a decrease in fertility rate when the concentration fell to less than 30 \( \times 10^6 \) and normal morphology (Tygerberg strict criteria) less than 4 \( \times 10^6 \). There remains a need for further studies in larger populations and different demographics before a consensus can be reached on the necessity of resetting current values to increase the predictiveness and utility of the semen analysis.

Additional tests (nonroutine)

Sperm–mucus interaction

Also known as the postcoital test (PCT), this test can assess cervical environment as a cause of infertility. Accurate timing is crucial, as it has to be conducted when the cervical mucus is thin and clear just before ovulation. In this test, cervical mucus is examined 2 to 8 hours after normal intercourse. Progressively motile sperm greater than 10–20/hpf is designated as normal. Practical guidelines of American Society of Reproductive Medicine (ASRM) (2004) recommend PCT in the setting of hyperviscous semen, unexplained infertility, or low volume semen with normal sperm count. Medical history and semen analysis can predict PCT results in half of the infertile couples [41]. Poor-quality semen most likely will have poor PCT, and it is not recommended routinely for males who have abnormal semen analyses. Couples who will show defective sperm mucus interaction may be advised to proceed with IUI, as additional tests are unlikely to impact the management [39]. This test has fallen out of favor in general infertility practice [10]. It may be useful in patients who are unable to produce an ejaculate or are unwilling because of religious proscriptions.

Antisperm antibodies

The tight Sertoli-cell junction provides the testis with a barrier that prevents the immune system from coming in contact with the postmeiotic germ cells. In certain conditions such as testicular torsion, vasectomy and testicular trauma, this unique barrier can be violated resulting in an immune response to sperm, displayed as antisperm antibodies (ASA). These antibodies affect fertility by blocking spermatozoal penetration of the cervical mucus or by preventing sperm binding and penetration of the zona pellucida. Approximately 10% of infertile men will present with ASA (versus 2% of fertile men) [40]. Excessive sperm agglutination or an abnormal PCT can suggest the presence of ASA. Often, sperm parameters are normal [42], thus leading some to suggest that this be tested routinely in all men undergoing infertility work-ups [8,32]. Techniques for this assay are described in the WHO manual [4]. Indirect testing detects the biological activity of circulating ASA, and false positives can come from nonimmunologic factors [43]. Direct ASA detects sperm-bound immunoglobulins. IgG-MAR (mixed antiglobulin reaction) and SpermMAR (Conception Technologies, San Diego, California) tests are more economical and readily available and thus may be recommended for screening. Immunobead Test (IBT), which measures IgG, IgA, and IgM, is employed widely and may be additionally recommended when either of the previous tests gives a positive result to determine if IgA are bound to the sperm surface. Fewer than 10% (IgG MAR) or 20% (IBT) of spermatozoa with adherent
particles have acceptable normal values by WHO (1992) standards. A weakly positive IgG MAR/IBT in men who have low motile sperm rules out immunologic factors, and no further testing is needed [43].

Clinical implications of ASA on male infertility are varied. ASA are present in 34% to 74% of vasectomized men and persist in 38% to 60% after vasectomy reversal [43,44]. Most clinicians do not test for ASA routinely in this setting, because they are of uncertain significance and usually do not affect the decision to do a vasectomy reversal. After orchidopexy for cryptorchidism, there are conflicting reports regarding ASA levels [45]. In genito–urinary infections, ASA is thought to be a consequence of the inflammatory process rather than cross-reactivity to the microorganism [43].

Management options include corticosteroid therapy in cyclic doses to increase antibody free spermatozoa and the selection of ICSI over IUI and IVF. Corticosteroid treatments are not always successful, and the adverse reactions associated with their usage should be considered [45]. There are reports of successful pregnancies with IUI, with 64.3% pregnancy rates after four IUI cycles [46] in superovulated females with partners who are IgG-MAR/IBT positive. The decision to proceed with IUI versus ICSI in immunologic infertility can be aided by a zona pellucida (ZP) test. If the sperm exhibit inability for ZP binding, ICSI is the ART procedure of choice. Presently, flow cytometry techniques are being developed to quantify ASA in individual spermatozoa [47]. These techniques also are being explored to identify sperm surface antigens for possible immunocontraceptive development.

**Acrosome reaction**

After capacitation, the sperm fuses with the ova’s plasma membrane and releases acrosomal enzymes that will allow sperm penetration and fertilization. Transmission electron microscopy, although the procedure of choice to detect acrosome reaction defects, is labor-intensive and expensive. Other techniques such as fluorescence microscopy and beads coated with antiacrosomal antibodies have been developed, but these tests are not readily available. This test may be recommended in cases of profound abnormalities of head morphology or in the setting of unexplained fertility in patients with poor IVF pregnancy rates.

**Sperm penetration assays and sperm zona binding tests**

The sperm penetration assay (SPA) uses zona-free hamster oocytes to measure fertilization capability. The zona pellucida is stripped, to allow cross species fertilization. Normally, 10% to 30% of ova are penetrated [4]. The ZP test uses oocytes that failed to fertilize in IVF clinics. Oligozoospermic and severely teratospermic men have a higher number of defective sperm ZP interactions, which may account for their low fertility potential in both spontaneous and IVF pregnancies [48]. Sperm capacitation index (SCI) is a variant of the SPA test, assessing the mean number of penetrations per ovum. It has been suggested that ICSI should be offered to couples with a SCI less than 5 instead of doing standard IVF procedures [46]. Meta-analysis of sperm function assays by Oehninger and colleagues [49] showed a high predictive power of sperm–zona pellucida-binding assays over SPA for fertilization and IVF outcome. The need for human oocyte supply, however, remains a limitation to the use of this test. SPA, although with low predictive power, is correlated positively with spontaneous pregnancy outcomes [50].

**Biochemical tests**

Acrosin is a serine protease-like enzyme that exhibits a lectin-like carbohydrate binding activity to the zona pellucida glycoproteins. Low acrosin activity has been associated with low sperm density, motility, poor normal morphology [51], HOST (hyper osmotic test) [51,52], and increased ROS [52] in subfertile men. Assays for this include a gelatinolyis technique and a spectrophotometric assay. Its activity has been correlated inversely with low fertility rates in IVF and has been suggested as a predictor of IVF success, independent of sperm morphology [52].

Citric acid, zinc, alpha glutamyl transferase, and acid phosphates are biochemical substances associated with the prostate. These have antioxidative properties that neutralize ROS in seminal plasma. Zinc is necessary for chromatin stability and decondensation, and a possible role in head–tail detachment in fertilization. It is measured by colorimetric methods with a reference value of 13 μmol per ejaculate [4]. Reports on the effects of zinc in sperm function and semen parameters are conflicting. Mankad and colleagues [53] reported positive correlations between seminal zinc levels, alpha glucosidase, and sperm count; however there are other reports that showed no significant
changes in count and motility [54–56]. Zinc levels in seminal plasma are decreased significantly in asthenozoospermic and oligoasthenozoospermic men, but spermatozoal zinc levels are increased [57]. Low zinc–calcium ratio is associated with better motility [54] than high ratio. Dietary supplementation of zinc, however, did not improve semen variables [58].

The seminal vesicles contribute the bulk of seminal fluid that serves as the transport medium and nutrition in the form of fructose. There is a positive correlation between sperm motility and seminal fructose levels [56]. Low or absent fructose is seen in ductal obstruction and congenital conditions like congenital absence of vas deferens (CBAVD). Semen fructose testing may be requested when hypofunctioning seminal vesicles are suspected, although morphometric analysis of seminal vesicles using transrectal ultrasound has gained popularity.

L-carnitine is secreted by the epididymis and is concentrated in the seminal plasma to 10 times serum levels. It has a role in sperm maturation. Low L-carnitine levels are found in oligoasthenozoospermic men [59,60]. The levels of carnitine possibly can serve as indicators to the level of obstruction in the ductal system. Extremely low concentrations of L-carnitine are found in azoospermic men who have postepididymal obstructions, while normal levels are found in azoospermic men who have intratesticular obstructions [59]. Administration of L-carnitine supplements did not improve sperm density, but contrasting results have been reported for sperm motility [60]. L-carnitine determinations remain far from becoming mainstream tests in male infertility until significant well-designed studies are conducted. Alpha glucosidase, tested by fluorimetric methods, has been used to distinguish nonobstructive from obstructive azoospermia. It is used as a specific marker for epididymal function and believed to play a role in sperm maturation in the epididymis. A cut-off value of 12 mIU/mL distinguishes ductal obstruction from primary testicular failure [61]. The usefulness of this test was questioned by Krause and Bohring (1999), but Comhaire and colleagues [61], in their review, showed a strong association between alpha glucosidase and semen parameters. The cut off level (12U l⁻¹) had 95% specificity in identifying obstructive azoospermia. This suggests that the test can predict IUI response (higher pregnancy rate greater than 78 U per ejaculate) as high levels indicate better zona-binding capacity [61]. The presence of commercial test kits using colorimetric methods promises to make testing accessible and affordable.

Other tests

Reactive oxygen species

Small amounts of ROS are normal and in fact necessary for the hyperactivation and capacitation of spermatozoa. In large amounts, it causes spermatozoal damage by lipid peroxidation of the plasma membrane, germ cell apoptosis, and DNA strand breakage [62]. Leukocytes or WBCs are the main source of ROS. Abnormal spermatozoa are a minor source of ROS, and they are caused by retention of cytoplasmic droplets during defective spermiogenesis. Smoking, alcohol abuse, and exposure to radiation and toxic chemicals have been associated with increased seminal ROS [60,61]. The oxidants are in hydroxyl (OH⁻), superoxide (O₂⁻), hydrogen peroxide (H₂O₂), and hypochlorite forms, as well as nitrogen-derived forms of nitric (NO⁻) and nitrous oxide (N₂O). Increased ROS levels have negative correlation with sperm concentration, motility, morphology, and overall normal semen parameters [63–66]. In addition, patients who have unexplained infertility may have increased levels [62,64]. There is an inverse relationship between ROS and in vivo fertilization [67]. Meta-analysis on ROS levels and IVF revealed an inverse relationship between the two [68].

Measurement of ROS is done by several methods, the most common of which is chemiluminescence, which measures total seminal ROS (from WBC, abnormal spermatozoa, and seminal fluid). Leukocytospermia is associated with increased ROS levels and can serve as indirect measurement of ROS [23].

Normal or reference values are not established at this point. A study by Shekarriz and colleagues [64] showed 0–5.5 × 10⁵ cpm at a sperm concentration of 20 × 10⁶ in normal fertile donors. The use of seminal WBC levels as basis of ROS levels, although proven and well-accepted, has yet to establish definite cut-off points. Even low WBC levels (below the WHO cut-off) are associated with ROS [22]. The seminal fluid contains antioxidants (zinc, glutathione) that neutralize the detrimental effects of ROS. Oxidative stress (OS) describes a condition in which there is greater ROS than the total antioxidant capacity (TAC). Measurement of oxidative stress (ROS-TAC score) has been proposed to be a more accurate determination of the total effectual ROS, and a higher score (greater than 30)
can help in the prediction of pregnancy outcomes [25]. As the standardization of testing and the availability of these tests remain limited, it will be some time before this test will become a mainstream investigation in the evaluation of male infertility.

**DNA fragmentation**

DNA fragmentation initially was described in 1993 and has since been researched as a test to aid fertility predictions in subfertile males. The spermatozoal chromatin is a tightly packed structure because of the disulfide cross linkages between protamines that allow compaction of the nuclear head and protect the DNA fragments from stress and breakage. DNA damage is multifactorial and theories on its etiology include protamine deficiency and mutations that may affect DNA packaging or compaction during spermiogenesis [62,69,70]. Factors associated with increased sperm DNA damage are tobacco use, chemotherapy, testicular carcinoma, and other systemic cancers. DNA damage is correlated positively with poor semen parameters (low sperm concentration and low sperm motility), leukocytospermia, and high ROS levels [62,69,71,72]. Approximately 8% of subfertile men who have normal semen parameters will have high abnormal DNA [67].

Direct methods for DNA damage assay include single cell electrophoresis (COMET) and terminal deoxynucleotidyl transferase medicated 2-deoxyuridine 5-triphosphate (TUNEL). Indirect methods are sperm chromatin structure assay (SCSA), which measures sperm chromatin integrity, and DNA intercalating dyes (acridine orange) that differentiate single and double stranded DNA. The sperm DNA denaturation test and the sperm chromatin dispersion test are other tests reported in literature [73,74].

A cut off rate of greater than 30% was associated with a significant decrease in in vivo fertilization rates [71,75]. A DNA fragmentation index (DFI) of greater than 30% has a sensitivity of 15% and a specificity of 96% [76]. Meta-analyses by Evenson and Wixon [77] and Li and colleagues [78] showed that couples are twice as likely to become pregnant with regular IVF methods if the DFI is less than 30%. In IUI, DFI also has been found to be a useful pregnancy predictor, and an odds-ratio of DFI greater than 30% correlates with lower rates of clinical pregnancy, biochemical pregnancy, and delivery [79].

Based on the previous studies, testing for DNA fragmentation defects can help couples decide on what fertility modality and possible lifestyle modifications they can employ that may increase their chances of conception. ICSI is advised when DFI is above cut-off levels. There is a higher rate of DNA damage in ejaculated or epididymal sperm than intratesticular spermatozoa, hence use of intratesticular spermatozoa from high DFI men is recommended for ICSI [80,81]. One study has demonstrated a higher miscarriage rate after ICSI in men who have high DFI [82]. Contrasting reports, however, have failed to show significant correlation between DNA damage and idiopathic infertility [83,84]. In addition, significant intraindividual variation exists (using SCSA) making conclusions problematic [70]. Treatment employed to counteract or decrease DNA fragmentation defects are likewise varied. The ASRM (2006) best summarizes the current viewpoint on DNA integrity testing, concluding that there are not enough data to make DNA testing routine in infertility testing and that treatments have yet to prove their clinical value. Still, its applications to research can provide greater insights to infertility and andrology. If tests on this become standardized, inexpensive, accessible, and reliable in their application, the possibility of their use in clinical practice will be highly likely.

**Electron microscopy**

Ultrastructural details of the sperm only can be seen under the electron microscope (EM). Patients who have low sperm motility (less than 5% to 10%) with high viability (HOST or Eosin-Nigrosin staining) and density may be appropriate candidates for EM assessment. These spermatozoa may test positive for viability even with the ultrastructural defects [10]. Mitochondrial and microtubular defects that are not visible under the usual Papaniculau smear will be evident. Subfertile men will have more serrated and blurred circular sulcus, less intact acrosome membrane, a bigger proportion of the spermatic head, and more droplets attached to acrosome membrane [85].

**What is in the future of sperm analysis? (emerging technologies)**

**Microarray**

This method analyzes the transcriptome of cells and tissues. Comparison of the transcriptomes at the different stages of spermatogenesis may provide clues to molecular mechanisms to genetic infertility (ie, Yq microdeletions) and
potential biochemical markers for infertility. The primary application at this time in the field of andrology is in uncovering the still unknown genes, pathways, and mechanisms in sperm production. Creation of mRNA profiles possibly can distinguish spermatogenic infertility from other causes. The use of ICSI bypasses the natural selection of preventing transmission of defective genes, and clues in men who have genetic infertility may be provided for by these profiles [86].

Proteomics

Body fluids with complex proteins are ideal candidates for studies as they have the potential to contain biomarkers. The advent of electrospray ionization (ESI) and matrix-associated laser desorption/ionization (MALDI) has pushed this field into sequencing peptides and proteins of body tissues at different biological states, including seminal fluid [87]. Seminal fluid has been found to have 923 proteins [88], and at least 20 proteins have altered expressions in infertile men [89]. This noninvasive technique not only provides the potential to detect causes of infertility, but may play a role in the development of male contraception [90]. It has been shown that there are possible biomarkers for normal cellular function in in vivo embryonic development [91]. Insight to signaling complexes in physiologic process is a good start in understanding the biologic functions of these proteins, and the future of developing biomarkers does not seem to be far from becoming a reality [36].

Metabolic profiling (metabolomics)

This technology brings together microassay and proteomic technologies. Metabolites are formed or released by cell processes. These
biochemical intracellular substances can provide both qualitative and quantitative data for a glimpse of the network processes in vivo [92]. In male infertility, the production of oxidative stress byproducts and naturally occurring antioxidants can serve as biomarkers to potentially differentiate fertile from subfertile men with idiopathic infertility [93]. The same principle can be applied to assisted reproductive techniques, as oxidative stress can affect pregnancy outcomes markedly. The noninvasiveness of this test is its main advantage should it be developed and proven helpful for use in the clinical setting.

**Atomic force microscopy**

In 1986, a high-resolution type of scanning microscope with resolutions in the fractions of a nanometer was invented. Its main advantage over EM is the three-dimensional images it can provide, and the simplicity of the sample preparation (air drying). It also allows observation in an air or liquid milieu and thus the potential of observing biomolecules in vivo. The disadvantage is that the image quality is limited by the radius of curvature of the probe tip, and an incorrect tip can result in image artifacts. Spermatozoa have been reported as good subjects because of their small size and rigidity [87]. Studies in sperm plasma membrane during maturation and capacitation have identified new areas with phosphorylated proteins, and large aggregates of lipid did not cross postacrosome and equatorial segments [94]. Although this method for andrology testing requires expensive equipment at the present time, its use in research will provide information on the intricate processes and structures in sperm and uncover some of the unknown causes of male infertility.

**Summary**

In the primary care level, a proper diagnosis on male infertility can be made with comprehensive and properly performed semen analyses in conjunction with a thorough history and physical examination. Fertility, however, is not dependent on a single test but is often a combined sum of different factors.

Fig. 1 presents algorithms in sperm assessment. It should be remembered that the values set for each are not absolute and that there is still much to learn about sperm, its biochemical processes, and its interaction with environment and physical stresses.

The advent of new tests should be geared toward better understanding of the intricacies in this haploid cell. The emergence of home kits for sperm assessment can be seen not as deterrent to seeking adequate advice but rather an information tool to promote awareness of this problem.

One must continue to carefully assess the male factor component and to continue to attempt to cure infertility and use assisted reproductive technologies judiciously. Although a carefully performed semen analysis remains the initial choice in the evaluation of male infertility, exciting new developments in semen testing promise continued advances in the targeted diagnosis and management.

**Acknowledgments**

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**References**


ASSESSING SPERM FUNCTION


Female Fertility: What Every Urologist Must Understand

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Infertility is a disease of couples, and it affects 2.1 million married couples, many of whom seek fertility assistance [1]. Once thought to be a disease of women, it is clear that the cause of infertility in couples seeking advanced reproductive technology can be found in the male partner (18.5%), female partner (50%), both partners (18.4%), or neither partner (unexplained infertility, 12%). In 2006, 8% of married childless couples sought infertility services, and 15% of all women report having used some infertility service in the past. It behooves urologists who care for infertile men to be acquainted with female fertility. This article provides an overview of the causes for and treatment of female factor infertility.

Female reproductive physiology

In contrast to spermatogenesis, oogenesis begins in utero. By the fifth month of gestation, mitosis is complete and the peak number of oocytes is achieved. Barring any causes of ovarian failure, a pubertal girl has approximately 400,000 oocytes resting in primordial follicles containing a single layer of granulosa cells. During embryologic oogenesis, oocyte development arrests in the diplotene phase of the first meiotic division and does not resume meiosis until after ovulation. The process of ovulation requires that the follicle develop from a primordial follicle to a tertiary follicle over approximately three menstrual cycles. Only the final phase of folliculogenesis is hormone dependent. The process continues throughout postpubertal life even in the presence of cycle disruptors such as oral contraception or pregnancy. Follicles that reach the hormone-dependent stage at a time asynchronous with the menstrual cycle become atretic. Subsequently, only 300 to 400 follicles ovulate in a woman’s lifetime.

The first day of the menstrual cycle is marked by the first day of bleeding. At this point, ovarian and pituitary hormone productions are at their nadir, and multiple morphologically identical antral follicles are present. Under the control of gonadotropin-releasing hormone from the hypothalamus, the anterior pituitary begins to release follicle-stimulating hormone (FSH) to begin hormone-dependent follicular growth. Cumulus expansion of the granulosa cells occurs as the oocytes compete to be the dominant follicle. Although selection of the dominant follicle is poorly understood, it is clear that the intrafollicular environment plays a significant role. The follicular fluid of the dominant follicle has significantly higher levels of estrogen than its competing cohort. There is also a greater concentration of luteinizing hormone (LH) receptor on the theca cells and FSH receptor on the granulosa cells. Several authors have theorized that the ability to recruit steroid receptors and produce estrogen is the major determinant of dominance [2]. Human animal data suggest that insulin-like growth factor (IGF) plays a major role in dominance selection; the Igf null mouse does not undergo hormone-dependent folliculogenesis [3,4]. In the bovine model, the dominant follicle has a low concentration of IGF binding proteins and an elevated concentration of pregnancy-associated plasma protein-A and insulin-like growth factor binding protein (IGFBP) proteolytic enzyme [5].
A significant association between intrafollicular pregnancy-associated plasma protein-A and estradiol levels suggests that pregnancy-associated plasma protein-A plays a role in dominance selection in humans [6]. IGF is synergistic with FSH to augment estradiol production [7]. Data suggest that increase in intrafollicular-free IGF concentration is associated with dominance.

Clearly, the induction of estradiol is a key component of the follicular cycle. Initially, this estrogen has a negative feedback on the hypothalamus and pituitary. After follicular dominance is determined, however, estradiol exerts positive feedback that leads to the surge of LH. Again, the mechanisms involved in this surge are unclear. In addition to inducing ovulation, LH is required for the resumption of meiosis [8]. At the time of ovulation, the oocyte completes meiosis I and arrests in the metaphase of meiosis II. Meiosis is completed at the time of fertilization. The mechanisms involved in meiosis resumption are under intense study by researchers interested in in vitro maturation of follicles for preservation of fertility.

After ovulation, the ruptured follicle forms a corpus luteum and produces progesterone to support the endometrium for implantation. Implantation typically occurs 5 to 7 days after ovulation. The early trophoblast cells produce human chorionic gonadotropin to maintain the corpus luteum. In the absence of human chorionic gonadotropin, the corpus luteum involutes and the resultant decrease in estrogen and progesterone results in menses. Corpus luteal hormone production is critical to maintaining the pregnancy during the first 8 weeks of gestation; loss of corpus luteal progesterone production results in abortion.

Evaluation of the female partner

History

A focused history can evaluate for risk factors of infertility and guide further diagnostic modalities and treatment. Risk factors include advancing age, a history of pelvic infections (including salpingitis and appendicitis), severe pelvic pain or endometriosis, and irregular menstrual cycles. One also must ascertain the frequency of intercourse and the use of vaginal lubricants that may be sperm toxic. Of these risk factors, age is the strongest predictor of pregnancy either with or without advanced reproductive techniques. Fecundability begins to decline at age 30, sharply declines after age 35, and is less than 5% after age 40 (Fig. 1). Patients of advancing age are often counseled to begin with more aggressive treatment.

Menstrual history

Characterization of a woman’s menstrual regularity may provide insight into the normalcy of ovulation. Normal menstrual cycles range from 24 to 25 days, and menses occurs for 3 to 7 days. In a woman younger than age 35, a history of regular menstrual cycles is highly correlated with the presence of ovulation. This association is strengthened when menses are accompanied by monthly moliminal symptoms, including breast tenderness, bloating, or mood changes. A long cycle is often associated with anovulation. In contrast, a short cycle may be associated with ovulation, with an inadequate follicular phase leading to poor endometrial development or luteal phase deficiency.

Assessment of ovulation

Several tools are available for the assessment of ovulation, including basal body temperature charts (Fig. 2), assessment of mid-luteal progesterone, endometrial biopsy, and urinary LH prediction kits. Each of these tests, with the exception of urinary LH, can only retrospectively inform the user of ovulation. Basal body temperature is effective because progesterone production from the corpus luteum raises core body temperature by approximately 0.6°F, which provides a “biphasic” pattern of temperature. Temperature should be taken every morning, however, which
reminds the woman of infertility before starting her day. This reminder is cumbersome and may be emotionally taxing.

Measurement of mid-luteal progesterone is another retrospective method of determining ovulation. Clinicians often use this value to determine the adequacy of the luteal phase; however, data do not support a specific value that correlates with a luteal phase defect. Endometrial biopsy on cycle has long been considered the “gold standard” determinant of ovulation. After ovulation, the endometrium undergoes classic morphologic changes that correlate with the day of the menstrual cycle. It has been suggested the endometrial dysynchrony of two or more days, as determined by endometrial biopsy, is associated with luteal phase defect [9]. Significant inter- and intraobserver variability is present in the evaluation of endometrial biopsies, however [10,11]. The National Institutes of Health/National Institute of Child Health and Human Development–funded National Cooperative Reproductive Medicine Network recently completed a study on the use of endometrial biopsy. This multicentered trial concluded that the endometrial biopsy cannot discriminate between fertile and infertile women and should not be a routine part of the infertility evaluation [12].

Urinary LH testing uses an enzyme-linked immunoassay against the beta subunit of LH, which rises abruptly for approximately 18 hours before it peaks. Ovulation typically occurs approximately 36 hours after the onset of the surge. Because the hormone needs to be conjugated before it is excreted, urinary LH predicts ovulation approximately 24 hours in advance, which provides a prospective assay of ovulation that also can be used to time intercourse.

**Ovarian reserve testing**

Research has established that age is the best predictor of fertility potential. Ovarian reserve testing is a measure of “ovarian aging.” Although studies have used these tests to predict the time to menopause, most studies have had in vitro fertilization (IVF) pregnancy as the outcome measure. Normative measures of ovarian reserve in a non-infertility population have not been established. Tests of ovarian reserve include the measurement of basal hormone levels, dynamic ovarian testing, and sonographic assessment of the ovaries.

Basal hormones that have been measured to estimate ovarian reserve include FSH, estradiol, inhibin, and anti-müllerian hormone. FSH is inversely proportional to the production of the follicular hormones estradiol and inhibin. It follows that as the cohort of developing follicles is reduced, the basal levels of FSH increase. Over the past 20 years, many studies have evaluated the predictive value of FSH, and the results of these studies are highly conflicting. Although most of these studies agree that the FSH level indicates the number of oocytes retrieved from an IVF cycle, several large studies have failed to demonstrate the test’s ability to predict pregnancy, especially in a young patient population. Estradiol traditionally has been measured in conjunction with FSH to ensure that the FSH was drawn during the cycle nadir. Several studies have demonstrated that elevated estradiol level on day three may be independently predictive of poor stimulation, however [13,14]. Because neither of these hormones is highly predictive of pregnancy, investigators have tested the predictive value of additional hormones. Inhibin B, a hormone made by the granulosa cells, regulates FSH production by negative feedback. Studies suggest that inhibin B levels decrease earlier than changes in estradiol. Although results are conflicting, this hormone may be an earlier predictor of IVF response [15,16].

Dynamic tests of ovarian reserve involve challenging the ovaries with a fertility medication, such as clomiphene citrate or gonadotropin [17,18]. Although predictive of response to IVF, these tests have not been demonstrated to be superior to basal hormone levels in predicting pregnancy. Likewise, ultrasound ovarian assessments, including ovarian volume and antral follicle...
counts, may predict response to medications. It is important to re-emphasize that although each test of ovarian reserve may help to counsel patients regarding their fertility potential, age remains the strongest predictor of pregnancy.

**Pelvic infections and anatomic abnormalities**

Pelvic infections, especially gonorrhea and chlamydia, may irreversibly damage the fallopian tubes. These infections are endemic in the United States and have an annual incidence of approximately 1 million new reported cases. Most of the cases are diagnosed in women between the ages of 15 and 24, with an annual incidence of approximately 1 in 35 women [19]. Unfortunately, because chlamydia is asymptomatic in 75% of cases, a large amount of chlamydial infections are not diagnosed. Up to 20% of women with undiagnosed chlamydia develop infertility. Although gonorrhea and chlamydia are the most common infectious causes of tubal damage, any significant pelvic inflammatory condition can result in adhesion formation that affects tubal integrity.

Severe dysmenorrhea may be associated with uterine anomaly, such as subserosal fibroids or outflow tract obstruction. It also may be associated with endometriosis or pelvic adhesions. This finding also leads to specific diagnostic evaluation.

**Anatomic assessment**

Assessment of the structural integrity of the reproductive tract is an essential component of the fertility evaluation. It can consist of radiologic imaging or surgical evaluation. The most common test for evaluating the uterine cavity and checking tubal patency is the hysterosalpingogram, which is performed by injecting radiopaque dye into the uterus and tubes under fluoroscopic visualization. Uterine abnormalities are outlined by the dye, and tubal obstruction is noted by the absence of free-spill into the peritoneal cavity. In addition to the diagnostic value of the hysterosalpingogram, the test may be therapeutic [20,21].

Intrauterine abnormalities also can be identified with saline-infusion sonography (Fig. 3). Because the uterus is a potential space, traditional ultrasonography is not sensitive enough to determine if a lesion is intracavitary. Saline-infusion sonography is performed by injecting saline into the uterus to provide a sonographic window within the endometrial cavity. The sensitivity and specificity of saline-infusion sonography have both been estimated to be 100% when surgery was used as a gold standard [22,23]. The advent of three-dimensional ultrasonography has improved the diagnostic capabilities of ultrasonography, and several publications have reviewed its technical aspects [24–26]. Briefly, the operator

![Fig. 3. Biphasic basal body temperature chart from an ovulatory patient.](image-url)
selects a region of interest, and specialized probes scan through the region in multiple planes. Computer reconstruction assembles the images to create a structure that can be manipulated and viewed in multiple planes. This technique is highly accurate for diagnosing uterine anomalies and intrauterine pathologic conditions, such as septum and synechiae [27].

MRI is an excellent modality for viewing soft tissues, thereby surpassing the diagnostic ability of CT for imaging uterine abnormalities. It has been reported to have 100% specificity and 80% to 100% sensitivity for evaluating pelvic anomalies [28,29]. Because it images in multiple planes, MRI is an excellent preoperative assessment before reproductive gynecologic surgeries such as myomectomy and metroplasty.

Clearly, endoscopy can be used to visualize pelvic anatomy. Although radiologic imaging provides information about the pelvic structures, it provides little information about peritubal adhesions, pelvic infection, or endometriosis. The decision to perform laparoscopy as an initial diagnostic modality is based on the clinician’s suspicion of pathologic condition. For example, in a patient with a history of cyclic pelvic pain that suggests endometriosis, laparoscopy may be the best initial evaluation.

Anatomic cervical abnormalities may result in abnormal cervical mucus production. Cervical mucus protects the sperm from the acidic milieu of the vagina and is critical for introducing sperm into the upper genital tract. Patients with extensive cervical surgery have lower fecundity. Some clinicians evaluate preovulatory cervical mucus with a postcoital test. Cervical mucus is evaluated for elasticity, ferning, and the presence of adequate numbers of motile sperm. The postcoital test has not been associated with improving pregnancy outcome, however.

Treatment strategies

Surgery

The use of tubal reconstruction has been highly debated in the infertility literature. In the early to mid-1990s, several authors reported that pregnancy rates after salpingostomy or fimbrioplasty were equivalent to those associated with in vitro fertilization [30–32]. Variables that predict success include a patient’s age, unilateral versus bilateral tubal disease, density of the adhesions, and thickness of the tubal wall. It should be noted that although pregnancy rates approximate 30%, ectopic pregnancy rates approximate 14% [33]. As the pregnancy rates from IVF continue to improve, the value of surgical intervention, with its increased surgical risk, has diminished. The exception to this may be tubal anastomosis after voluntary sterilization. Anastomosis is highly successful when at least 4 cm of tube are available for repair [34]. Although this allows the patient to have “natural conception,” the cost and success are similar to those for IVF. For the couple who only wants to have one child or an additional child, anastomosis restores fertility and results in the need for birth control.

Laparoscopic surgery continues to be used for the treatment of endometriosis. Endometriosis can be found in 10% of all women and 30% to 40% of women with infertility [35,36]. Severe endometriosis can lead to peritoneal adhesions and a distortion of pelvic anatomy. Endometriosis also has been associated with an induction of peritoneal inflammation and oxidative stress, however, which has been associated with luteal dysfunction, poor embryonic development, and implantation failure [37]. Randomized clinical trials have been performed to determine if surgery improves outcome in patients with endometriosis [38,39]. Multiple meta-analyses have concluded that there is a benefit to surgical ablation of endometriosis, independent of the stage of disease [35,38]. The per-cycle pregnancy rate after IVF for women with endometriosis is higher than after surgery, however, despite the finding that endometriosis is associated lower peak E2 concentration, a fewer number of oocytes retrieved, a lower fertilization rate, and a lower implantation rate [35]. Several studies have investigated the use of surgery to treat patients with endometriomas before infertility treatment. Although conflicting, most of these data do not demonstrate any clear advantage of surgical intervention [40–42].

Ovulation induction

Ovulation induction is the treatment of choice for patients with anovulation or unexplained infertility. For anovulatory patients, monofollicular development is the desired outcome. For unexplained infertility, superovulation is desired to increase the probability of conception. Medications used include selective estrogen receptor modulators, aromatase inhibitors, and gonadotropins, each of which has distinct advantages and disadvantages.
Clomiphene citrate is the most commonly used medication for the treatment of infertility. A selective estrogen receptor modulator, it is a competitive antagonist of estradiol at the level of the cytoplasmic nuclear receptor complex. The drug binds to estrogen receptors in the hypothalamic arcuate nucleus, disrupts the negative feedback, and augments gonadotropin-releasing hormone production. Endogenous production of FSH is augmented and hyperstimulation is achieved [43]. The main side effects of clomiphene therapy are related to the brain’s decreased perception of estrogen. Patients often complain of hot flashes, headaches, and visual changes. Clomiphene citrate can compete with estrogen at receptor sites outside the brain, including the uterus and cervix. Lowering the effect of estrogen in the uterus can result in poor endometrial development and low implantation rates [44,45]. Estrogenized cervical mucus is necessary to provide an environment to support sperm survival and transport [46]. Clomiphene citrate has been associated with decreases in cervical mucus score that result in poor sperm-mucus interactions [47,48]. Some clinicians advocate performing postcoital tests in patients undergoing clomiphene citrate induction cycles or routinely performing intrauterine inseminations.

The aromatase inhibitors—letrozole and anastrozole—recently were reported to be beneficial for ovulation induction [49]. These drugs are competitive reversible inhibitors of testosterone aromatization and decrease circulating estrogen by more than 97%. Similar to clomiphene, the reduction in estrogen affects the hypothalamic feedback and induces greater levels of FSH. Because there is no suppression of the estrogen receptor, researchers have postulated that aromatase inhibitors would not negatively affect the uterus or cervical mucus, and several randomized controlled trials have demonstrated improved endometrial development with the use of letrozole [50,51]. Recent concern has been raised about the association of aromatase inhibitors with birth defects. This association seems unlikely because the medications are not present during the time of organogenesis because of their short half-lives (approximately 45 hours) [52]. A recent multicenter trial did not report any increased risk of birth defects in a cohort of 514 children born after letrozole stimulation [53].

The mainstay of ovulation induction, especially for assisted reproductive technologies, is exogenous gonadotropins. Gonadotropins were first purified from pregnant mares’ urine more than 70 years ago. Although effective, the medication was highly antigenic and could only be used for limited cycles. Human menopausal gonadotropin became clinically available in the 1960s. Initially, human menopausal gonadotropin contained a 1:1 mixture of FSH:LH [54], and these initial preparations contained a large amount (> 90%) of protein impurities [55]. As purification techniques improved, the amount of LH in the preparations decreased. Currently, most FSH is pure FSH because it is produced in Chinese hamster ovary cells that contain genes inserted with recombinant technology [54]. Debate has arisen regarding the requirement of LH supplementation for folliculogenesis. During ovulation induction for patients without hypothalamic amenorrhea, a significant need for LH is unlikely [56]. Although most patients respond adequately to FSH alone after pituitary down-regulation for in vitro fertilization, there is likely to be a subset of patients who would benefit from LH supplementation. Additional research is necessary to identify this subgroup, however.

Typical starting doses of gonadotropin range between 75 IU and 225 IU, depending on a patient’s age, diagnosis, and prior stimulation history. Patients should be monitored frequently with ultrasound and estradiol levels to assess follicular development and maturity, and the dose of gonadotropin should be adjusted to avoid overstimulation. When two to four follicles are approximately 18 mm in mean diameter, ovulation can be induced with human chorionic gonadotropin.

Ovulation induction and ovarian cancer risk

It has been hypothesized that use of fertility medications may increase a patient’s risk for ovarian cancer. One of the first studies to make this association was by Whittemore and colleagues in 1993 [57]. This study was an analysis of data collected from 12 case-controlled studies of ovarian cancers diagnosed over a 30-year period. They reported a 2.7-fold increase in ovarian cancer in women who had taken fertility drugs and conceived and a 27-fold increase in ovarian cancer in women who took drugs and remained nulligravid. This study did not identify a precise “fertility drug” and included numerous drugs used to treat infertility. Their conclusion was met with numerous editorials that challenged the validity of their results.
Cohen and colleagues [58] concluded that the association between ovarian cancer and fertility drugs was caused by a confounding effect of gravidity; once gravidity was controlled, the association between drugs and cancer was not significant. The first major cohort study was published by Rossing and colleagues [59], who detected 11 ovarian tumors, of which 4 were invasive epithelial cancers, in 3837 women and reported a significant association with fertility drug use. The strongest association was in users of clomiphene citrate after 12 consecutive cycles (11-fold increase). Since that time, numerous epidemiologic studies have been published arguing for [60] and against [61,62] this association. The bulk of this evidence does not support the association between ovarian cancer and fertility drugs. The American Society of Reproductive Medicine concluded that “Although initial reports suggested that women who use fertility drugs have an increased risk for ovarian cancer, numerous recent studies support the conclusion that fertility drugs are not linked to ovarian cancer. Nevertheless, there is still uncertainty whether a risk exists and research continues to address this question” [63].

In vitro fertilization with or without intracytoplasmic sperm injection (ICSI)

Some researchers believe that in vitro fertilization is the most successful treatment for infertility, regardless of diagnosis. In 2004, 411 fertility clinics in the United States performed 127,977 IVF cycles that resulted in the births of 49,458 infants [64]. This statistic represents more than approximately 1.2% of all births in the United States. For women younger than age 35, the live birth rate/transfer is 43%, which is considerably higher than the live birth rate of approximately 30%/transfer in 1995. The improved pregnancy rates are largely attributable to improvements in embryo handling in the laboratory and at the time of transfer. In the laboratory, culture media have been improved by reducing the amount of glucose [65], reducing oxidative stress, and supplementing the media with amino acids [66] and growth factors [67]. Reports also suggest improved embryo development by culturing embryos in physiologic oxygen tension [68]. Atraumatic transfer catheters and ultrasound-guided transfer have contributed to improved pregnancy rates [69]. These improvements are coupled with better abilities to select the best embryos for transfer at the cleaved and blastocyst stages [68,70]. A more detailed description of ICSI can be found in the article by Alukal and Lamb elsewhere in this issue.

Summary

Treatments for infertility have been improving dramatically, and an increasing number of couples are seeking treatment. This is likely to translate to an increase in the number of infertility visits to urologists. Because abnormalities are frequently noted in both partners and treatment of the female partner can affect the treatment of the male partner, it is critical that urologists understand the basics of female infertility. This understanding also facilitates improved communication between the treating urologist and reproductive endocrinologist.

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Evolving Approach to the Varicocele
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The varicocele is the most common cause of male infertility world wide (Table 1). Varicoceles have been found in 15% of the normal male population and in up to 40% of patients with male infertility [1]. In approximately 70% of patients with secondary infertility, a varicocele is an underlying cause [2]. Understanding of the pathophysiology, treatments, and outcomes of a varicocele and varicocele repair has evolved significantly over the past several decades. Our goal is to discuss the approach to its diagnosis and treatment that has evolved.

Pathophysiology of the varicocele

In 1978, Greenberg and colleagues [6] found not only that varicoceles were associated with testicular atrophy but also that the testicular damage associated with a varicocele was progressive with age. Infertile patients with varicoceles have been found to have semen with decreased density, decreased motility, and abnormal morphology. Varicoceles also have been associated with abnormal testosterone and follicle-stimulating hormone levels [7].

Many theories have been suggested to explain why varicoceles lead to impaired spermatogenesis and subsequent infertility. The prevailing theory is that poor venous drainage leads to disruption of the countercurrent exchange of heat from the spermatic cord, which elevates scrotal temperatures. The elevated scrotal temperature leads to impaired spermatogenesis. Thus, a unilateral varicocele may have effects on both testicles [8,9]. Increased scrotal temperatures have been shown to result in decreased testosterone synthesis by Leydig cells, injury to germinal thermolabile cell membranes, decreased protein biosynthesis, decreased amino acid transport, and altered Sertoli cell function and morphology [10–14].

Other theories that are less commonly ascribed to as to why varicoceles result in impaired spermatogenesis include oxygen deprivation, poor venous drainage that leads to impaired drainage of gonadotoxins from the testis, and increased oxidants within the semen. Allamaneni and colleagues [15] reported a positive correlation between seminal reactive oxygen species (ROS) or “oxidant” levels and varicocele grade. They demonstrated that higher seminal ROS levels are seen in men with grade 2 and 3 varicoceles compared with men with grade 1 varicoceles. Another recent study demonstrated that levels of oxidants are

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significantly higher in semen of infertile men than in semen of fertile men [16]. This study demonstrated that varicoceles significantly increase the oxidant levels within semen of infertile men and that repairing the varicoceles in these infertile men led to a significant decrease in the semen’s oxidant levels. In reality, it is likely that a varicocele causes testicular damage through multiple simultaneous mechanisms, all of which result in male infertility.

Beneficial effects of varicocele repair

Our understanding of how a varicocele repair affects overall semen and hormone parameters has evolved over the past several decades. For example, correction of varicoceles has been shown to improve not only sperm motility, density, and morphology but also specific functional sperm defects [7,17–20]. Improvements in the sperm penetration assay [18], oxidant determination (ROS) [19], and DNA fragmentation [17,20] have been achieved after a varicocele repair. In 1987, Kass and Belman [22] were the first to demonstrate a significant increase in testicular volume after varicocele repair in adolescents. Finally, pregnancy rates after varicocele repair have been shown to increase with intrauterine insemination despite the absence of significant changes in gross semen analyses [23]. It is believed that improved functional factors not measured on routine semen analysis may explain these increased intrauterine insemination success rates.

The evolution of the varicocele repair in patients who have azoospermia

In 1955, Tulloch [24] was one of the first surgeons to report a varicocele repair in a patient who had azoospermia. He reported that a varicocele repair resulted in restoration of spermatogenesis and subsequent pregnancy in a patient who had azoospermia. Since then, many other studies have demonstrated the return of motile sperm after varicocele repair in patients who have azoospermia. Matthews and associates [25] found that 55% of men with azoospermia and 69% of men with zero motile sperm before surgery had motile sperm in their ejaculate after varicocele repair. Kim and colleagues [26] demonstrated that varicocele repair can result in sperm in the ejaculate of men who have azoospermia when severe hypospermatogenesis or late maturation arrest is identified histologically. Some studies have demonstrated that the best chance of having motile sperm return in the ejaculate occurs when sperm or spermatids are present on preoperative testis biopsy [25–27]. Other investigators have found that men with nonobstructed azoospermia rarely have adequate sperm in their ejaculate after varicocele repair to avoid testicular sperm extraction, however [28].

Table 1
Distribution of final diagnostic categories found in male fertility clinic [1]

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<th>Category</th>
<th>Number</th>
<th>Percent (%)</th>
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<td>Varicocele</td>
<td>603</td>
<td>42.2</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>324</td>
<td>22.7</td>
</tr>
<tr>
<td>Obstruction</td>
<td>205</td>
<td>14.3</td>
</tr>
<tr>
<td>Normal/female factor</td>
<td>119</td>
<td>7.9</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>49</td>
<td>3.4</td>
</tr>
<tr>
<td>Immunologic</td>
<td>37</td>
<td>2.6</td>
</tr>
<tr>
<td>Ejaculatory dysfunction</td>
<td>18</td>
<td>1.3</td>
</tr>
<tr>
<td>Testicular failure</td>
<td>18</td>
<td>1.3</td>
</tr>
<tr>
<td>Drug/radiation</td>
<td>16</td>
<td>1.1</td>
</tr>
<tr>
<td>Endocrinopathy</td>
<td>16</td>
<td>1.1</td>
</tr>
<tr>
<td>Others (all &lt; 1%)</td>
<td>31</td>
<td>2.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1430</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>


In 1987, Kass and Belman [22] were the first to demonstrate a significant increase in testicular volume after varicocele repair in adolescents. Finally, pregnancy rates after varicocele repair have been shown to increase with intrauterine insemination despite the absence of significant changes in gross semen analyses [23]. It is believed that improved functional factors not measured on routine semen analysis may explain these increased intrauterine insemination success rates.

Diagnosis

Varicoceles are diagnosed primarily by physical examination. Patients should be examined in standing and supine positions. While standing, patients should be asked to perform the Valsalva maneuver so the physician can assess reversal of venous flow. The duplex ultrasound has significantly improved our ability to diagnose varicoceles. Since its introduction, the use of other radiographic tools, such as venography, the Doppler stethoscope, and radionucleotide scans such as technetium 99m pyrophosphate, has greatly decreased. It should be noted, however,
that the diagnosis of a varicocele is made primarily by physical examination, and the duplex ultrasound should be used only to corroborate or confirm results of the physical examination. Most clinicians would agree that the diagnosis of a varicocele by ultrasound should be based on a patient having veins dilated between 2 and 3 mm and reversal of venous flow with Valsalva maneuver. There are currently no standard and clearly defined criteria for diagnosing a varicocele by ultrasound, however.

Subclinical varicoceles are not palpable on physical examination but rather are diagnosed radiographically. Most authors agree that subclinical varicoceles are varicoceles less than 3 mm in diameter [29]. There has been much debate as to the clinical relevance of subclinical varicoceles. Studies have shown that subclinical varicoceles have no significant impact on fertility and that repairing subclinical varicoceles has no significant impact on improving fertility rates [30]. Radiographic testing to diagnose a varicocele should be performed only when there is uncertainty on a physical examination or to identify recurrent or persistent varicoceles.

**Indications for treatment**

The Male Infertility Best Practice Policy Committee of the American Urological Association recommended that a varicocele repair should be offered to the male partner of a couple attempting to conceive when all four of the following conditions are present [31]:

1. The female partner has normal fertility or a potentially correctable cause of infertility
2. The couple has documented infertility
3. A varicocele is palpable or, if suspected, is corroborated by ultrasound
4. The male partner has one or more abnormal semen parameters or sperm function test results

Adult men who have a varicocele and abnormal semen parameters and do not wish to conceive currently but might in the future could be considered for a varicocele repair. Adolescent boys with varicoceles should be considered for a varicocele repair if there is testicular pain or a reduction in the ipsilateral testicular volume. If there is no identifiable reduction in ipsilateral testicular volume, these young men can be followed with annual physical examinations or a semen analysis. These patients and their families should be fully apprised of the concerns and controversies surrounding adolescents with a varicocele.

**Evolution of the surgical treatments of varicocele**

The scrotal approach was one of the first operations used for varicocele repairs. In 1904, Hartman was the first to describe radical resection of the scrotum and external clamping of varicoceles [32]. The scrotal approach is no longer performed because of the increased risk of injury to the testicular artery and its high rate of recurrence. Currently, there are three main surgical approaches to a varicocele repair: inguinal, subinguinal, and retroperitoneal (Fig. 1). The retroperitoneal approach can be performed either open or laparoscopically. Each of these approaches has its own pros and cons, which are discussed briefly.

The open retroperitoneal approach (Palomo) involves a muscle-splitting approach. The peritoneum is retracted medially and the spermatic vessels are ligated lateral to the ureter. A laparoscopic retroperitoneal approach also has been described with artery-sparing and non-artery-sparing techniques. There has not been any increased efficacy with the laparoscopic approach over the open approach, and the laparoscopic approach is performed with decreased frequency [33].

The inguinal approach (Ivanissevich) involves making an incision superior to the external inguinal ring and incising the external oblique fascia. A 3- to 4-cm “mini” inguinal incision can

---

**Fig. 1. Open surgical approaches to varicocelectomy.**
be made. Varicoceles in this region generally present with a typical vascular pattern in which the artery is next to, or adheres to, several veins. There is generally a separate isolated vein nearby. The testicular artery adheres to the undersurface of a large vein in approximately 50% of cases.

The subinguinal approach does not require a facial incision and theoretically offers less postoperative pain and faster recovery. The veins tend to branch at this level, however, and a higher number of smaller caliber veins need to be ligated. Hopps and colleagues [35] demonstrated that internal spermatic arteries at the subinguinal level were more than three times as likely as those identified at the inguinal level to be surrounded by a network of adherent veins. The arteries at this level tend to be end arteries, and inadvertent injury to these arteries carries a higher rate of testicular injury. Chan and colleagues [36] reported 19 cases of testicular artery injury in a series of 2102 varicocele repairs during microsurgical inguinal varicocelectomy. Subsequent testicular atrophy occurred in only one patient. Thus, the overall risk of testicular atrophy was less than 1%.

**Percutaneous embolization of varicoceles**

The first reports of percutaneous embolization of varicoceles occurred in 1978 [37]. Since that time, advancements have been made in this technique with the use of coils, balloons, and sclerotherapy. Sclerotherapy has been particularly useful for occlusion of smaller collateral veins [38]. Percutaneous embolization of varicoceles typically is generally not the initial treatment for varicoceles because of higher recurrence rates and failed procedure rates. Pryor and Howards [39] found that the overall success rate, taking into account failed primary attempts and the recurrence rate, was 68%. Percutaneous varicocele embolization should be reserved for recurrent or persistent varicoceles when the anatomy causing the varicocele needs to be radiographically defined. A less commonly used percutaneous approach to treating varicoceles is antegrade scrotal sclerotherapy. The success rate for this technique in the few published series varies between 87% and 95% [40]; however, the initial reflux grade and the number of collateral vessels of the spermatic vein are the most important factors for predicting success of this technique.

**Advances in surgical repair of the varicocele**

Two major advances in the surgical repair of the varicocele have been the surgical microscope and the intraoperative Doppler ultrasound. The main advantage of microsurgical (microscopic) repair over nonmicrosurgical (nonmicroscopic) repair is the significant reduction in postoperative complications, such as testicular artery injury, hydrocele formation, and varicocele recurrence. The complication rates for hydrocele formation with nonmicrosurgical technique range from 3% to 39% [41,42], whereas hydrocele formation is rarely reported with a microsurgical technique [43]. These improved results are thought to be caused by the greater ability to identify and preserve individual lymphatics. The recurrence rate for microscopic inguinal varicocelectomy has been reported between 1% and 2% compared with 9% and 16% for nonmicroscopic inguinal varicocele repair [43–45]. The recurrence rate for nonmicroscopic subinguinal varicocele repair is reported to be between 5% and 20% [42,46].

The micro Doppler is another advance that has improved the outcomes in varicocele repair. We currently use the micro Doppler probe with a disposable tip and a low signal-to-noise ratio Doppler amplifier (Vascular Technology, Inc., Nashua, NH) (Fig. 2). Hallak and colleagues [47] found that microsurgical varicocelectomy combined with intraoperative Doppler ultrasound improved preservation of the testicular artery and increased the number of veins ligated. In this study, 60 patients underwent a microsurgical varicocele repair (103 clinical varicoceles). In Fig. 2. The micro Doppler probe assesses testicular artery flow.
40 patients (73 clinical varicocele repairs), an intraoperative 9.3-Mhz vascular Doppler (Vascular Technology Inc.) was used. Twenty patients (30 clinical varicoceles) underwent a microsurgical varicocele repair without the use of a Doppler. In the Doppler group, 1.7 arteries were identified compared with 1.1 arteries in the non-Doppler group \( (P = .001) \). The total number of ligated veins was higher in the Doppler group than in the non-Doppler group (seven veins versus five veins, respectively) \( (P = .02) \). The Doppler is a valuable tool in helping reduce intraoperative complications and improving the number of testicular veins ligated, because by definitively excluding the artery, more veins can be taken without concern for arterial injury. Although no studies thus far have demonstrated the use of the Doppler causing a more significant improvement in semen parameters or overall outcomes, a recent study demonstrated that the number of veins ligated during varicocele repair does correlate positively with an increase in total sperm motility [48].

Complications

The most common complications after a varicocele repair are the formation of a hydrocele, varicocele recurrence, and testicular artery damage. The rates of these complications are highly contingent upon the surgical approach and a surgeon’s skills. The complication rates after a varicocele repair have declined significantly since the introduction of the microscope and intraoperative Doppler.

Outcomes of varicocele repair

Most studies report improved semen parameters after varicocele repair [5]. Conflicting data exist on improved pregnancy and fertility outcomes, however [49–51]. A meta-analysis of 22 studies with 2989 patients who underwent varicocele repair showed that 71% of patients had improvements in their postoperative semen parameters, and 37% achieved pregnancy [52]. A recent Cochran review evaluated all of the randomized controlled trials assessing the efficacy of a varicocele repair [53]. Only three of the eight randomized controlled trials included patients with abnormal semen analysis and palpable varicocele. In the three studies, there were overall 117 patients in the control group and 120 patients in the treatment group. The analysis demonstrated a significant increase in pregnancy rates in patients who underwent varicocele treatment (36.4%) compared with rates in the control group (20%) \( (P = .009) \). The methodologic quality and statistical power of the studies were considered to be poor, however.

Predicting successful repairs

There has been much interest in identifying predictive markers to assess which male patients would most benefit from varicocele repair. Marks and colleagues [54] reported four preoperative factors associated with an increased likelihood of postoperative pregnancies: (1) a lack of testicular atrophy, (2) sperm density more than 50 million per ejaculate, (3) sperm motility of 60% or more, and (4) and serum follicle-stimulating hormone values less than 300 ng/mL. Kamal and colleagues [55] found that men with more than 5 million sperm per milliliter had a spontaneous pregnancy rate of 61% after varicocele repair compared with an 8% spontaneous pregnancy rate in men with less than 5 million sperm per milliliter. Although previous reports have suggested that the gonadotropin-releasing hormone stimulation test may be useful in predicting clinical outcomes after microsurgical varicocelectomy, recent studies have found this not to be the case [56]. The gonadotropin-releasing hormone stimulation test is not commonly used in clinical practice.

Summary

Since the initial recognition of the varicocele in the first century, our understanding of the pathophysiology, treatments, and outcomes of a varicocele and varicocele repair has evolved significantly. It is clear that varicoceles impair male fertility through many mechanisms, such as decreasing sperm motility, morphology, and count and increasing ROS levels and DNA fragmentation rates. Repair of varicoceles has been shown to improve sperm quality and overall fertility rates. Repair of varicoceles has evolved from scrotal to subinguinal and inguinal repairs with the use of microscopes and intraoperative Dopplers. The use of the intraoperative microscopic and Doppler-guided procedures has significantly reduced intraoperative complications and varicocele recurrence rates. We know that varicocele repairs have been beneficial even in some men who have azoospermia. Because varicoceles are
the leading cause of male infertility in the world, it is not surprising that we continue to search for better ways to diagnose and treat this vascular abnormality.

References


Outcomes of Varicocelectomy Treatment: An Updated Critical Analysis

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In an extensive 1994 review of treatment outcomes after varicocelectomy [1] the authors lamented the lack of prospective, randomized, double-blind, controlled studies to evaluate the impact of varicocele repair on male infertility. They acknowledged that the criteria for blinding probably would not be met when studying a surgical procedure or treatment of infertility, and they chronicled the controlled studies that were available at the time. They found that, in general, the results of the studies were imperfect because of flawed study design and reporting. They were able to discern an improvement in sperm density with an associated increase in motility and morphology after varicocelectomy and concluded that, although a definitive statement regarding the efficacy of varicocelectomy could not be made, the results from many studies did support a beneficial effect.

This article provides an updated analysis of the literature published since 1994. The present authors have followed the format of the previous review [1] and have included a summary of the results from the 1994 article at the end of each section.

Controlled studies after 1994

Since the authors’ 1994 review was published, only six new controlled trials have been published in peer-reviewed journals (Tables 1–4) [2–11].

Un fortunately, the results of these studies have not quelled the controversy surrounding the varicocele and the benefit of its repair. In 1998, Nieschlag and colleagues [2] published an update of their previously reported controlled study (1995) [12] in which they randomly assigned 120 infertile couples to receive surgical ligation, radiologic embolization, or no treatment. All male patients had a varicocele and abnormal semen parameters. The mean age of the men was 32 years, and the mean age of the female partners was 31 years. Patients who had other known causes of infertility (eg, cryptorchidism, infections, anovulation, tubal blockage) or any chronic comorbidities were excluded. Ninety-five patients completed the study. Pregnancy rate was the key outcome assessed; semen parameters and hormone concentrations were secondary variables. After 1 year there was no significant difference in pregnancy rates (25.2% in the treatment group versus 27.1% in the counseling group). Nevertheless, sperm concentration did increase significantly in the treated patients, whereas no significant changes in semen parameters occurred in the nontreatment group. Both study arms, however, included a large percentage of patients who had grade I varicoceles (48% in the treatment group and 57% in the control group). This fact is potentially significant, because several studies have noted that larger varicocele size may be associated with greater degrees of improvement after varicocele treatment [13–16]; thus the larger numbers of patients who had grade I varicoceles may have blunted any effect of treatment in this study. Nieschlag and
<table>
<thead>
<tr>
<th>Study</th>
<th># of Cases</th>
<th>Mean age of males (years)</th>
<th>Mean age of females (years)</th>
<th>Clinical grade</th>
<th>Inclusion semen criteria (million/mL)</th>
<th>Pregnancy rate</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Treatment (n)</td>
<td>Control (n)</td>
</tr>
<tr>
<td>Nieschlag et al 1998 [2]</td>
<td>125</td>
<td>32.8</td>
<td>30.4</td>
<td>I–III</td>
<td>0 → 20</td>
<td>29% (62)</td>
<td>25% (63)</td>
</tr>
<tr>
<td>Madgar et al 1995 [3]</td>
<td>45</td>
<td>28.7</td>
<td>Not reported</td>
<td>II–III</td>
<td>&gt; 5 → 20</td>
<td>60% (20)</td>
<td>40% (25)</td>
</tr>
<tr>
<td>Krause et al 2002 [4]</td>
<td>67</td>
<td>32.2</td>
<td>Not reported, 29.7</td>
<td>I–III</td>
<td>&gt; 0 → 20</td>
<td>16% (31)</td>
<td>18% (33)</td>
</tr>
<tr>
<td>Grasso et al 2000 [5]</td>
<td>68</td>
<td>Not reported</td>
<td>Not reported</td>
<td>I</td>
<td>&lt; 20</td>
<td>3% (34)</td>
<td>6% (34)</td>
</tr>
<tr>
<td>Unal et al 2001 [6]</td>
<td>42</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Subclinical</td>
<td>No restrictions</td>
<td>10% (21)</td>
<td>5% (21)</td>
</tr>
<tr>
<td>Yamamoto et al 1996 [7]</td>
<td>85</td>
<td>32</td>
<td>Not reported</td>
<td>Subclinical</td>
<td>No restrictions</td>
<td>7% (45)</td>
<td>10% (40)</td>
</tr>
<tr>
<td>Breznik et al 1993 [8]</td>
<td>79</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Subclinical–III</td>
<td>No restrictions</td>
<td>34% (38)</td>
<td>54% (41)</td>
</tr>
<tr>
<td>Vermeullen et al 1986 [9]</td>
<td>115</td>
<td>28–29</td>
<td>Not reported</td>
<td>Subclinical–III</td>
<td>&lt; 20</td>
<td>47% (90)</td>
<td>64% (25)</td>
</tr>
<tr>
<td>Baker et al 1985 [10]</td>
<td>651</td>
<td>Not reported</td>
<td>Not reported</td>
<td>I–III</td>
<td>&gt; 0 → no restrictions</td>
<td>47% (283)</td>
<td>21% (36)</td>
</tr>
<tr>
<td>Nilsson et al 1979 [11]</td>
<td>96</td>
<td>30–31</td>
<td>Not reported</td>
<td>III</td>
<td>&gt; 0 → no restrictions</td>
<td>8% (51)</td>
<td>18% (45)</td>
</tr>
</tbody>
</table>
colleagues [2] subsequently published an update that included data on 30 more couples who completed the study with the same follow-up and outcomes. The additional participants did not affect the outcomes reported in the initial study. The population of this study was quite small, losing most patients (78) after randomization with a 38% drop-out rate. Nevertheless, this was a well-designed, carefully described, single-center study demonstrating a beneficial effect of varicocelectomy on semen parameters without an effect on pregnancy rates.

The study published by Madgar and colleagues [3] is part of a multicenter trial (84,902) by the World Health Organization (WHO) that has yet to be published in its entirety. This prospective, randomized, controlled trial is well designed but differs significantly from the Neischlag study in that only men who had grade II or III varicoceles and sperm counts between $5 \times 10^6$ and $20 \times 10^6$/mL

### Table 2
Randomized varicocelectomy trials: sperm density

<table>
<thead>
<tr>
<th>Study</th>
<th># of Patients</th>
<th>Comment</th>
<th>Intake sperm density (million/mL)</th>
<th>Follow-up sperm density (million/mL)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krause et al 2002 [4]</td>
<td>14</td>
<td>Sclerotherapy</td>
<td>11.7 ± 21.0</td>
<td>10.8 ± 22.5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Controls</td>
<td>6.6 ± 33.1</td>
<td>8.8 ± 32.7</td>
<td>NS</td>
</tr>
<tr>
<td>Unal et al 2001 [6]</td>
<td>21</td>
<td>Varicocelectomy</td>
<td>47.9 ± 35.7</td>
<td>59.8 ± 50.1</td>
<td>$P = .038$</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>Clomiphene citrate</td>
<td>51.6 ± 39.4</td>
<td>59.1 ± 46.0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>Controls</td>
<td>16.17</td>
<td>15.83</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>Controls</td>
<td>15.1 ± 20.1</td>
<td>13.4 ± 16.8</td>
<td>NS</td>
</tr>
<tr>
<td>Madgar et al 1995 [3]</td>
<td>25</td>
<td>Varicocelectomy</td>
<td>15a</td>
<td>32a</td>
<td>$P &lt; .05$</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Controls</td>
<td>15a</td>
<td>15a</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>Controls</td>
<td>59 ± 43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>Controls</td>
<td>17</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviation:* NS, not significant.

* Data from graph.

### Table 3
Randomized varicocelectomy trials: motility

<table>
<thead>
<tr>
<th>Study</th>
<th># of Patients</th>
<th>Comment</th>
<th>Intake motility</th>
<th>Postoperative motility</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krause et al 2002 [4]</td>
<td>14</td>
<td>Treated with sclerotherapy</td>
<td>NA</td>
<td>$-5.4 \pm 22.1^a$</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Controls</td>
<td>NA</td>
<td>$-2.1 \pm 25.3^a$</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>Clomiphene citrate</td>
<td>43.8 ± 20.5</td>
<td>58.9 ± 13.6</td>
<td>NS</td>
</tr>
<tr>
<td>Grasso et al 2000 [5]</td>
<td>34</td>
<td>Varicocelectomy</td>
<td>22.06 ± 2.83</td>
<td>22.99 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>Controls</td>
<td>19.53 ± 5.12</td>
<td>20.49 ± 4.31</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>Controls</td>
<td>21.7 ± 13.2</td>
<td>21.5 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Controls</td>
<td>30%</td>
<td>33%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>Controls</td>
<td>31 ± 7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations:* NA, not available; NS, not significant.

* Data reported as change in motility.
were included in this study. Although 210 couples were evaluated within the study period, only 45 couples were assigned randomly to treatment with surgical ligation or to no treatment. The patients were followed for 12 months with pregnancy as the primary outcome variable. Patients in the observation group who did not achieve a pregnancy after 12 months underwent varicocelectomy. All patients were followed for 36 months after surgery. Semen parameters did not change in the nontreatment group during the observation period, but these patients experienced a significant improvement in sperm count, motility, and morphology after varicocelectomy. Only two pregnancies (10%) occurred in the nontreatment group during the observation year, but eight conceptions (44.4%) occurred in this group during the first year after surgery. The group of patients who underwent immediate surgery achieved 15 pregnancies (60%) in the first year. In the immediate-surgery group the sperm count, motility, and morphology improved significantly. At 36 months' follow-up, the overall pregnancy rate in the group receiving immediate surgical treatment was 76%, whereas the delayed-treatment group achieved a pregnancy rate of 66.7%. This study is perhaps one of the best published regarding varicocele repair: the criteria for inclusion were strict regarding infertility and the presence of varicocele, and the authors simply compared surgical treatment and observation. As discussed later, few other studies have met these standards. Although this study did not use a true cross-over study design, the significant improvements in semen parameters and pregnancy outcomes after surgery in the observation group are remarkable. Unfortunately, the study population was small and had a high drop-out rate, a common problem in this young, busy, and mobile population [17].

In 2002, Krause and colleagues [4], in another prospective, randomized, controlled multicenter study, compared the efficacy of sclerotherapy versus no treatment of varicoceles. All subjects had abnormal semen parameters and had been infertile for at least 1 year. Varicoceles were diagnosed by palpation and Doppler sonography. Patients who had subclinical or symptomatic varicoceles were excluded, as were men who had comorbidities or sperm density below 2 × 10^6 mL^-1. Patients were assigned randomly to treatment or observation groups. The authors provided an intention-to-treat analysis. Unfortunately, this study suffered from poor recruitment and difficult-to-understand shifts between the groups. An intention-to-treat analysis demonstrated that 300 patients were required for the study to be powered sufficiently, but only 67 patients were randomized. More than half the patients were lost to follow-up at 6 months. The authors acknowledge this study flaw and present an “as-treated analysis.” This analysis demonstrated that testicular volume increased significantly in treated groups, but there were no significant alterations in sperm parameters. The conception rate was 30% within 12 months after intervention in the treated

### Table 4
Randomized varicocelectomy trials: morphology

<table>
<thead>
<tr>
<th>Study</th>
<th># of Patients</th>
<th>Comment</th>
<th>Intake morphology (% normal)</th>
<th>Postoperative morphology (% normal)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unal et al 2001 [6]</td>
<td>21</td>
<td>Varicocelectomy</td>
<td>69.1 ± 16</td>
<td>70.4 ± 12.4</td>
<td>NS</td>
</tr>
<tr>
<td>Yamamoto et al 1996 [7]</td>
<td>40</td>
<td>No treatment</td>
<td>30.3 ± 8.5</td>
<td>30.5 ± 9.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Abbreviations:** NA, not available; NS, not significant.
patients compared with 16.2% in the untreated patients \( (P = .189) \). Interestingly, the authors reported a cure rate (absence of reflux) of only approximately 50% in their patients. “Two high ligations of the varicocele were performed” with no explanation as to why or how these particular patients were analyzed in the study. Clearly, this study suffers from many methodologic flaws that prevent one from drawing any valid conclusions.

Several other controlled trials have been published since 1994. Unfortunately, these studies included patients who had subclinical varicoceles or normal semen analyses. Although the current guidelines do not recommend the treatment of varicoceles in these patients \[18\], these studies certainly are worthy of analysis in any critical analysis of varicocele outcomes. Yamamoto and colleagues \[7\] studied 85 patients diagnosed by thermography (infrared imaging) with thermal asymmetry \(( > 0.3^\circ C)\) of the testes. Patients were diagnosed as having subclinical varicoceles (no varicocele was found on examination). Male patients had a mean age of 32 years and had been infertile for at least 1 year. Patients were assigned randomly to treatment with high ligation of the internal spermatic vein or to no treatment. There were no restrictions on semen parameters. Sperm density was the only variable that showed a significant increase \( (P < .006) \) in the treatment group compared with controls. The authors conclude that subclinical varicocele repair is not warranted because it does not improve postoperative pregnancy rate. The authors, however, did not restrict the study participants to men who have abnormal semen parameters; indeed, the mean preoperative morphology was normal based on WHO standards \( (> 30\%) \) \[19\]. In addition, because scrotal thermography lacks specificity as a diagnostic tool, it has not been embraced as a diagnostic modality.

Grasso and colleagues \[5\] also performed a randomized, controlled study comparing surgical treatment versus observation in patients who had grade I varicoceles diagnosed by bidirectional Doppler velocimetry. Sixty-eight patients 30 to 38 years old who had been infertile for more than a year were assigned randomly to spermatic vein ligation or to a 12-month observation period. Although patients included in this study did have abnormal semen parameters, the exclusion criteria were not described. Additionally, the age and fertility status of the female partners were not detailed. The authors discovered a significant decrease in sperm concentration in the first 6 months, but the count normalized to baseline at 12 months. There was no demonstrable trend in semen parameters in the group that underwent observation. The authors state that “paternity was verified in one patient in group 1 (surgery) and two patients in group 2 (observation).” It was not clear whether these were pregnancies or live births. The authors conclude that left spermatic vein ligation “has no influence on fertility in patients over the age of 30 years with grade I left varicocele.” This study has several difficulties. Again, the study population included only patients who had grade I varicoceles, and the sample size is quite small. In addition, because no information was provided regarding evaluation of the partners, one cannot be sure the persistent infertility was solely the result of male infertility, without other confounding factors.

Another trial by Unal and colleagues \[6\] also studied subclinical varicoceles. This study randomly assigned 42 men who had subclinical varicoceles to high ligation of the spermatic vein or to 6 months of clomiphene citrate treatment. These men had a mean age of 32.7 ± 6.1 years, had been infertile for more than 1 year, and had a normal hormonal profile and normal testicular size. The fertility evaluation of the patients or partners was not described. Exclusion criteria also were not described. With the exception of motility, the baseline semen parameters for both groups were normal, based on WHO criteria \[17\]. In the surgery group sperm density and motility percentage were improved significantly at 6 months compared with baseline. The clomiphene citrate group had no improvement in any parameter. There also was no statistical difference in semen parameters between the groups. Only two pregnancies were achieved in the surgery group, versus one in the clomiphene group, a difference that was not statistically significant. As in the report by Grasso \[5\], the fertility assessment of the study participants was not detailed. Additionally, there was no control group in this study. Participants were assigned randomly to one of two treatment arms. Although the evaluation of alternatives to invasive treatment methods of varicoceles is important, it is difficult to determine the value of an intervention or dismiss a treatment as ineffective without comparing it with a true control.

Two additional randomized trials have been presented in abstract form but have yet to be published. A 2003 abstract by Dohle and colleagues \[20\] randomly assigned 72 couples who had more than 1 year of infertility to treatment
Men who had ultrasound-confirmed varicoceles and abnormal semen parameters were included. Men who had azoospermia were excluded. Female partners had normal gynecologic evaluations and were younger than 36 years old. After 1 year of follow-up there was an improvement in sperm density and motility. After 1 year the rate of spontaneous pregnancies was 36% in the treatment group compared with 9% in the control group. These results certainly are promising; one hopes that, when the results are published in a peer-reviewed journal, the study design and statistical analysis withstand critical review.

The WHO task force on the management and prevention of infertility began a prospective clinical trial to compare varicocele ligation and observation in 1984. The study included 248 couples in 12 countries. Men included in the study had a palpable varicocele and abnormal semen measurements; female partners were demonstrated to ovulate and to have proven fallopian tube patency. The study has not yet been published, but the results have been presented at several meetings. When the study was submitted for publication, it was determined to be too flawed to proceed with publication in a peer-reviewed journal [21]. In this study the pregnancy rate was 34.8% in the intervention group compared with 16.7% in the observation group ($P < .003$). There also were significant improvements in sperm concentration in the immediate-treatment group compared with the observed group, and this difference persisted for the entire first year of the study. The results of the study showed that surgical varicocele repair was 2.5 times more effective than delayed treatment. The study suffered from extensive loss to follow-up and bias, however. Not all patients were enrolled formally in the study before treatment. There also were procedural mistakes in the randomization of patients. The major flaw of this multi-institutional study was the varied interpretation of the study protocol by the different centers across the world [22].

**Meta-analyses and statistical reviews**

After reviewing the available randomized clinical trials in the literature, it is clear that difficulties with methodology and reporting persist. Only one of the published studies presents positive results. Is there, however, sufficient evidence to conclude that treatment of varicocele is not warranted? Evers and Collins [22,23] arrived at this conclusion after their own review of the varicocele literature. They conducted an extensive search for controlled trials in the literature, including proceedings of annual meetings and hand searches through andrology journals. After reviewing eight studies (they included two studies previously described in the 1994 review by Schleginger et al [1]), they concluded the Peto odds ratio was 1.10 (95% confidence interval [CI], 73–1.68) favoring treatment over observation. When only the three trials that included patients who had clinical varicoceles and abnormal semen analyses were analyzed, the Peto odds ratio was 1.75 (95% CI, 0.60–4.25) in favor of treatment. Although this analysis showed a trend in favor of treatment, it was not statistically significant ($P = .06$). The authors state that their review “fails to offer evidence that treatment of a varicocele … improves the couple’s spontaneous pregnancy chances.”

A meta-analysis combines the results of several studies that address a set of related research hypotheses. A well-conducted analysis can provide a more precise estimate of a treatment effect. On the other hand, poorly conducted meta-analyses may be biased because of the inclusion of inadequate studies [24]. Evers and Collins themselves state that the studies included for analysis were not of “high quality,” were significantly heterogeneous, and included studies with poor methodology. Ficarra and colleagues [25] conducted their own review of the three randomized, controlled trials that included patients who had clinically diagnosed varicoceles and subnormal semen parameters. They acknowledged the high rate of drop-out and patients lost to follow-up and therefore searched the studies to perform an “as-treated” analysis. They demonstrated a pregnancy rate of 36.4% in the treatment group versus 20% in the control group ($P = .009$). Although these numbers are favorable and contradict the conclusions of the Cochrane review [22], they again are drawn from data and patients from clinically and statistically heterogeneous studies.

Marmar and colleagues [26] sought to improve the prior published meta-analysis by excluding studies that included patients who had subclinical varicoceles. They also attempted to decrease heterogeneity by including only patients treated with surgical varicocelectomy. They excluded any patients who had undergone assisted reproductive techniques (ART). They included only patients who had at least one abnormal semen
parameter. Of 101 articles retrieved from a search that contained pregnancy data, only five studies were sufficiently free of bias to pass the authors' rigorous review. The reviewers were blinded during evaluation: the methods, results, tables, and figures were separated from qualitative or quantitative reports of the results. Furthermore, each study was evaluated for four different categories of bias. These studies included randomized and observational studies. The odds of spontaneous pregnancy after varicocelectomy compared with no treatment were 2.87 (95% CI, 1.33–6.20). Tests for heterogeneity were not significant. The number needed to treat to achieve spontaneous pregnancy was 5.7. The statistical methodology in this study is more meticulous and thorough than in the other meta-analyses described previously. The authors excluded studies with large numbers of individual dropouts after randomization. The “scoring system” used to detect bias in the studies included in the meta-analysis was not clearly delineated, however. Each of the four categories of bias—selection, confounding, information, and “other”—was assigned a threshold score, and articles with scores below this threshold were excluded. This threshold score, however, was different for each category and was ill defined. Based on their meta-analysis techniques, the authors concluded that varicocelectomy improved spontaneous conception rates.

Varicocelectomy: “stacking therapies”

The Cochrane review [22] included only studies that reported pregnancy rates as an outcome measure. The pregnancy rate is, of course, the most important variable when considering an intervention to improve fertility, but pregnancy rates are affected by a multitude of other factors that often are not well studied or characterized by the published studies. Additionally, today ART is becoming more common and more accessible to infertile couples. As described previously, in their review of the literature the present authors often found that individual semen parameters often improved, but pregnancy rates did not. Indeed, semen analysis has a limited ability to distinguish fertile from infertile males, and pregnancies credited to increases in semen parameters may be unrelated to these increases [27]. Similarly, the lack of pregnancy does not in itself indicate that varicocelectomy did not have a beneficial effect. Improvements in individual semen parameters may not be insignificant. Galarneau and Nagler [28] have coined the term “progressive stacking of therapy” to capture the full beneficial effect of varicocelectomy. The concept of “stacking of therapy” states that although semen parameters may not improve enough after varicocelectomy to result in spontaneous pregnancy, they may be sufficient for ART in patients who before surgery did not have adequate semen parameters to access these treatments. One study showed that in 31% of patients semen parameters improved enough for the couples to shift from being candidates for in vitro fertilization candidates to being eligible for intrauterine insemination or achieving spontaneous pregnancy [29]. The overall pregnancy rate achieved after varicocele treatment was 36.6%. The treatment of male infertility with varicocelectomy followed by intrauterine insemination has been shown to be more cost effective than in vitro fertilization and intracytoplasmic sperm injection [28,30].

Effect of varicocelectomy on individual semen parameters

Most of the studies demonstrating improved semen quality and pregnancy rates are uncontrolled [27]. Although these studies are not the most powerful tools with which to evaluate the efficacy of varicocele treatment, they do contain valuable information. Noncontrolled studies may be closer to real clinical practice than randomized, controlled trials in which more patients may refuse randomization [25]. These studies generally have larger series of patients with longer follow-up. The information provided by these studies is summarized and discussed in the following sections.

Effect of varicocelectomy on sperm density

In addition to the randomized, controlled trials described previously in this article (see Tables 1–4), 19 studies between 1997 and 2007 included an assessment of sperm density (Table 5) [13–15, 31–46]. The data comprised 2988 patients. The types of varicocele repair differed across the studies. infertility was the indication for most repairs, but one study [41] included only patients who had persistent or recurrent varicocele. Indications for varicocelectomy in other studies included pain and testicular atrophy [31]. Three of the randomized, controlled trials demonstrated an improvement in sperm density after treatment of varicocele. The majority of the uncontrolled
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th># of Patients</th>
<th>Comment</th>
<th>Intake sperm density (million/mL)</th>
<th>Postoperative sperm density (million/mL)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Kandari et al [31]</td>
<td>2007</td>
<td>40</td>
<td>Ivanisevich</td>
<td>22.4 ± 4</td>
<td>40 ± 6</td>
<td>( P &lt; .01 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>Laparoscopic</td>
<td>21 ± 5</td>
<td>41 ± 6</td>
<td>( P &lt; .01 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>Microsurgical subinguinal</td>
<td>20 ± 5</td>
<td>42 ± 7</td>
<td>( P &lt; .01 )</td>
</tr>
<tr>
<td>Hsieh et al [32]</td>
<td>2006</td>
<td>254</td>
<td>Ivanisevich</td>
<td>24.2 ± 18</td>
<td>41 ± 28</td>
<td>( P &lt; .05 )</td>
</tr>
<tr>
<td>Marmar and Benoff [33]</td>
<td>2005</td>
<td>60</td>
<td>Marmar</td>
<td>11.92 ± 8.80</td>
<td>19.93 ± 12.00</td>
<td>( P &lt; .01 )</td>
</tr>
<tr>
<td>Libman et al [34]</td>
<td>2006</td>
<td>157</td>
<td>Bilateral varicocectomy</td>
<td>20.7 ± 2.0</td>
<td>27.3 ± 2.5</td>
<td>( P = .005 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>212</td>
<td>Unilateral varicocectomy</td>
<td>19.0 ± 1.7</td>
<td>24.8 ± 2.4</td>
<td>( P = .002 )</td>
</tr>
<tr>
<td>Hussein et al [35]</td>
<td>2006</td>
<td>104</td>
<td>Microsurgical subinguinal</td>
<td>13 ± 17.9</td>
<td>17.4 ± 13</td>
<td>( P &lt; .0001 )</td>
</tr>
<tr>
<td>Zucchi et al [36]</td>
<td>2006</td>
<td>22</td>
<td>Ivanisevich</td>
<td>13.4 ± 4.4</td>
<td>14.0 ± 4.5</td>
<td>( P &lt; .5 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>Antegrade sclerotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orhan et al [37]</td>
<td>2005</td>
<td>82</td>
<td>Microsurgical inguinal</td>
<td>30 ± 8.3</td>
<td>33 ± 8.9</td>
<td>( P = .01 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65</td>
<td>Microsurgical subinguinal</td>
<td>29 ± 8.5</td>
<td>32 ± 10.0</td>
<td>( P = .05 )</td>
</tr>
<tr>
<td>Zini et al [38]</td>
<td>2005</td>
<td>37</td>
<td>Marmar</td>
<td>34.6 ± 6.0</td>
<td>38.4 ± 7.6</td>
<td>( P = .54 )</td>
</tr>
<tr>
<td>Gat et al [39]</td>
<td>2005</td>
<td>101</td>
<td>Embolization</td>
<td>0.22 ± 0.30</td>
<td>9.28 ± 1.2</td>
<td>( P &lt; .001 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>Azoosperma</td>
<td>0</td>
<td>3.81 ± 1.69</td>
<td>( P &lt; .03 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31</td>
<td>Virtual azoosperma</td>
<td>0.054 ± 0.007</td>
<td>10.31 ± 1.87</td>
<td>( P &lt; .001 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38</td>
<td>Severe oligozoosperma</td>
<td>0.54 ± 0.04</td>
<td>12.11 ± 1.85</td>
<td>( P &lt; .001 )</td>
</tr>
<tr>
<td>Watanabe et al [40]</td>
<td>2005</td>
<td>50</td>
<td>High ligation</td>
<td>15.9 ± 19.8</td>
<td>28.8 ± 32.5</td>
<td>( P &lt; .01 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33</td>
<td>Laparoscopic</td>
<td>21.9 ± 22.2</td>
<td>52.3 ± 55.4</td>
<td>( P &lt; .01 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61</td>
<td>Marmar</td>
<td>23.5 ± 29.7</td>
<td>59.4 ± 50.2</td>
<td>( P &lt; .01 )</td>
</tr>
<tr>
<td>Grober et al [41]</td>
<td>2004</td>
<td>54</td>
<td>Recurrent or persistent</td>
<td>15.8</td>
<td>26.0</td>
<td>( P = .02 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>varicoceles/microsurgical</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>repair</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polito et al [13]</td>
<td>2004</td>
<td>426</td>
<td>Percutaneous srecroembolization</td>
<td>27.55 ± 4.41</td>
<td>34.08 ± 3.53</td>
<td>( P &lt; .01 )</td>
</tr>
<tr>
<td>Kibar et al [42]</td>
<td>2002</td>
<td>90</td>
<td>Subinguinal</td>
<td>22.1 ± 4.2</td>
<td>38.3 ± 6.1</td>
<td>( P = .00002 )</td>
</tr>
<tr>
<td>Onozawa et al [14]</td>
<td>2002</td>
<td>18</td>
<td>Observed</td>
<td>36.6 ± 31.9</td>
<td>41.9 ± 45.7</td>
<td>( P &gt; .05 )</td>
</tr>
<tr>
<td>Cayan et al [43]</td>
<td>2000</td>
<td>26</td>
<td>Palomo</td>
<td>26.0 ± 28.8</td>
<td>34.6 ± 29.2</td>
<td>( P = .09 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>232</td>
<td>High ligation</td>
<td>30.97 ± 2.46</td>
<td>34.57 ± 3.58</td>
<td>( P &lt; .001 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>236</td>
<td>Microsurgical high ligation</td>
<td>29.7 ± 1.24</td>
<td>36.62 ± 1.58</td>
<td>( P &lt; .001 )</td>
</tr>
<tr>
<td>Jungwirth et al [15]</td>
<td>2000</td>
<td>272</td>
<td>Subinguinal microsurgical</td>
<td>51.7 ± 4.2</td>
<td>64.3 ± 5.6</td>
<td>( P &lt; .0001 ) versus control</td>
</tr>
<tr>
<td>Scherr et al [44]</td>
<td>1999</td>
<td>26</td>
<td>Unilateral microsurgical</td>
<td>98.5 ± 94.8</td>
<td>167.6 ± 200.3</td>
<td>( P = .0519 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65</td>
<td>Bilateral microsurgical</td>
<td>69.6 ± 90.0</td>
<td>136.9 ± 157.2</td>
<td>( P = .00003 )</td>
</tr>
<tr>
<td>Vazquez-Levin et al [45]</td>
<td>1997</td>
<td>33</td>
<td>Microsurgical</td>
<td>35.2 ± 6.2</td>
<td>45.8 ± 8.0</td>
<td>( P &lt; .1 )</td>
</tr>
<tr>
<td>Seftel et al [46]</td>
<td>1997</td>
<td>30</td>
<td>Subinguinal</td>
<td>18.9 ± 15.1</td>
<td>41.7 ± 33.1</td>
<td>( P &lt; .0001 )</td>
</tr>
</tbody>
</table>
studies demonstrated an improvement in sperm density after treatment. Only two studies demonstrated no improvement [14,38]. Two studies had mixed results [37,44]. The controlled study by Onazawa and colleagues [14] compared 31 patients who underwent repair versus 18 who underwent conservative treatment. Although there was a control arm, this was not a randomized study because patients were allowed to choose their method of treatment. Therefore this controlled, nonrandomized study is included in this section for analysis. The patients undergoing varicocele repair had a significant improvement in sperm density in this study. The trial by Zini and colleagues [38] revealed an improvement in sperm density in 37 men who underwent repair, but this result did not reach statistical significance.

The 1994 review [1] included 16 studies comprising 1077 patients. Twelve of those studies showed a significant improvement in sperm density. Currently, the authors describe 19 studies from 1997 to 2007 comprising 2988 patients. Seventeen showed a significant improvement in sperm density after varicocelectomy. Only two of these studies had equivocal results. Varicocele repair has been shown consistently to improve sperm density.

Grade/size and location of varicoceles: sperm density

In their 1994 review Schlesinger and colleagues [1] were unable to conclude that the size of varicocele had a definite influence on sperm density. Several centers have revisited this issue in addition to studying the impact of laterality on semen parameters (Table 6) [13–16]. Jungwirth and colleagues [15] studied 272 patients who underwent microsurgical varicocelectomy and determined that sperm density had improved significantly regardless of grade. The study group was not well characterized, however, and seems to have included fertile patients and men who had normal semen parameters. Polito and colleagues [13] had similar results in 426 patients. Although reported sperm density improved for treated patients overall, the grade III varicoceles did not have a significant improvement versus baseline. This group was quite small compared with the others, however, and perhaps was not sufficiently powered to obtain a significant result. Conversely, Onozawa and colleagues [14] did find that more severe varicoceles had a greater impact on sperm density; the grade III varicocele group was the only group to show a significant improvement after treatment. Pasqualotto and colleagues [16] examined whether the number of veins ligated at varicocelectomy had an impact on seminal parameters in 61 patients. Only patients who had more than 10 veins ligated had significant improvement in sperm density. The number of veins ligated at surgery is difficult to compare with other types of repair. The authors included the distribution of grades in the study population, but for unexplained reasons they did not correlate varicocele grade with the number of veins ligated. In addition, almost half the patients in this study underwent “conventional inguinal technique,” and the other half underwent microsurgical repair. Certainly, one would expect that the technique employed would affect the number of veins ligated, further confounding the results.

The controversy regarding varicocele size also has been extended to the subclinical varicocele by the widespread availability of scrotal ultrasonography. Jarow and colleagues [47] found that the total motile sperm count is significantly lower following subclinical varicocelectomy than after repair of clinical varicoceles. Although 41% of patients who had subclinical varicoceles had significant postoperative improvement in semen parameters, an equal number were worse postoperatively. Unal and colleagues [6] compared only patients who had subclinical varicocele versus medical therapy. They found an improvement in sperm density overall, but the other randomized, controlled trials that studied subclinical varicoceles did not [5,7].

Laterality may affect sperm density. Historically, the prevalence of bilateral varicoceles has been reported as 10%, although recent evidence quotes the number at 30% to 50% [27]. The present authors found the prevalence of clinically diagnosed contralateral varicoceles to be 35% in 256 men [48]. It has been reported that larger varicoceles are associated with lower sperm densities than smaller varicoceles [47,49]. Scherr and colleagues [44] studied the impact of unilateral versus bilateral microsurgical varicocelectomy in men who had grade II or III left varicoceles associated with grade I right varicocele. They found that bilateral repair resulted in significantly greater improvement in postoperative semen parameters than unilateral repair. Libman and colleagues [34] also found a greater
sperm count response after bilateral repair than after unilateral repair, although the difference was not significant. Commenting on this article, Richardson and Nagler [50] pointed out that one would expect worse preoperative semen parameters in the bilateral varicocele group than in the unilateral varicocele group if the adverse effects of a varicocele were dose dependent (eg, large versus small or unilateral versus bilateral). This was not the case. Similarly, the studies that addressed higher-grade varicoceles (see Table 6) did not demonstrate that grade III varicoceles had worse preoperative semen parameters. In 1995 Grasso and colleagues [51] conducted a study similar to that of Scherr [44]. They did not find a significant difference in seminal improvement based on grade of the varicocele. In this study they diagnosed varicoceles according to the Hirsh classification rather than the Dubin and Amelar criteria [52]. Thus, the results may not be entirely comparable. The importance of the size and bilaterality of varicoceles remains controversial.

In their 1994 article, Schlesinger and colleagues [1] demonstrated mixed results: some studies indicated that the grade of varicocele had an effect on sperm density, but other studies showed no effect. The authors recommended that the size of varicocele should not influence the decision to repair varicoceles. In the present article several more parameters can be used to determine the impact of varicocele size. The impact of varicocele grade is still in dispute, with some studies showing that larger varicoceles have a greater detrimental effect on semen parameters. Laterality may have an impact on sperm density: a small number of studies demonstrate an increased benefit from the repair of bilateral versus unilateral varicoceles. Bilateral varicocelectomy, however, should not be performed without documentation of the presence of a bilateral varicocele.

### Sperm motility

Twenty-one studies evaluated the impact of varicocelectomy on sperm motility in 3676 patients (Table 7) [13–15,29,31–46]. Six of these studies did not demonstrate a significant improvement in sperm motility, although in each there was a trend toward improvement. Two had mixed results. Additionally, all these studies, with the exception of that by Zini and colleagues [38], also observed an improvement in sperm density. Nabi and colleagues [54] stratified improvements in sperm motility by preoperative semen density in patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Varicocele grade (treatment group)</th>
<th># of Patients</th>
<th>Intake sperm density (million/mL)</th>
<th>Postoperative sperm density (million/mL)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polito et al [13]</td>
<td>2004</td>
<td>Grade 1</td>
<td>216</td>
<td>24.12 ± 3.19</td>
<td>36.72 ± 4.28</td>
<td>P &lt; .01 versus baseline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grade 2</td>
<td>183</td>
<td>26.53 ± 2.81</td>
<td>33.04 ± 3.49</td>
<td>P &lt; .01 versus baseline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grade 3</td>
<td>27</td>
<td>23.04 ± 1.37</td>
<td>25.19 ± 1.54</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grade 1</td>
<td>91</td>
<td>42.7 ± 6.1</td>
<td>41.7 ± 6.6</td>
<td>P &lt; .01 versus control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grade 2</td>
<td>71</td>
<td>44.4 ± 7.8</td>
<td>52.0 ± 7.7</td>
<td>P &lt; .01 versus control</td>
</tr>
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<td></td>
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<td>Grade 3</td>
<td>107</td>
<td>55.2 ± 16.9</td>
<td>89.2 ± 34.7</td>
<td>P &lt; .01 versus control</td>
</tr>
<tr>
<td>Jungwirth et al [15]</td>
<td>2000</td>
<td>Grade I</td>
<td>91</td>
<td>33.6 ± 33.1</td>
<td>36.8 ± 33.6</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
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<td>Grade 2</td>
<td>71</td>
<td>13.4 ± 13.7</td>
<td>22.8 ± 20.3</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grade 3</td>
<td>107</td>
<td>13.7 ± 1.43</td>
<td>29.3 ± 23.7</td>
<td>P = .03</td>
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<tr>
<td>Onozawa et al [14]</td>
<td>2002</td>
<td>Grade I</td>
<td>6</td>
<td>38.87 ± 13.2</td>
<td>22.09 ±</td>
<td>P = .03</td>
</tr>
<tr>
<td></td>
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<td>P = .03</td>
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<td>Grade 3</td>
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<td>13.4 ± 4.6</td>
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<td>5–9 veins ligated</td>
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<td>≥ 10 veins ligated</td>
<td>12</td>
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<tr>
<td>Study</td>
<td>Year</td>
<td># of Patients</td>
<td>Comment</td>
<td>Intake motility</td>
<td>Postoperative motility</td>
<td>Statistical significance</td>
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<tr>
<td>Al-Kandari et al [31]</td>
<td>2007</td>
<td>40</td>
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<td>33 ± 4</td>
<td>48 ± 4</td>
<td>P &lt; .05</td>
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<tr>
<td></td>
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<td>50 ± 5</td>
<td>P &lt; .05</td>
</tr>
<tr>
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<td>34 ± 6</td>
<td>52 ± 6</td>
<td>P &lt; .05</td>
</tr>
<tr>
<td>Hsieh et al [32]</td>
<td>2006</td>
<td>254</td>
<td>Ivanisevich</td>
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<td>47 ± 16</td>
<td>P &lt; .05</td>
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<tr>
<td>Hussein [35]</td>
<td>2006</td>
<td>104</td>
<td>Microsurgical subinguinal</td>
<td>24 ± 13.9</td>
<td>28.6 ± 17.6</td>
<td>P &lt; .0001</td>
</tr>
<tr>
<td>Libman et al [34]</td>
<td>2006</td>
<td>157</td>
<td>Bilateral varicocelectomy</td>
<td>26.1 ± 1.4</td>
<td>34.1 ± 1.8</td>
<td>P = .0001</td>
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<td>29.6 ± 1.4</td>
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<td>Ramasamy and Schlegel [53]</td>
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<td>Microsurgical/testis delivery</td>
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<td>93 ± 5</td>
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<tr>
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<td>110</td>
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<td>Microsurgical/no testis delivery</td>
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<td>65 ± 11</td>
<td>P &lt; .01</td>
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<td>22</td>
<td>Ivanisevich</td>
<td>38.2 ± 33.4</td>
<td>48.0 ± 33.8</td>
<td>P &lt; .1</td>
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<tr>
<td>Marmar and Benoff [33]</td>
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<td>44.02 ± 9.51</td>
<td>P &lt; .001</td>
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<tr>
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<td>2005</td>
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<td>Microsurgical inguinal</td>
<td>29 ± 8.4</td>
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<td>2005</td>
<td>37</td>
<td>Marmar</td>
<td>20.9 ± 1.9</td>
<td>22.1 ± 2.6</td>
<td>NS</td>
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<tr>
<td>Gat et al [39]</td>
<td>2005</td>
<td>101</td>
<td>Embolization</td>
<td>8.78 ± 1.59</td>
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<td>P &lt; .01</td>
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<td>Virtual azooosperma</td>
<td>6.07 ± 2.69</td>
<td>35.8 ± 2.76</td>
<td>P &lt; .01</td>
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<td>38</td>
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<td>32.34 ± 3.13</td>
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<tr>
<td>Watanabe et al [40]</td>
<td>2005</td>
<td>50</td>
<td>High ligation</td>
<td>38.0 ± 22.1</td>
<td>39.1 ± 25.2</td>
<td>Not significant</td>
</tr>
<tr>
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<td></td>
<td>33</td>
<td>Laparoscopic</td>
<td>40.4 ± 18.8</td>
<td>42.5 ± 22.6</td>
<td>Not significant</td>
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<tr>
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<td>61</td>
<td>Marmar</td>
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<td>38.8 ± 26.2</td>
<td>Not significant</td>
</tr>
<tr>
<td>Polito et al [13]</td>
<td>2004</td>
<td>426</td>
<td>Percutaneous sclerotherapy</td>
<td>12.81 ± 1.08</td>
<td>13.07 ± 1.15</td>
<td>Not significant</td>
</tr>
<tr>
<td>Grober et al [41]</td>
<td>2004</td>
<td>54</td>
<td>Recurrent or persistent varicoceles/ micro-surgical repair</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cayan et al [29]</td>
<td>2002</td>
<td>540</td>
<td>Microsurgical varicocelectomy</td>
<td>19.04 ± 1.18</td>
<td>27.12 ± 1.49</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>Onozawa et al [14]</td>
<td>2002</td>
<td>26</td>
<td>Palomo</td>
<td>50.8 ± 28.2</td>
<td>61.3 ± 26.6</td>
<td>Not significant</td>
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<tr>
<td>Kibar et al [42]</td>
<td>2002</td>
<td>90</td>
<td>Subinguinal</td>
<td>23.2 ± 2.2</td>
<td>45.1 ± 1.9</td>
<td>P &lt; .01</td>
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<tr>
<td>Jungwirth et al [15]</td>
<td>2000</td>
<td>272</td>
<td>Subinguinal microsurgery</td>
<td>17.4 ± 0.7a</td>
<td>28.3 ± 1.3a</td>
<td>P &lt; .001</td>
</tr>
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<td></td>
<td></td>
<td>91</td>
<td>Grade I</td>
<td>19.9 ± 1.6a</td>
<td>30.9 ± 1.8a</td>
<td>P &lt; .01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71</td>
<td>Grade II</td>
<td>16.5 ± 1.5a</td>
<td>26.1 ± 1.7a</td>
<td>P &lt; .001</td>
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<td></td>
<td>107</td>
<td>Grade III</td>
<td>17.2 ± 5.8a</td>
<td>21.6 ± 60a</td>
<td>Not significant</td>
</tr>
<tr>
<td>Cayan et al [43]</td>
<td>2000</td>
<td>232</td>
<td>High ligation</td>
<td>28.1 ± 1.69</td>
<td>25.6 ± 1.16</td>
<td>Not significant</td>
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<td>236</td>
<td>Microsurgical high ligation</td>
<td>34.43 ± 3.03</td>
<td>43.47 ± 1.55</td>
<td>Not significant</td>
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<tr>
<td>Scherr et al [44]</td>
<td>1999</td>
<td>26</td>
<td>Unilateral microsurgical</td>
<td>35.7 ± 21.0</td>
<td>47.0 ± 14.2</td>
<td>P = .1115</td>
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<td>Bilateral microsurgical</td>
<td>29.3 ± 28.5</td>
<td>39.5 ± 19.2</td>
<td>P = .03</td>
</tr>
<tr>
<td>Vazquez-Levin et al [45]</td>
<td>1997</td>
<td>33</td>
<td>Microsurgical</td>
<td>26.4 ± 2.6</td>
<td>31.0 ± 2.1</td>
<td>Not significant</td>
</tr>
<tr>
<td>Seftel et al [46]</td>
<td>1997</td>
<td>30</td>
<td>Subinguinal</td>
<td>29.1 ± 17.1</td>
<td>39.1 ± 17.5</td>
<td>P &lt; .004</td>
</tr>
</tbody>
</table>

* Forward progressive motility.
who were treated with percutaneous embolization. They determined that the postoperative motility percentage improved significantly for the group only with a preoperative semen density of 10 to 30 million/mL. Contrary to observations noted in the 1994 review by Schlesinger et al [1], no studies demonstrated an improvement in motility without an improvement in density. The study by Madgar and colleagues [3], however, was the only randomized, controlled trial to show a postoperative improvement in motility.

Several groups also compared size of varicocele with regard to motility. Onozawa and colleagues [14] found no correlation with size and motility. Libman and colleagues [34] and Scherr and Goldstein [44] demonstrated a significantly greater improvement in motility in bilateral varicocelectomy patients versus unilateral.

In 1994 Schlesinger and colleagues [1] also demonstrated an improvement in motility after varicocelectomy in 5 of 12 studies. Motility rarely improved without improvements in sperm density. In the present review, 21 studies involving 3676 patients showed improvements in motility. Fifteen studies showed a significant improvement. There may be a correlative relationship between improvement in sperm motility and sperm density.

### Morphology

Fourteen studies reporting 2166 patients provided data on changes in morphology after varicocelectomy (Table 8) [13,14,29,31,33–35,37–39,42,44–46]. Six showed a significant improvement, seven did not, and one had mixed results. All studies, regardless of significance, had a trend toward improvement except for the study by Polito and colleagues [13], which did not demonstrate improvement in any overall seminal parameter. There were no improvements in morphology without improvements in density. The studies by Vazquez-Levin and colleagues [45] and by Seftel and colleagues [46] showed

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### Table 8

Uncontrolled varicocelectomy trials: morphology

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th># of Patients</th>
<th>Comment</th>
<th>Intake morphology (% normal forms)</th>
<th>Postoperative morphology (% normal forms)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Kandari et al [31]</td>
<td>2007</td>
<td>40</td>
<td>Ivanisevich</td>
<td>34 ± 2</td>
<td>36 ± 2</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Laparoscopic</td>
<td>33 ± 3</td>
<td>35 ± 3</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Microsurgical subinguinal</td>
<td>31 ± 4</td>
<td>32 ± 5</td>
<td>Not significant</td>
</tr>
<tr>
<td>Hussein [35]</td>
<td>2006</td>
<td>104</td>
<td>Microsurgical subinguinal</td>
<td>45.7 ± 25.3</td>
<td>58.6 ± 23.8</td>
<td>*P &lt; .001</td>
</tr>
<tr>
<td>Libman et al [34]</td>
<td>2006</td>
<td>157</td>
<td>Bilateral varicocelectomy</td>
<td>38.5 ± 1.9</td>
<td>40.0 ± 2.0</td>
<td>Not significant</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>Unilateral varicocelectomy</td>
<td>33.4 ± 1.5</td>
<td>35.2 ± 1.6</td>
<td>*P = .04</td>
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<td>Marmar and Banoff [33]</td>
<td>2005</td>
<td>60</td>
<td>Marmar</td>
<td>14.65%</td>
<td>25.28%</td>
<td>*P &lt; .001</td>
</tr>
<tr>
<td>Orhan et al [37]</td>
<td>2005</td>
<td>82</td>
<td>Microsurgical inguinal</td>
<td>9.8 ± 0.7%</td>
<td>18 ± 0.8%</td>
<td>*P &lt; .001</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Microsurgical subinguinal</td>
<td>9.0 ± 0.5%</td>
<td>17 ± 0.6%</td>
<td>*P &lt; .001</td>
</tr>
<tr>
<td>Zini et al [38]</td>
<td>2005</td>
<td>37</td>
<td>Marmar</td>
<td>21.2 ± 1.8%</td>
<td>23.4 ± 1.6%</td>
<td>*P = .08</td>
</tr>
<tr>
<td>Gat et al [39]</td>
<td>2005</td>
<td>101</td>
<td>Embolization</td>
<td>3.79 ± 0.74%</td>
<td>13.72 ± 1.37%</td>
<td>*P &lt; .005</td>
</tr>
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<td>Polito et al [13]</td>
<td>2004</td>
<td>426</td>
<td>Percutaneous sclerotherapy</td>
<td>64.7 ± 2.21</td>
<td>60.31 ± 2.49</td>
<td>Not significant</td>
</tr>
<tr>
<td>Kibar et al [42]</td>
<td>2002</td>
<td>90</td>
<td>Subinguinal</td>
<td>2.6 ± 0.5%</td>
<td>10.2 ± 0.9%</td>
<td>*P &lt; .0001</td>
</tr>
<tr>
<td>a Onozawa et al [14]</td>
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<td>Observed</td>
<td>4.2 ± 5.2</td>
<td>4.3 ± 5.1</td>
<td>Not significant</td>
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<tr>
<td>Cayan [29]</td>
<td>2002</td>
<td>540</td>
<td>Microsurgical varicocelectomy</td>
<td>58 ± 1</td>
<td>61 ± 1</td>
<td>*P &lt; .001</td>
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<tr>
<td>Scherr and Goldstein [44]</td>
<td>1999</td>
<td>26</td>
<td>Unilateral microsurgical</td>
<td>36.2 ± 23.1</td>
<td>36.3 ± 20.3</td>
<td>*P = .43</td>
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<tr>
<td>a Vazquez-Levin et al [45]</td>
<td>1997</td>
<td>33</td>
<td>Microsurgical</td>
<td>9.8 ± 1.0</td>
<td>14.5 ± 1.3</td>
<td>*P = .038</td>
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<tr>
<td>a Seftel et al [46]</td>
<td>1997</td>
<td>30</td>
<td>Subinguinal</td>
<td>5.1 ± 3.6</td>
<td>6.6 ± 4.3</td>
<td>*P &gt; .05</td>
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</tbody>
</table>

*a* Kruger morphology.
a statistically significant improvement in Kruger morphology. The study by Libman and colleagues [34] showed mixed results. Only patients who had unilateral varicoceles had significant improvement in morphology. Nabi and colleagues [54] reported a statistically significant improvement in morphology percentage for patients who had a preoperative semen density between 10 and 30 million/mL, the same group that showed an improvement in the other semen

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th># in Group</th>
<th># Pregnancies</th>
<th>Pregnancy rate (%)</th>
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<td>Donkol and Salem [60]</td>
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<td>107</td>
<td>27</td>
<td>25</td>
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<tr>
<td>Nabi et al [54]</td>
<td>2004</td>
<td>45</td>
<td>18</td>
<td>40</td>
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<tr>
<td>Al-Kandari et al [31]</td>
<td>2007</td>
<td>120</td>
<td>40</td>
<td>33</td>
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<tr>
<td>Grober et al [41]</td>
<td>2004</td>
<td>35</td>
<td>8</td>
<td>23</td>
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<tr>
<td>Ramasamy and Schlegel [53]</td>
<td>2006</td>
<td>94</td>
<td>47</td>
<td>50</td>
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<td>Libman et al [34]</td>
<td>2006</td>
<td>258</td>
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<td>2002</td>
<td>445</td>
<td>163</td>
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<td>56</td>
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<td>Pasqualotto et al [16]</td>
<td>2005</td>
<td>42</td>
<td>18</td>
<td>43</td>
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<td>Onozawa et al [14]</td>
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<td>28</td>
<td>6</td>
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<td>Perimenis et al [61]</td>
<td>2001</td>
<td>146</td>
<td>62</td>
<td>47</td>
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<td>Cayan et al [43]</td>
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<td>104</td>
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<td>Grasso et al [5]</td>
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<td>Segenreich et al [62]</td>
<td>1998</td>
<td>63</td>
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<td>Abdulmaaboud et al [63]</td>
<td>1998</td>
<td>222</td>
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<td>Seftel et al [46]</td>
<td>1997</td>
<td>30</td>
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<tr>
<td>Totals</td>
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<td>933</td>
<td>35</td>
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</table>

* Weighted average, 40.7.

Table 10
Effect of varicocelectomy on sperm density in patients who have azoospermia

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th># of Patients</th>
<th>Comment</th>
<th>Follow-up sperm density</th>
<th>Pregnancy rate (%)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
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<td>Gat et al [39]</td>
<td>2005</td>
<td>32</td>
<td>Azoospermia</td>
<td>3.81 ± 1.69</td>
<td>No information</td>
<td>$P &lt; .03$</td>
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<td></td>
<td>2005</td>
<td>31</td>
<td>Virtual azoospermia</td>
<td>10.31 ± 1.87</td>
<td>No information</td>
<td>$P &lt; .001$</td>
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<td>Esteves and Glina [56]</td>
<td>2005</td>
<td>17</td>
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<td>Pasqualotto et al [65]</td>
<td>2003</td>
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<td>Kim et al [66]</td>
<td>1999</td>
<td>28</td>
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<td>1.2 ± 3.6</td>
<td>(1 via intrauterine insemination, 1 via testicular sperm extraction)</td>
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<tr>
<td>Matthews et al [67]</td>
<td>1998</td>
<td>22</td>
<td>Azoospermia</td>
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<td>14</td>
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<tr>
<td>Pasqualotto et al [16]</td>
<td>2005</td>
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<td>$P = .04$</td>
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<tr>
<td></td>
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<td>Maturation arrest</td>
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<td>25</td>
<td>$P = .08$</td>
</tr>
<tr>
<td>Shik Lee et al [57]</td>
<td>2007</td>
<td>3</td>
<td>Hypospermatogenesis</td>
<td>0.50 ± 0.62</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>6</td>
<td>Maturation arrest</td>
<td>0.17 ± 0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>10</td>
<td>Germ cell aplasia</td>
<td>0.03 ± 0.1</td>
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</tbody>
</table>
parameters that were discussed in the previous section. This finding also suggests a density-dependent improvement in morphology, but without control groups it is difficult to make this comparison. Again, only the randomized, controlled trial conducted by Madgar and colleagues [3] (Table 1) showed an improvement in morphology.

In their 1994 review Schlesinger and colleagues [1] also determined that morphology rarely improved without an associated improvement in sperm density. An improvement in morphology was found in 5 of 10 studies. The results in the present article are similar: in 14 studies comprising 2166 patients, all studies except one showed a trend toward improvement, with six showing a significant improvement in morphology. There may be a relationship between improvement in sperm morphology and sperm density.

### Varicocelectomy and azoospermia

The incidence of azoospermia associated with varicocele ranges between 5% and 10% [55,56]. Azoospermia historically has been regarded as a contraindication to varicocelectomy [57]. Although in 1955 Tulloch [58] classically reported the initiation of spermatogenesis after varicocelectomy in an azoospermic man, only recently has there been renewed interest in varicocelectomy in the azoospermic patient. Tables 9–12 [2–7,14,16,29,31,32,34,37,39,41,43,46,53,54,56,57,60–67] present data from studies assessing the effect of varicocelectomy for azoospermic patients. Esteves and Glina [56] biopsied azoospermic men who underwent microsurgical varicocelectomy. Improvement was observed after varicocelectomy in five of six men who had hypospermatogenesis and in three of five men who had maturation arrest but not in the six men who had Sertoli cell–only syndrome. They hypothesized that testicular histology may be of value in predicting recovery of spermatogenesis. Pasqualotto and colleagues [65], however, found a more significant improvement in sperm density in patients who had germ cell aplasia than in those who had maturation arrest. They reasoned that the varicocele is a pathologic cause of germ cell aplasia and that its repair would be more beneficial in these patients. On the other hand, Shik Lee and colleagues [57] found an improvement in all semen parameters regardless of testicular histology. The differences among these observations may be explained by the

### Table 11

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th># of Patients</th>
<th>Comment</th>
<th>Postoperative motility</th>
<th>Statistical significance</th>
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<td>32</td>
<td>Azoospermia</td>
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<td></td>
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<tr>
<td>Esteves and Glina [56]</td>
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<td>17</td>
<td></td>
<td>0.8</td>
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<tr>
<td>Pasqualotto et al [65]</td>
<td>2003</td>
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<td>Microsurgical repair</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Kim et al [66]</td>
<td>1999</td>
<td>28</td>
<td>Microsurgical repair</td>
<td>19 ± 24</td>
<td>P &lt; .001</td>
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<td>Matthews et al [67]</td>
<td>1998</td>
<td>22</td>
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<td>2.2 ± 1.1</td>
<td>P &lt; .05</td>
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<tr>
<td>Pasqualotto et al [16]</td>
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<td>28</td>
<td>Germ cell aplasia</td>
<td>41.3 ± 19.6</td>
<td>P = .9</td>
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<tr>
<td></td>
<td></td>
<td>32</td>
<td>Maturation arrest</td>
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<td>P = .08</td>
</tr>
<tr>
<td>Shik Lee et al [57]</td>
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<td>3</td>
<td>Hypospermatogenesis</td>
<td>38.67 ± 41.79</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>Maturation arrest</td>
<td>24.50 ± 29.35</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>Germ cell aplasia</td>
<td>6.70 ± 21.19</td>
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</table>

### Table 12

<table>
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<tr>
<th>Study</th>
<th>Year</th>
<th># of Patients</th>
<th>Comment</th>
<th>Follow-up sperm morphology (%)</th>
<th>Statistical significance</th>
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<tr>
<td>Gat et al [39]</td>
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<td>101</td>
<td>Embolization</td>
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<td>Pasqualotto et al [65]</td>
<td>2003</td>
<td>15</td>
<td>Microsurgical repair</td>
<td>2</td>
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</table>
heterogeneity of testicular histology: a diagnostic biopsy may not be representative. Even with the appearance of sperm in the ejaculate, however, it is likely that the infertile couple with azoospermia treated with varicocelectomy will require ART [27]. Also, semen samples should be cryopreserved after the appearance of sperm in the ejaculate following varicocelectomy, because relapse to a state of azoospermia may occur [65].

Symptomatic varicocele

Although this article focuses on the effectiveness of varicocelectomy in the treatment of the infertile patient, the effectiveness of varicocelectomy in the treatment of the symptomatic patient is equally as controversial. Pain is an uncommon indication for varicocelectomy, with an incidence of 2% to 14% [68–70]. Although these reports did not assess the effect of varicocelectomy on semen parameters, symptomatic improvement was analyzed critically. Symptomatic varicoceles usually are very large [27]. Yaman and colleagues [71] reported that 72 of 82 patients (88%) who underwent microsurgical varicocelectomy had complete resolution of pain, 4 (5%) had partial resolution, and 5 (6%) reported no improvement. Karademir and colleagues [70] demonstrated that 101 of 121 patients reported improvement on their scrotal pain questionnaires after undergoing inguinal or subinguinal varicocelectomy for scrotal pain. Of these patients 75% had complete resolution of pain; the remaining 25% had partial resolution. Ribe and colleagues [59] demonstrated improvement in 20 of 25 patients (88%) who had chronic testicular pain and varicocele as the only established diagnosis. Al-Buheissi and colleagues [68] attempted to determine predictors of success after surgical ligation of painful varicoceles. Again, the majority of patients (76.5%) experienced a marked or complete resolution. Analysis of postoperative questionnaires completed by 82% of patients indicated that the quality of pain—a dull ache—was the only predictor of successful pain resolution. Patients who had a sharp pain failed to benefit from varicocele ligation (P < .01). Patient age, varicocele grade or laterality, and duration of pain had no effect on success. As demonstrated by this study, and despite the marked improvement in many patients, repair may not always bestow adequate relief of pain. Conservative measures should be taken, and other causes of scrotal and inguinal should be eliminated before offering repair for symptomatic varicoceles.

Pregnancy

The average pregnancy rate in the 24 reviewed studies is 39.35% (see Table 9). These results are comparable to those of the 1994 review [1]. The percentages across the studies vary widely, however. The reasons for this variation are not easily explained. As Schlesinger and colleagues [1] surmised in 1994, fertility and fecundity are multifactorial. More recent studies have done no better in delineating the evaluation of female partners.

Onozawa and colleagues [14] found that patients who underwent varicocelectomy had a 60% pregnancy rate versus 28% in the conservatively treated patients (P = .04). Although postoperative sperm density improved significantly after treatment, they found no statistical relationships between the presence or absence of pregnancy and the alterations in semen analysis. Nabi and colleagues [54] also attempted to correlate pregnancy with seminal improvement. The quality of the semen in the 18 patients who achieved pregnancy was not significantly different from that of the 27 patients who did not establish a pregnancy, despite an improvement in semen parameters in all 45 treated patients.

Interestingly, the randomized clinical trials have the widest range of pregnancy rates, from 2.9% [6] to 76% [3]. As stated previously, these studies are methodologically and clinically heterogeneous and are of poor quality statistically, and the wide disparity in pregnancy rates certainly reflects this fact. The three lowest pregnancy rates were from trials that studied only subclinical varicoceles, perhaps suggesting a dose effect of varicoceles. Donkol and Salem [60] reported a 36.6% pregnancy rate in 55 patients who had clinically detected varicoceles versus 16% in the 25 patients who had subclinical varicoceles. In addition, they reported a significantly higher pregnancy rate for patients who had bilateral varicoceles than in those who had unilateral varicoceles (P = .0099). In their large series, Libman and colleagues [34] also found a significantly greater spontaneous pregnancy rate (49%) in patients who underwent bilateral varicocelectomy than in those who had unilateral varicoceles (36%). Nonetheless, as Richardson and Nagler stated [50], the higher pregnancy rates in the bilateral group may indicate that contralateral varicoceles were overlooked in the unilateral group. The
failure to correct the overlooked contralateral varicoceles would prevent complete recovery of function, and consequently lower pregnancy rates, in the unilateral group.

The pregnancy rates in Table 9 do not include any pregnancies aided by ART. As discussed earlier, varicocelectomy repair can improve seminal parameters enough to allow patients to downgrade the method of ART or to bypass ART altogether [29,30]. Meng and colleagues [72] compared the cost effectiveness of varicocelectomy versus ART. In men who had lower total motile sperm counts (<10 million) surgical repair was more efficacious than ART when a surgeon could achieve a pregnancy rate of greater than 14%. In patients who had better initial semen parameters (>10 million total motile sperm), however, an individual center should be able to assure at least a 45% pregnancy rate to be more cost effective than ART. Although Meng and colleagues [72] provide a guide for determining the costs related to infertility treatments, these parameters may not be applicable to all infertility patients at all centers.

Several studies were able to demonstrate pregnancy in azoospermic patients. Pasqualotto and colleagues [16] demonstrated a 43% pregnancy rate. Patients who had maturation arrest had an even higher rate of pregnancy despite an insignificant improvement in sperm concentration and motility percentage. These results certainly are remarkable, given the lower rates of pregnancy in other studies of patients who had maturation arrest, including studies that included normospermic patients. The authors, however, did not indicate whether the patients used ART.

The average pregnancy rate across 65 studies comprising 6983 patients was 32.34% in the 1994 review [1]. This article reviews 24 studies with an average pregnancy rate of 39.35%.

Summary

Despite the lack of good randomized data, many uncontrolled studies support the efficacy of varicocelectomy. Improper study protocols and inappropriate reporting still hamper the understanding of the effect of varicoceles on fertility and the efficacy of varicocelectomy. This update reports on additional studies that have been published since the 1994 review by Schlesinger and colleagues [1] that demonstrate improved semen parameters after varicocelectomy. Unfortunately, these studies do not provide any further insight into the factors that predict success after varicocelectomy. Pregnancy rates reported before and after 1994 are comparable and remain around 30% to 40%.

As previously stated, fecundity may not be correlated only to changes in semen variables. Some men who have abnormal semen parameters may have no difficulty reproducing, and others who have more normal analyses may be infertile. Other parameters are being studied to evaluate varicocele efficacy. Reactive oxygen species have been implicated as mediators of the abnormal spermatogenesis observed in the presence of the varicocele [73]. Acrosome reaction via the Acrobear test (FUSO Pharmaceutical Industries, Osaka, Japan) has been used to assess sperm quality [74]. Human sperm DNA damage or fragmentation may affect fertility outcomes adversely [75]. No additional testing has been established as predictive of response to varicocelectomy, however.

Twenty years ago Pryor and Howards [76] published a review on varicoceles in this journal. At the time they reported that more than 1000 reports on varicoceles had been published within the previous 10 years. Although the number of studies has increased, there is a paucity of good studies assessing the impact of varicocelectomy on semen parameters and pregnancy rates. Until the enigma of the pathophysiology of the varicocele has been explained, it is unlikely that it will be possible to predict which patients will benefit from varicocelectomy. Until that time, varicocelectomy will probably remain an important part of the armamentarium in the treatment of the infertile male.

References


Anejaculation and Retrograde Ejaculation

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Many normal physiologic functions need to come together to allow a man to father a child. The components of normal reproductive function include hormonal homeostasis, spermatogenesis, epididymal sperm transport and storage, normal erectile function, and finally, the ability to ejaculate intravaginally to deliver the sperm. Infertility may result from dysfunction on any of these levels.

Ejaculatory dysfunction is a relatively uncommon, but not rare, cause of infertility. Without adequate sperm delivery during intromission, the sperm and oocyte never meet. In this article we discuss the normal physiology of ejaculation and review the classification and causes of ejaculatory dysfunction. The evaluation of a patient who presents with possible ejaculatory dysfunction is presented, followed by a review of treatment modalities.

Anatomy of normal ejaculation

The ejaculatory reflex results from the interaction of anatomic structures that are under control of cerebral and peripheral neural pathways. The ejaculate is composed of fluids from various reproductive organs, which are presented here in order of decreasing volumetric contribution. The seminal vesicles supply approximately two thirds of the ejaculate volume and fructose and seminogellin, which contribute to semen coagulation. The prostate, which supplies approximately one third of the ejaculate volume from prostatic secretions, also secretes prostate-specific antigen, a proteolytic enzyme that cleaves seminogellin, effecting liquefaction [1]. The testicular component, the sperm, comprises only approximately 1%–2% of the ejaculate volume. There is also a small contribution of fluid from the bulbourethral glands, and much of their output, a clear mucoid discharge, is released during sexual stimulation, before ejaculation. The bulbourethral glands are located in the pelvic floor striated muscle [2].

Fluids from the testicles and seminal vesicles enter the prostatic urethra via the ejaculatory ducts, which are formed from the coalescence of the ampulla of the vas deferens and the distal ducts of the seminal vesicles [3]. The ejaculatory ducts course through the prostate as separate structures and enter the prostatic urethra lateral to the verumontanum. The arrangement of sphincter muscles in relation to the entry of the ejaculatory ducts is important. The internal sphincter/bladder neck is located proximal to the ejaculatory ducts, and because it contracts during seminal emission, it is able to prevent retrograde
ejaculation into the bladder. The external sphincter, which is located distal to the ejaculatory duct openings, remains continent during emission and opens during ejaculation.

Normally during sexual stimulation, penile erection occurs to allow vaginal penetration. Erection is stimulated by parasympathetic fibers coursing to the corpus cavernosum. Erection is not a requirement for ejaculation but typically occurs before climax. In normal situations, erectile dysfunction naturally leads to infertility by preventing intromission. Erectile dysfunction–related infertility can be treated by obtaining a semen specimen by masturbation and using assisted reproductive techniques, such as artificial insemination.

Ejaculation itself results from coordination of various nervous system components. Cerebral input comes from erotic imagery and sensory and visual stimulation; cerebral influence in the ejaculatory process is poorly understood. Signals from upper centers travel down the thoracolumbar sympathetic nerves, resulting in the contraction of the prostatic smooth muscle and contraction of the seminal vesicles and vas deferens. The effector nerves that cause seminal emission and bladder neck closure are sympathetic fibers that arise from spinal levels T10-L2, coursing through the sympathetic ganglia, hypogastric plexus, and peripheral pelvic nerves [4]. This bladder neck closure occurs simultaneously with the deposition of the seminal fluid in the posterior urethra.

Sensory input from penile stimulation is also important in the ejaculatory process. Penile stimulation courses through the dorsal nerves of the penis, paired in the dorsum of the penis glans and inserting into the pelvis, ultimately entering the spinal cord at S2-4. Efferents that arise from S2-4 travel via the pudendal nerves to pelvic floor and perirectal muscles. The well-known bulbocavernosal reflex travels this route. Stimulation of the glans results in a signal carried by dorsal nerves, enters the S2-4 spinal segment, and elicits a reflex that exits S2-4 and stimulates the pelvic floor skeletal muscle, which causes contraction.

**Physiology of ejaculation**

The events that occur during normal ejaculation include seminal emission, bladder neck contraction, and projectile ejaculation. During sexual stimulation, and most notably peripheral genital stimulation, there is involuntary tonic, low-level contraction of the periurethral/pelvic floor skeletal muscle. This contraction may be responsible for the “pre-ejaculate” that results from expulsion of bulbourethral gland secretions, due to muscular compression of the glands. With ongoing sexual stimulation there is persistent periurethral contraction, which eventually rises to high pressure within the prostatic urethra. The high pressure rise within the external sphincter rise seems to be a necessary prelude to the ejaculatory reflex [5].

After a rather rapid rise to peak contraction pressures of the external sphincter, internal sphincter pressure rises rapidly over a few seconds. It is possible the sensation of genital orgasm results from the high pressure contraction of these sphincter muscles. When the internal sphincter pressure rises, external sphincter pressure drops, and at the same time the ejaculatory organs contract, expelling their contents into the urethra, which is the process of emission. With the internal sphincter tightly closed in tonic contraction, a sequence of involuntary rhythmic contractions of the perirectal muscles occurs, leading to a pulsatile, projectile ejaculation phase. The internal sphincter remains tightly closed during this time to prevent retrograde ejaculation [6]. The rhythmic contractions were previously thought to be a reflex response to distension of the posterior urethra during emission, but this supposition does not seem to be the case, because these contractions take place as part of the ejaculatory reflex, even in medical conditions in which seminal emission is absent. A timeline showing the sequence of events during ejaculation is found in Fig. 1.

Sperm are transported from the storage site in the cauda epididymis in response to sexual stimulation and proceed rapidly through the vas deferens during seminal emission. After ejaculation, vasal sperm are transported back to the cauda epididymis, where they are stored in a friendly environment [7]. Contrary to common thought, sperm are not stored in the seminal vesicles, which is a hostile environment. In certain clinical conditions, such as ejaculatory duct partial obstruction [8], prolonged abstinence, and spinal cord injury (SCI) [9], sperm may be stored in the seminal vesicles, and poor sperm motility results.

**Types of ejaculatory dysfunction**

There are several broad categories of ejaculatory dysfunction, which are listed in Box 1.
In this issue of the *Urologic Clinics*, the topic of ejaculatory duct obstruction is covered in a separate article and is not discussed further here. Idiopathic aperistalsis of the vas deferens has been described. It is treated with sympathomimetic agents [10] but remains poorly characterized and also is not discussed further here.

Premature ejaculation (PE) may be the most common male sexual dysfunction and has been reported to affect up to 31% of men aged 18 to 59 [11]. It leads to significant distress in many couples. PE may be lifelong or acquired. It is thought that men with lifelong PE likely suffer from a physiologic difference in the ejaculatory threshold when compared with normal men [12,13] and may need medical therapies. Men with acquired PE are better candidates for cognitive-behavioral therapy [14]. Medical therapy in the past has consisted of chronic selective serotonin reuptake inhibitors, and on-demand selective serotonin reuptake inhibitors. A rapid-onset, short-acting selective serotonin reuptake inhibitor, dapoxetine, has undergone US Food and Drug Administration review but has not been approved to date [15].

Prolonging the intravaginal ejaculatory latency time gives significant relief and improvement of the sexual experience. Because ejaculation in patients who have PE usually occurs intravaginally, however, it rarely causes infertility. If ejaculation occurs before intromission, however, couples can perform home-based intravaginal insemination to circumvent the fertility problem. Because PE does not usually lead to infertility, it is not discussed further in this article.

**Neurogenic anejaculation**

SCI is the most common cause of neurogenic anejaculation. Men who have SCI suffer from erectile and ejaculatory dysfunction. Many men with SCI, especially those with upper motor neuron lesions, have reflex erections and some degree of ability to engage in vaginal intercourse. Even men with short-lived reflex erections usually respond to oral erectogenic agents or penile injection of vasoactive agents. The greater problem that leads to infertility in men with SCI is absence of ejaculation, which affects most men with spinal lesions [16].

A frequent component of the treatment plan for testicular cancer is a retroperitoneal lymph node dissection (RPLND). Although this treatment has contributed to the current excellent survival from this previously lethal disease, we currently have good fortune of treating infertility in the survivors. The surgical field in the classical operation removes the postganglionic sympathetic nerves exiting the sympathetic chains and the hypogastric plexus, effectively removing the efferent stimulation for seminal emission and bladder neck closure [17].

Nerve-sparing RPLND procedures have been devised to preserve antegrade ejaculation [18]. Crucial areas to preserve during such procedures include the hypogastric plexus anterior to the aorta below the inferior mesenteric artery and the postganglionic sympathetic nerves from T10-L2. Templates designed to unilaterally preserve at least one side are successful, but when

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**Box 1. General classification of ejaculatory dysfunction**

- Premature ejaculation
- Neurogenic anejaculation
- Retrograde ejaculation
- Iatrogenic causes
- Idiopathic anejaculation/anorgasmia
- Aperistalsis of the vas deferens

---

**Fig. 1.** Representation of ejaculation events. (A) Sexual stimulation and contraction of external sphincter. (B) Ongoing external sphincter contraction with simultaneous rise in internal sphincter pressure. (C) Ejaculation occurs at peak internal sphincter contraction and relative relaxation of external sphincter. (D) Rhythmic contractions of the external sphincter produce projectile ejaculation. (E) Resolution. (*Data from Sonksen J, Ohl DA, Wedemeyer G. Sphincteric effects during penile vibratory ejaculation and electroejaculation in men with spinal cord injuries. J Urol 2001;165(2):426–9.*)
combined with meticulous dissection of bilateral sympathetic nerves, the preservation rate of antegrade ejaculation approaches 100%. Some situations still exist in which surgery results in ejaculatory dysfunction. Large tumor burden or RPLND surgeries performed after chemotherapy give high risk for ejaculatory dysfunction. The risk of surgical sympathectomy is not limited to RPLND. Any surgery that is performed in the periaortic or pelvic regions can effect ejaculation, including aortic aneurysm surgery and aortic bypass surgeries [19]. Any retroperitoneal lymph node samplings or trauma surgeries also can result in problems.

Patients with post-RPLND ejaculatory dysfunction present with a normal feeling of orgasm and ejaculation. This presentation leads many clinicians to refer to post-RPLND ejaculatory dysfunction as “retrograde ejaculation,” although this is not the case. A study by Kedia and colleagues [20] suggested that if a dry orgasm is noted after RPLND, there is a high likelihood that this symptom represents total absence of ejaculation and not simply a retrograde ejaculation situation. These patients experience a failure of emission (see previous discussion) and thus experience a “dry ejaculate.”

Although it is commonly recognized that men with diabetes mellitus are at risk for complications of retinopathy, vasculopathy, and neuropathy, the effect of diabetes on sexual function is less well known. Erectile dysfunction is the most common sexual issue related to diabetes, but ejaculatory problems are also common [21]. Control of blood sugar (or lack thereof) is directly related to the risk of complications [22]. Men with ejaculation changes caused by diabetes exhibit a slowly progressive decline in ejaculatory function [23]. Typically, the first symptom is a decrease in the amount of ejaculate, which progresses to retrograde ejaculation, with postclimax urine cloudiness, to loss of the cloudiness, which is consistent with loss of emission.

Congenital spinal anomalies, such as spina bifida, also can impair ejaculation. Occasionally men may present with lifelong or acquired anejaculation and can be found to have an occult dysplasia of the lower spinal cord, possibly with a tethered cord syndrome. Men with lifelong problems may have poorer sperm quality than men with acquired SCI. Other neurologic conditions that affect spinal cord function or its sympathetic outflow can result in ejaculatory dysfunction; examples include multiple sclerosis, transverse myelitis, and vascular spine injuries. These disorders resemble the SCI group in their dysfunction.

**Retrograde ejaculation**

In retrograde ejaculation, all the components of the ejaculatory reflex are present, except for bladder neck closure. As discussed earlier, bladder neck closure occurs because of high pressure. If this closure cannot occur because of anatomic reasons (eg, after prostate resection) or physiologic reasons (eg, diabetes mellitus), the result is retrograde ejaculation. In the absence of adequate internal sphincter contraction, the emitted semen takes the path of least resistance and flows backward into the bladder. The patient might notice that the postorgasm urine is cloudy because of the presence of semen.

Because seminal emission and bladder neck closure are both controlled by alpha-adrenergic neurons, all of the causes listed for neurogenic anejaculation may cause retrograde ejaculation instead of absent emission. It is a matter of the degree of the neural dysfunction. Some other causes warrant mention, however, because they more commonly cause retrograde ejaculation, with little or no diminution of the amount of emission.

**Iatrogenic causes of ejaculation dysfunction**

Although not specifically neurogenic, medications can prevent climax and therefore ejaculation. Selective serotonin reuptake inhibitors and tricyclic antidepressants are the most common. Because these drugs are used to treat PE, administration to a normal individual can result in dysfunction. When all types of antidepressants are considered, the rate of male sexual dysfunction with antidepressants is as high as 62.4% [24]. Certain medications usually do not limit the ability to achieve climax and seminal emission but can specifically impair bladder neck contraction. The most common class is the alpha-adrenergic antagonists. These drugs may be either nonselective, such as terazosin, which is given for systemic hypertension, or genitourinary selective, such as tamsulosin or alfuzosin, which are for prostatic obstruction. Usually there is some diminution in seminal emission, but most of these individuals who have dysfunction from alpha blockers have retrograde ejaculation [25].

Anatomic opening of the bladder neck is usually postsurgical. In the past, Y-V plasty of the bladder neck was a common treatment for
Idiopathic anejaculation/anorgasmia

The word “idiopathic” is used frequently when we cannot determine the accurate physiologic cause of a problem, but it is also used frequently when describing conditions that are functional. With idiopathic anejaculation, the latter use of the word seems appropriate. Although there may be some undiagnosed neuropathic/physical problem in these patients, most practitioners in the field believe that idiopathic anejaculation/anorgasmia is a psychogenic problem [28].

The characteristics of the condition support this conclusion. There are no demonstrable neurologic derangements. Several reports link inability to achieve orgasm in otherwise healthy individuals to other psychogenic issues, such as anxiety. Men with this condition tend to have a history of religiously strict upbringing [29]. The history of these men is often revealing. Some are able to climax and ejaculate with masturbation but not with intercourse. More often, however, there has never been a climax while awake. Notably, most men with this condition have intermittent nocturnal emissions [30], and some awaken during the event. The fact that ejaculation occurs during sleep suggests that this condition is psychogenic, much the same as nocturnal erectile function supports a psychogenic source of erectile dysfunction while awake.

Recently, Perelman described a subset of men with acquired inability to climax during sex that is related to frequent masturbation or use of idiosyncratic masturbation techniques. He believes that these men are conditioned to respond to the masturbatory technique and not to the sensation of vaginal intercourse. Changing the stimulation technique and decreasing the frequency of masturbation have been successful in reversing the problem [31]. It is reasonable to treat men with idiopathic anejaculation with sex therapy techniques. Success rates other than the subset reported by Perelman, however, are poor. Men with refractory inability to ejaculate, despite a course of sex therapy, may be candidates for ejaculation induction procedures [29,30,32,33].

For men interested in fertility, electroejaculation (EEJ) or surgical sperm retrieval techniques can be used.

Evaluation of ejaculatory dysfunction

The medical history is the most important part of the evaluation process, including the length of time of ejaculatory dysfunction and whether any inciting events caused the problem (ie, medical or psychosocial issues). The physician often needs to help the patient define the problem, because many patients cannot differentiate erectile and ejaculatory dysfunction. Many men who have lifelong problems have difficulty communicating the nature of their dysfunction. Some men believe they are ejaculating when “pre-ejaculate” is ejected from the urethra. Describing actual symptoms and sensations may be more helpful than using terminology.

Past medical and surgical history should define presence or absence of risk factors for ejaculatory problems. The patient’s list of medications should be surveyed for antidepressants and alpha blockers. The review of systems and urinalysis occasionally can uncover a previously undiagnosed underlying condition, such as diabetes. Physical examination should verify normal size and consistency of the testicles and determine the presence or absence of the vas deferens. Blood testing for follicle-stimulating hormone and testosterone can be helpful, in conjunction with the testis examination, in determining if sperm production is likely to be present. The position of the urethral meatus should be noted to ensure that infertility is not caused by poor delivery from hypospadias.

If there is any antegrade semen output, a semen analysis is performed. If there is low-volume azoospermia and the consistency is mucoid, this fluid may represent bulbourethral gland secretions only and should be correlated to a detailed patient description of the symptoms. If no ejaculate is produced or if the ejaculate is of low volume (< 1.5 mL) despite a normal orgasm, a postorgasm urine sample should be checked. The bladder should be emptied before climax and then emptied again as soon as possible afterward for analysis. If retrograde ejaculation is confirmed, the bladder can be catheterized before climax to instill media (such as modified human tubal factor medium with human serum albumin, both from Irvine Scientific) and after to retrieve a complete specimen for insemination purposes. For screening...
purposes, however, catheterization is probably unnecessary.

Management of ejaculatory dysfunction-related infertility

Medications

Alpha blockers and antidepressants should be discontinued, if possible, to see if resolution of the ejaculatory dysfunction results. Just as alpha blockade can cause retrograde ejaculation or anemission, administration of sympathomimetic agents may be of value in certain clinical situations [34,35]. One goal of these agents is to convert retrograde ejaculators into antegrade ejaculators by assisting the contraction of the smooth muscle. This approach is most likely to work in individuals with a slowly progressive dysfunction, such as in diabetic neuropathy. Administration of drugs may “reverse” the course for a finite period of time, but most people eventually progress to the point at which medication is not helpful [36]. If it is effective in producing an antegrade ejaculate, the drugs might be taken cyclically for a week before ovulation and then discontinued for the remainder of the cycle. Another goal is to convert persons with anemission to antegrade ejaculators or at least to produce a retrograde specimen. This approach is less likely to be effective because of the more extreme dysfunction. If a person is successful in producing a retrograde ejaculate, artificial insemination may be possible (see later discussion).

Adverse effects of adrenergic agonists include dryness of the nose and mouth and hypertension. Because persons who have diabetic neuropathy are at risk for concomitant cardiovascular disease, these drugs need to be administered with caution (Box 2).

Artificial insemination for retrograde ejaculation

In men who have persistent retrograde ejaculation despite medical therapy, sperm retrieval from the bladder and intrauterine insemination is possible. One has to consider that the urine contact is toxic to the sperm because of acidity and osmolality [37], and preparation of the bladder environment needs to be considered. The authors recommend administration of sodium bicarbonate, 500 mg (one-half teaspoon baking soda), given 12 and 2 hours before the sperm retrieval to counteract the acidity. Some clinicians have recommended fluid loading to decrease osmolality. There is no consensus agreement on these issues, but the urine pH can be checked at the time of retrieval to guide the future dosing and use of bicarbonate in individual patients.

To maximize the yield of motile sperm, catheterization before ejaculation, with bladder emptying and instillation of buffered sperm-friendly media into the bladder, can be performed [38]. The retrograde specimen can then be retrieved with postejaculation catheterization and media rinse. The specimen should be washed promptly in the laboratory and prepared by standard techniques for intrauterine insemination.

Penile vibratory stimulation

A vibrator placed on the penis can create enough stimulus to produce an antegrade ejaculation in some men with anejaculatory infertility. The best candidates for this procedure are men with SCI who have all parts of the ejaculatory reflex system in place. Because components of the reflex arc are essential for normal ejaculation, we can predict, to some extent, which men with SCI will respond [39–41]. Essential components are sacral efferents and afferents, thoracolumbar sympathetic outflow, and communication between the sacral and thoracolumbar segments. The best candidates for penile vibratory stimulation (PVS) should be men who have SCI and complete upper motor neuron lesions above T10. This prediction is corroborated by clinical studies [42–44].

Vibratory stimulation depends on a basic reflex response to penile vibration. Cortical inhibition of
this reflex can prevent ejaculation. Men with incomplete spinal injuries and men with other anejaculatory infertility causes generally respond poorly to PVS because of cortical inhibition. The vibration parameters are key in maximizing ejaculation rates with PVS. In a landmark paper, Sønksen determined that a vibration amplitude of 2.5 at 100 Hz was optimal for inducing vibratory ejaculation in men with SCI [45]. The only commercially available vibrator approved by the US Food and Drug Administration, the FertiCare, uses these vibration settings (Fig. 2).

The vibrator is placed on the penile frenulum until ejaculation occurs (Fig. 3). The vibration is discontinued at the time of ejaculation to limit the possibility of autonomic dysreflexia. In men prone to autonomic dysreflexia, sublingual nifedipine, 10 to 20 mg, 10 minutes before vibration can blunt the blood pressure rise [46]. Monitoring of blood pressure during the procedure is essential. Tonic muscular contraction immediately precedes ejaculation, followed by several spasms and a fairly normal ejaculatory reflex that results in projectile ejaculation. If no ejaculation occurs within 3 minutes, a rest period of 1 minute is allowed, followed by further stimulation cycles. If ejaculation does occur, multiple ejaculations are possible in a single session. There is typically little, if any, retrograde fraction, because all the components of a normal reflex are present, including bladder neck closure.

Adverse events associated with PVS include autonomic dysreflexia and penile skin changes. The procedure is safe in the absence of autonomic dysreflexia and, with selected patients, can be performed by the patient in the home setting. Specimens obtained by couples at home can be used for simple vaginal insemination if the sperm quality is adequate.

**Electroejaculation**

Rectal probe EEJ is offered to men with SCI who have failed a trial of PVS. This procedure is uniformly successful in obtaining a semen specimen. **Box 3** shows causes of anejaculation that have been treated with EEJ. The only US Food and Drug Administration–approved device for EEJ, the Seager Electroejaculator (Dalzell Medical Systems, The Plains, VA), is shown in Figs. 4 and 5.

Before EEJ, sublingual nifedipine, 10 to 30 mg, is given to men who are prone to autonomic dysreflexia [46]. The bladder is catheterized before stimulation to empty the bladder and instill “sperm-friendly” buffered medium. Rectoscopy is performed before and after the stimulation to rule out pre-existing lesions or postprocedural complications. The rectal probe is inserted in the lateral decubitus or dorsal lithotomy position, and stimulation is delivered in a wave-like pattern. The antegrade ejaculate is captured during stimulation, and the retrograde fraction is collected via postprocedural catheterization. A plastic catheter and nonspermicidal lubricant are used for the catheterization.

**Box 3. Causes of anejaculation in which electroejaculation has resulted in seminal emission**

- Spinal cord injury
- Retroperitoneal lymphadenectomy
- Diabetic neuropathy
- Multiple sclerosis
- Postrectal surgery
- Spina bifida
- Senile anejaculation
- Psychogenic anejaculation
- Situational anejaculation
- Pediatric cancer patients

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**Fig. 2.** FertiCare personal vibratory (Multicept Corporation, Copenhagen, Denmark).

**Fig. 3.** Position of the penile vibrator on the frenulum during a PVS procedure.
The stimulation pattern is as follows: initial stimulation is at 5 V, with the current delivered for 5 seconds, followed by abrupt discontinuation of all current. After each stimulation, a waiting period of approximately 20 seconds ensues, during which ejaculation occurs. The ejaculate may drip out or actual rhythmic periurethral muscle contraction may occur, with a more projectile pattern. After the contractions stop, the next stimulation proceeds. The voltage is increased by 2.5 to 5 V per stimulation, to a peak of 20 to 30 V. Discontinuation of current and the waiting period follows each 5-second stimulation. The pattern described is the result of physiologic studies performed on sphincter activity during EEJ [5,47].

Surgical sperm retrieval

Men who experience anejaculation can have sperm retrieved by percutaneous or surgical methods. These methods are covered elsewhere in this issue.

Choice of sperm retrieval technique

PVS should be first-line therapy for men with SCI who are seeking fertility treatment. This recommendation is based on greater patient acceptance [48] and better semen quality than alternative procedures, such as EEJ [48,49]. For men with SCI who fail PVS trials, EEJ can be offered. Although EEJ has been used in many clinical situations in which anejaculation is present, it may not be the best choice for all patients. A cost-benefit analysis, published in 2001 [50], concluded that EEJ coupled with intrauterine insemination is cost effective in men in whom the procedures can be performed without anesthesia. This means that men with complete spinal cord lesions are candidates for the procedure. If anesthesia is necessary to perform EEJ (ie, for men who do not have SCI and men with incomplete spinal cord lesions), however, then intrauterine insemination was not cost effective and patients should proceed directly to in vitro fertilization. In the latter group, it makes more sense to perform local anesthetic surgical sperm retrieval and in vitro fertilization with intracytoplasmic sperm injection (ICSI). Although the cost analysis seems clear, a recent survey suggested that many centers are not following these recommendations [51].

Summary

The physiologic process of ejaculation is a complex occurrence that represents coordinated anatomic, neurologic, and psychologic events. Men who suffer from the uncommon problems of anejaculation and retrograde ejaculation can be offered various medical treatments to assist with their goal of fathering children. In the specific scenarios that involve anejaculatory men with SCI who wish to father children, the penile vibratory procedure can be offered confidently to many of these men. Although advanced assisted reproductive techniques may be necessary, the options of EEJ and PVS combined with artificial insemination have the potential to avoid more extensive and costly assisted reproductive options. In the absence of being able to correct ejaculatory dysfunction or elicit an ejaculate, sperm retrieval techniques may be used to treat couples who are infertile as a result of these abnormalities.
References


Ejaculatory Duct Obstruction
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First described by Farley and Barnes [1] in 1973, ejaculatory duct obstruction (EDO) underlies 1% to 5% of male infertility [2]. Although the diagnosis of EDO can be complex, treatment is well established and can be very effective [3,4]. In the past decade, transrectal ultrasound (TRUS) has replaced vasography as the mainstay of diagnosis. Although originally described in azoospermic men harboring complete blockage, it now is clear that EDO may present in several ways, including azoospermia and oligoasthenospermia. Excellent information provided by TRUS has improved the ability of the urologist to diagnose EDO.

Because a significant proportion of men who undergo treatment for EDO do not respond, the limitations of a TRUS-based diagnosis also have become apparent. This experience has led to the development of further diagnostic procedures to confirm EDO in select patients. Several adjunctive techniques now have been described, including seminal vesicle aspiration, seminal vesicle chromotubation, and seminal vesiculography. This article reviews these diagnostic techniques and their limitations and proposes an evidence-based, algorithmic approach to the diagnosis and treatment of this underdetected clinical condition.

Ejaculatory duct anatomy and physiology

Understanding the anatomy and physiology of the ejaculatory ducts is important for the diagnosis and management of EDO. Anatomically, the ejaculatory ducts are paired, collagenous, tubular structures that commence at the junction of the vas deferens and seminal vesicle, course through the prostate, and open into the prostatic urethra at the verumontanum (Fig. 1). The duct has three regions: a proximal, extraprostatic portion, a middle intraprostatic segment, and a short distal segment within the verumontanum near the urethra (see Fig. 1) [5]. Contrary to popular belief, there is no valvelike, muscular “sphincter” at the ejaculatory duct orifice, because the duct sheds its muscular layer in the middle, intraprostatic segment [5]. Instead, ejaculatory continence is maintained and urinary reflux prevented by the acute angle of duct insertion into the prostatic urethra.

The physiology of the ejaculatory duct complex also is well understood. Animal model studies indicate that a relationship exists between the seminal vesicle and ejaculatory duct that is quite similar to that of the bladder and urethra [6]. Specifically, the compliance and contractile properties of the smooth, muscle-lined, viscous organ that is the seminal vesicle are very similar to those described for the bladder (Fig. 2) [6]. Just as bladder outlet obstruction can result from prostatic or other blockage, so too can physical blockage of the ejaculatory ducts cause EDO. By the same reasoning, “functional” or neurologic dysfunction of the seminal vesicle probably exists that is similar to voiding dysfunction caused by bladder myopathy. Like the neurogenic bladder, this pathologic state could result in functional EDO [3,7].

Given the physiologic similarity between the organs of the ejaculatory duct complex and those of the urinary tract, several implications become...
apparent. “Static” anatomic imaging with TRUS or MRI may be unable to distinguish between functional and physical EDO. Indeed, it has been demonstrated that TRUS alone leads to a false-positive diagnosis of EDO, defined by lack of clinical benefit, in 50% of clinical cases [8] Thus, although it has simplified the diagnosis, TRUS is sensitive but not specific for EDO and may be even less informative in partial or functional forms of the disease. The clinical implications are (1) the evaluation of EDO should include a review of the use of medications and medical conditions that might predispose the patient to seminal vesicle dysfunction, and (2) “static” anatomic imaging such as TRUS may not be sufficient to distinguish among all the forms of EDO that can exist.

Definition of ejaculatory duct obstruction

Based on the current understanding of the anatomy and physiology, it is clear that EDO takes several forms (Table 1). Low-volume azoospermia defines complete or classic EDO and represents the physical blockage of both ejaculatory ducts. Unilateral complete or bilateral partial physical obstruction results in incomplete or “partial” EDO. Both these types of EDO are associated with one or more symptoms of low ejaculate volume, postejaculatory pain, or hematospermia. Partial EDO is uniquely associated with oligoasthenospermia. Currently, the diagnosis of functional EDO is one of exclusion and is made when anatomic evidence of obstruction is ruled out.

Diagnosis of ejaculatory duct obstruction

The causes of EDO are divided into congenital and acquired disorders (Fig. 3). It can result from seminal vesicle calculi, Müllerian duct (utricular) or Wolffian duct (diverticular) cysts, postsurgical or postinflammatory scar tissue, calcification near the verumontanum, or congenital atresia of the ducts [9]. The issue of prostatic cysts as a cause of EDO is interesting, because they are found in the prostate relatively commonly, but most are unlikely to cause obstruction. The incidence of prostatic cysts involving the ejaculatory ducts in infertile men has been reported to be as high as 17%, compared with a screening population in which the prevalence was 5% [10]. In cases of congenital blockage, the urologist should consider a genetic evaluation for cystic fibrosis gene mutations, because men who have idiopathic obstruction have a high prevalence (50%) of cystic fibrosis gene mutations [11].
Clinical presentation

Clinically, EDO classically presents as hematospermia, painful ejaculation [12], or infertility (see Fig. 3). Persistent or recurrent hematospermia indicates the need for TRUS, which may delineate abnormalities, including dilated seminal vesicles, ejaculatory duct cysts, calculi, absence of the vas, and intraprostatic Müllerian duct remnants [13]. Evaluation of hematospermia by TRUS reveals a malignant source in fewer than 4% of cases, however [14]. A prior urinary tract infection, epididymitis, perineal trauma, orchalgia, and perineal pain also may indicate the possibility of EDO. It is important to discontinue medications that may impair ejaculation (Box 1). Although unusual, a digital rectal examination demonstrating enlarged, palpable seminal vesicles may suggest the diagnosis of EDO. Although no pathognomonic findings exist on seminal evaluation for EDO, azoospermia in a centrifuged semen sample, associated with a low ejaculate volume (<2.0 mL), a pH below 7.2, and absence of fructose in the seminal fluid suggest EDO (see Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Incomplete or partial obstruction</th>
<th>Complete obstruction</th>
<th>Functional obstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejaculate volume</td>
<td>Low or low-normal</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Sperm count</td>
<td>Low</td>
<td>Absent</td>
<td>Absent or low</td>
</tr>
<tr>
<td>Sperm motility</td>
<td>Low</td>
<td>Absent</td>
<td>Absent or low</td>
</tr>
<tr>
<td>Ejaculate fructose</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent or low</td>
</tr>
</tbody>
</table>

Table 1
Classification of ejaculatory duct obstruction by semen analysis parameters

Fig. 3. Algorithm for diagnosis and management of EDO. NL, normal; TRUS, transrectal ultrasound; TURED, transurethral resection of the ejaculatory ducts. (From Walsh TJ, Turek PJ. Ejaculatory duct obstruction: New approaches to diagnosis and treatment. Contemporary Urology 2006; September. Copyright © 2006, Advanstar Communications Inc. All rights reserved.)
The finding of low ejaculate volume in association with impaired sperm concentration and motility and a positive clinical history also may warrant further evaluation for EDO (partial or functional). Part of the reason that this condition probably is underdiagnosed is because of its rarity, subtle presentation, and the concomitantly low index of suspicion held by clinicians.

**Evaluation**

The essential step in the evaluation of EDO is TRUS (see Fig. 3). In general, cystoscopy is uninformative. Seminal vesicle width greater than 1.5 cm or ejaculatory duct diameter greater than 2.3 mm on TRUS, particularly when combined with a cyst or calcification along the duct, helps confirm the diagnosis [3]. In suspicious cases with negative TRUS findings, T2-weighted pelvic MRI with an endorectal coil can provide excellent resolution and detect small obstructing cysts [14,15].

Because of the relatively invasive nature of the procedure, formal vasography is not considered a first-line diagnostic procedure unless it is obtained in the operating room as part of the comprehensive evaluation and treatment of obstructive azoospermia. In all men suspected of having EDO, a hormonal evaluation of testosterone and follicle-stimulating hormone levels should be normal. In cases of azoospermia associated with low ejaculate volume, TRUS evaluation can precede a testis biopsy that confirms spermatogenesis.

The presentation of partial or functional EDO, in contrast with complete or classic EDO, may be asymptomatic infertility with reduced ejaculate volume (see Table 1). Oligoasthenospermia, defined as a sperm concentration of less than 20 million/mL and motility less than 30%, usually is present. Fructose usually is present [8,16,17]. Although TRUS findings for partial EDO may be similar to those for classic EDO, asymmetry in seminal vesicle or ejaculatory duct size may suggest unilateral rather than bilateral obstruction.

Not all patients who have EDO have dilated seminal vesicles, and not all patients who have dilated seminal vesicles have EDO. Basing choice of treatment solely on TRUS findings leads to unnecessary surgery in as many as half of cases [8]. Recent refinements in diagnostic techniques have improved the specificity of the diagnosis, however (see Fig. 3). Using seminal vesicle aspiration, the finding of more than three sperm/high-powered field in the aspirate is considered positive and suggestive of obstruction [18]. For best results, the aspiration procedure should be performed within 24 hours of ejaculation [19]. This adjunctive technique confirms ongoing spermatogenesis and can suggest epididymal obstruction (if sperm production is normal but there is no sperm in the seminal vesicle), but it does not localize the site of blockage.

Seminovesiculography can provide excellent anatomic detail similar to formal scrotal vasography but relies on the transrectal injection of non-ionic contrast (50% renograffin) under TRUS guidance followed by a pelvic plain film or fluoroscopy. In 50% of cases, it provides a retrograde vasogram on the injected side as well. The newest adjunctive technique, seminal vesicle chromotubation, provides visual evidence of EDO similar to vasography after direct injection of diluted indigo carmine or methylene blue (1:5 dilution with saline) into the seminal vesicle with TRUS guidance (Fig. 4). When performed with cystoscopy, it assesses the patency of the ejaculatory ducts to the prostatic urethra.

Given that TRUS and several other techniques now are available to diagnose EDO, which technique is preferred? This issue was addressed in a prospective study of all three adjunctive techniques in a series of patients who had EDO [8]. As outlined in Table 2, patency with chromotubation was deemed the most accurate way to diagnose complete or incomplete ejaculatory duct obstruction [8]. At this time, however, no reference standard measure of EDO is available for comparison.

Differentiating between physical and functional obstruction in patients who present with EDO is important, because patients in whom no physical obstruction is present (ie, those who have functional obstruction) will not respond to

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**Box 1. Medications associated with impaired ejaculation**

**Antihypertensive agents**
- Adrenergic blockers (prazosin, phentolamine)
- Thiazides

**Antipsychotic agents**
- Thioridazine (Mellaril)
- Haloperidol (Haldol)

**Antidepressants**
- Imipramine
- Amitriptyline
also referred to as “seminal vesicle dysfunction,” functional obstruction can occur in patients taking the medications listed in Box 1 and in patients who have diabetes mellitus, multiple sclerosis, or spinal cord injury. In contrast, patients who have confirmed physical obstruction of the ejaculatory ducts are optimal candidates for surgical therapy to unblock the ejaculatory ducts.

**Treatment of ejaculatory duct obstruction**

Indications for treatment of EDO include coital discomfort or dyspareunia, recurrent hematospermia, and infertility. The discontinuation of the medications listed in Box 1 may improve states of ejaculatory dysfunction. The time-tested treatment for EDO, however, is transurethral resection of the ejaculatory ducts (TURED), which can be performed in an outpatient setting under general or regional anesthesia [9]. The technique combines cystourethroscopy with resection (using a 24-Fr electrocautery loop) of the verumontanum in the midline (for complete obstruction) or laterally (for unilateral obstruction) (Fig. 5). Incisions with cutting current in the peri-verumontanum area also can be performed with similar results. The role of unilateral resection (hemi-TURED) is defined by adjunctive diagnostic testing and can spare resection of most of the verumontanum in cases of unilateral partial EDO (Fig. 6). Several passes of the electrocautery loop often are required to unobstruct the ejaculatory ducts. At the correct level of resection, cloudy, milky fluid usually is seen refluxing from the opened ducts. Pure cutting current should be used to minimize cauterization of the ductal system. Hemostasis should be performed meticulously to avoid fulguration of the ejaculatory duct orifices. Although injuries to the rectum or rhabdosphincter are extremely uncommon, constant vigilance with the resectoscope and electrocautery loop helps minimize these complications. Postoperatively, a small Foley catheter is placed for 24 hours and is removed on an outpatient basis. After treatment for infertility, the patient may resume intercourse after 5 days. He should provide semen for a formal analysis as early as 2 weeks and then at regular intervals thereafter until semen quality stabilizes.

Success is more likely when the milky ejaculatory duct fluid that is observed after resection contains sperm. After aspiration of this fluid, bright-field microscopy confirms the presence of sperm. Intraoperatively, TRUS assists with localization of the obstruction and confirmation of the depth of resection. TRUS can guide the transrectal instillation of methylene blue or indigo carmine

Table 2

<table>
<thead>
<tr>
<th>Technique</th>
<th>Seminal vesicle aspiration</th>
<th>Chromotubation</th>
<th>Vesiculography</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Obstructed</td>
<td>No obstruction</td>
<td>Obstructed</td>
</tr>
<tr>
<td>Transrectal ultrasound</td>
<td>12/25 (48%)</td>
<td>13/25 (52%)</td>
<td>9/25 (36%)</td>
</tr>
<tr>
<td>obstructed (n = 25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminal vesicle aspiration</td>
<td>—</td>
<td>—</td>
<td>6/12 (50%)</td>
</tr>
<tr>
<td>obstructed (n = 12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromotubation</td>
<td>6/9 (67%)</td>
<td>3/9 (33%)</td>
<td>—</td>
</tr>
<tr>
<td>obstructed (n = 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vesiculography</td>
<td>8/13 (62%)</td>
<td>5/13 (38%)</td>
<td>9/13 (69%)</td>
</tr>
<tr>
<td>obstructed (n = 13)</td>
<td></td>
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</tbody>
</table>

into the seminal vesicles to guide resection by colorimetric response. Visualization of the dye after resection ensures that the obstruction is relieved. Typically, TURED can treat intraprostatic lesions within 1 to 1.5 cm of the verumontanum successfully. Lesions further from the urethra may not be amenable to resection or, if successfully resected, are more likely to scar and obstruct, probably because of the lack of mucosal lining.

Outcomes

Several large retrospective series in which patients were treated for infertility provide convincing evidence that at least a 20% to 30% pregnancy rate is achieved after TURED [8,16,17,20–22]. In one series, men treated for complete or partial EDO were equally likely (65% to 70%) to show improvements in semen quality after TURED [4]. In contrast, in another series, 16 patients who had partial EDO responded better by semen analysis to TURED (94% improved) than did men who had complete EDO (59% response) [17]. Partial and complete obstruction caused by congenital or acquired cysts responds better to TURED than does obstruction caused by calcification [17]. Long-term relief of postcoital and perineal pain after TURED can be expected in 60% of patients [1,8]. Although hematospermia has been treated effectively with TURED, this literature remains anecdotal [23,24].

Complications

Complications from TURED surgery occur in 10% to 20% of patients and include watery ejaculate, hematuria, epididymitis, seminal vasculitis, and a low risk of incontinence or rectal perforation [4,17]. Self-limited hematospermia and hematuria not requiring recatheterization are relatively common after TURED. Epididymitis and "watery" ejaculate occur much less frequently but typically are a cause of greater concern. High-volume watery ejaculate may be caused by the reflux of urine through the ejaculatory ducts into the seminal vesicles or into unroofed cysts, because in this circumstance the ejaculate often contains creatinine [25].

The patient should understand several potential outcomes of TURED surgery before...
undergoing the procedure. Roughly 10% to 15% of men treated by TURED for low-volume azoospermia convert to normal-volume azoospermia. This condition may be caused by secondary obstruction at the level of the epididymis requiring epididymovasostomy. Epididymal obstruction may reflect the effects of time and blockage on other portions of the delicate male ductal system. Notably, 4% of patients treated for partial EDO may become azoospermic after TURED, presumably from scar formation [4]. It may be prudent to advise preoperative sperm cryopreservation if TURED is planned for this indication.

Summary

Based on the current understanding of ejaculatory duct anatomy and physiology, EDO can take several forms, including conditions that represent functional and not physical obstruction. In addition, although it is a valuable diagnostic technique, TRUS is sensitive but not specific for the diagnosis. Sperm aspiration, seminal vesiculography, and chromotubation can increase the specificity of the diagnosis and further refine the surgical approach to treatment.

References

Restructuring Reconstructive Techniques—Advances in Reconstructive Techniques

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Microsurgical reconstruction to correct male infertility, although usually performed for vasectomy reversal, also is performed to correct other types of iatrogenic, congenital, and postinflammatory obstruction. An estimated 500,000 to 750,000 vasectomies are performed annually in the United States, and approximately 2% to 6% of men who undergo a vasectomy request a reversal [1]. In an effort to improve success rates and facilitate performance of these complex microsurgical procedures, modifications are continually suggested. This article reviews some of these proposed modifications. The modifications can be divided into five general categories: (1) use of biomaterials/sealants, (2) laser soldering, (3) use of absorbable and nonabsorbable stents, (4) new intussusception vasoepididymostomy (VE) anastomotic techniques, and (5) use of robotics.

Biomaterials/sealants

Fibrin glue (FG) has been used in experimental models and in one clinical study in urologic microsurgery. Fibrin sealant, as used in vaso-vasostomy (VV) and VE, stimulates the coagulation cascade and produces a fibrin seal around the anastomosis. Fibrinogen, when mixed with thrombin and calcium, is converted to fibrin monomer. The fibrin monomer is converted to a stable cross-linked fibrin polymer [2]. The rationale for using sealants such as FG is to decrease operative time and to simplify the procedure without compromising success rates.

Several investigators have used FG for VV in animals and reported patency rates of 90% or greater. In some instances the patency rate was similar to standard microsurgical anastomoses. Silverstein and Mellinger [3] described a FG-assisted anastomotic technique using only two transmural sutures and FG. The operative time was shorter, and the patency rate for the FG-assisted anastomosis (90%) was comparable to the formal two-layer technique (83%). It should be noted, however, that the patency rate of 83% for microsurgical VV is lower than most reported series. Vankemmel and colleagues [4] described a FG VV with three transmural sutures and reported a patency rate of 92%, similar to the 85% patency rate for the animals in which conventional modified one-layer anastomoses were used.

The major concern about the use of FG in clinical practice is the potential contact of the glue with the vasal lumen and resultant obstruction. Also, because FG is derived from pooled plasma, there has been concern about transmission of viral disease [5]. Niederberger and colleagues [6] demonstrated that FG could be prepared from a single human source and that a vasovasal anastomosis with FG could be performed with patency comparable to a standard microsurgical anastomosis in a rabbit model.

Schiff and colleagues [5] tested a biomaterial sealant and a biomaterial wrap (either derived from amniotic membranes or an acellular dermal matrix) for VV in a rat model. VV was performed using one of five different techniques: (1) standard multilayer VV; (2) the control technique using three full-thickness sutures; (3) three full-thickness sutures plus biomaterial wrap; (4) three full-thickness sutures plus biomaterial wrap and copolymer...
sealant; and (5) three full-thickness sutures plus copolymer sealant. Operative times were shorter for methods 2 through 5, and patency for group 3 was comparable to that of multilayer VV. The group hypothesized that the results in the sealant groups were inferior because an air-assisted delivery system may have forced sealant into the lumen and caused obstruction.

Ho and colleagues [2] performed the first study with FG for VV in humans using three transmural 9-0 sutures. They reported an 85% patency rate in patients with sufficient follow-up and a 96% patency rate when sperm was present in the vesic fluid. The definition of patency was not clearly stated, but the median sperm concentration at 9 and 12 months was greater than 50 million/mL. The mean follow-up was 6.2 months, the mean obstructive interval was 7.9 years, and the mean operative time was 79 minutes. VE was not performed in this study, and, as expected, patency declined with increasing obstructive interval. The pregnancy rate was 23% (9 of 39) with a mean follow-up of 6.2 months. Because the follow-up was short, the frequency of secondary azoospermia is not known. This technique could represent a viable alternative for the surgeon who performs only an occasional vasectomy reversal.

The use of FG also has been investigated for VE. Shekarriz and colleagues [7] described a new VE technique using FG and compared it with conventional end-to-side VE in a rat model. The conventional end-to-side two-layer VE was performed with 11-0 and 10-0 nylon sutures, and the FG-assisted anastomosis used an invagination technique, using two 11-0 nylon sutures for the inner layer and 10-0 nylon for the outer layer. Patency was 79% in the FG-assisted VE group and 63% in the conventional end-to-side VE group (P = .29). Operative time was shorter in the FG-assisted group than in the standard end-to-side group (15.3 ± 1.3 versus 33.2 ± 4.2 minutes, respectively; P < .001).

Although FG studies are encouraging, this technique has not been adopted for clinical practice.

Laser soldering

Like sealants, lasers have been used in microsurgery in animal models to improve tissue bonding and limit anastomotic leakage. Seaman and colleagues [8] compared a diode laser–assisted anastomosis with a conventional sutured microsurgical anastomosis for VV and VE in rats. Laser soldering (ie, laser tissue welding using a protein solder) was used for the experimental group. In this technique, the laser was used to activate a protein solder composed of albumin, sodium hyaluronate, and indocyanine green dye. Operative times were shorter for the laser-assisted group, and the patency rates were similar for both groups. Laser-assisted VV and conventional VV patency rates were 90% and 80%, respectively. Laser-assisted VE and conventional VE patency rates were 82% and 73%, respectively.

Mingin and Ditrolio [9] described laser-assisted VV using an argon laser and an albumin protein solder in five men, two of whom were undergoing repeat VV after a failed microsurgical VV. The investigators placed two intraluminal 9-0 nylon sutures followed by laser welding of a 10% albumin solution around the anastomotic site. Two 5-0 polyglactin sutures were placed in the perivasal tissues. All five men experienced patency with sperm concentrations of at least 17.5 M/mL, and three of the five established a pregnancy. Total operative times ranged from 37 to 60 minutes.

Shanberg and colleagues [10] reported their results with carbon dioxide laser–assisted VV in 32 patients. For obstructive intervals shorter than 10 years, the patency rate was 95%, but the pregnancy rate was only 35%. For obstructive intervals longer than 10 years, the patency and pregnancy rates were 36% and 9%, respectively. No explanation was provided for the marked decrease in success rates after 10 years’ obstruction. The authors concluded that the procedure was easier to perform than conventional microsurgical techniques and that results were comparable. The latter point could be disputed, because the pregnancy rate of 35% for obstructive intervals shorter than 10 years is inferior to most published series.

Stents

Stents are used widely in urinary tract reconstruction, so it is not surprising that investigators have explored their use for microsurgical reconstructive procedures. Absorbable and non-absorbable stents have been used in clinical studies and animal models, respectively, to improve the alignment of the two vasal ends, to make the procedure easier to perform, and potentially to improve results.

Rothman and colleagues [11] conducted a randomized trial comparing a conventional two-layer microsurgical closure with a modified anastomosis
using an absorbable polyglycolic acid stent without intraluminal sutures. The modified anastomosis was performed with the intraluminal stent and 9-0 nylon sutures placed in the muscularis. The patency rate was lower for the stented group than for the conventional anastomosis group, but the difference did not reach statistical significance (81% versus 89.6%, respectively; \( P = .2 \)). The operative time was significantly shorter in the stented group (118.1 ± 32.3 minutes versus 137.5 ± 24.9 minutes; \( P < .001 \)), but the pregnancy rate for the stented group was inferior to that for the conventional two-layer closure (22% versus 51%; \( P = .002 \)). The total motile sperm counts for the two groups were similar. The authors concluded that patency and pregnancy rates were inferior in the stented group and did not recommend use of the absorbable stent used in this study [11].

Vrijhof and colleagues [12] investigated a nonabsorbable polymeric stent for VV in rabbits. The animals were assigned randomly to either a one-layer anastomotic technique with interrupted 8-0 polypropylene suture or a stented anastomosis with three polypropylene sutures (8-0) placed in the muscularis over the stent. All the vasa were patent at 39 to 47 weeks. Inflammatory reaction was seen around the stent, but more animals in the unstented group had partial obstructions. Total sperm count was higher in the stented group \( (P = .05) \). The authors concluded that this type of stent warrants more study with application in human studies. Although the use of stents is intriguing, they have not found their way into clinical practice. One potential difficulty with stents is disparate lumen diameters of the testicular and abdominal ends.

**Intussusception vasepididymostomy anastomotic techniques**

Of all the modifications discussed in this article, intussusception VE anastomotic techniques have had the greatest impact on clinical practice and are now used widely by urologic microsurgeons. Single-tube anastomosis for VE was reported first by Lespinasse in 1918 [13]. Microsurgical end-to-end anastomosis of the epididymal tubule to the vas lumen was reported by Silber in 1978 [14]. The end-to-side technique was reported in the early 1980s and became the most popular technique for VE [15–17].

In the early 1990s intussusception techniques were described in animals. Stefanovic and colleagues [18,19] reported their results with both an end-to-end and end-to-side intussusception techniques in adult rats. Patency rates were 97% and 100%, and sperm granuloma rates were 10% and 23% for the end-to-end and end-to-side techniques, respectively. Shekarriz and Pomer [20] compared an end-to-side invagination technique with the standard end-to-side anastomosis. At 4 months postoperatively, patency rates were 80% and 63%, respectively, for the end-to-side invagination technique and the standard end-to-side anastomosis. The authors concluded that this technique could save operative time and was easier to learn.

Berger [21] first described the use of an invagination VE in clinical practice. He described a triangulation intussusception technique using three double-armed 10-0 nylon sutures, which would be equivalent to six luminal sutures. In a series of 12 men who underwent bilateral VE with this technique, the patency rate was 92%. Operative time was 156 ± 14 minutes. This report generated a great deal of enthusiasm for intussusception VE and led many other investigators to adopt this technique (Fig. 1).

Marmar [22] then described a two-suture intussusception VE technique that many regarded as another significant advance. Seven (77%) of his initial series of patients achieved patency. Operative time was 35 to 45 minutes per side. This report contributed further to the enthusiasm for intussusception VE. Many have adopted this technique as well, appreciating the added simplicity of preplacing two, rather than three, sutures in the epididymal tubule (Fig. 2).

Since these initial reports, others have published confirmatory reports and suggested further modifications in the intussusception VE technique. McCallum and colleagues [23] reported superior patency rates in rats for intussusception VE than with conventional end-to-side VE (91.7% versus 54.2%; \( P = .004 \)). Schiff and colleagues [24] and Chan and colleagues [25] reported comparable or improved outcomes with triangulation intussusception VE and two-suture longitudinal intussusception VE compared with end-to-end and end-to-side VE. In the series by Chan and colleagues [25], the patency rate was 84%, and 60% achieved patency at 1 month. The natural pregnancy rate achieved by men with at least 1 year of follow-up was 40%.

In summary, the intussusception techniques provide an easier, faster, and more successful approach to VE and are now widely used.
Fig. 1. Triangulation intussusception vasoepididymostomy as described by Berger. (A) Epididymal tunic is sutured to muscularis of the vas. (B) Three double arm sutures are placed in distended epididymal tubule. (C and D) Epididymal tubule has been opened and sutures are then placed in the corresponding positions in the vas lumen. (From Berger RE. Triangulation end to side vasoepididymostomy. J Urol 1998;159:1951; with permission.)

Fig. 2. Two-suture intussusception vasoepididymostomy as described by Marmar. (A) Two double arm sutures are placed in distended epididymal tubule and then in corresponding position in vas lumen. (B) As sutures are tied, epididymal tubule is intussuscepted into vas lumen. (From Marmar JL. Modified vasoepididymostomy with simultaneous double needle placement, tubulotomy, and tubular invagination. J Urol 2000;163:484; with permission.)
Robotics

Robotics has been employed in minimally invasive urologic surgery, particularly with laparoscopic prostatectomy, which now is performed routinely at most large centers. This technology also might facilitate urologic microsurgery by eliminating tremor, improving dexterity, and aiding in the use of small instruments and fine suture [26]. Three studies have investigated the potential application of robotics in urologic microsurgery.

In a pilot study, Schoor and colleagues [27] demonstrated that robotic technology could be applied to microsurgical VV using 10-0 nylon suture in a rat model. Surgeons in this study were able to manipulate the vas and suture with the robotic graspers and noted ease of suture placement, elimination of tremor, and enhanced comfort. Patency of the anastomoses was not tested in this study. In the study by Kuang and colleagues [26], the feasibility of robotic-assisted VV was examined in segments of human vas, and performance measures were compared with conventional modified one-layer microsurgical VV (MAVV). Although robotic-assisted VV took longer to perform and was associated with more adverse haptic events, tremor was eliminated, and the patency rate was comparable to MAVV. Finally, Schiff and colleagues [28] compared standard microsurgical VV and VE with robotic VV and VE in rats. Robotic VV was faster than microsurgical VV and had a lower rate of sperm granuloma formation. Robotic VE and microsurgical VE operative times were not significantly different. Patency rates were similar for robotic and conventional VV (100% versus 90%, respectively; \( P = 0.23 \)) and robotic and conventional microsurgical VE (100% versus 90%, respectively; \( P = 0.16 \)).

It is unlikely that robotics will be adopted widely in urologic microsurgery. Specialized skills are required to use the robot, and it could be argued that it does not offer any advantage to the experienced microsurgeon. Specialized instrumentation would have to be developed to work with 10-0 and 9-0 suture. An increased working distance from the endoscope would help create more working space for movement and minimize thermal effects on the vas and epididymis. Finally, the robotic system costs more than $1 million, and maintenance costs are over $100,000 per year [26].

Summary

Several modifications have been introduced to improve the success rates for VV and VE and to make them easier to perform. Given that VV has patency rates of 90% or better in appropriately selected patients, it is difficult to improve these success rates significantly [29]. Use of FG may make VV easier to perform and improve the patency rates for surgeons who do not perform this procedure regularly. To date, none of these modifications for VV have been adopted widely. Robotics may be used more extensively for VV and VE in the future, but this technology may not offer any significant advantage to the experienced microsurgeon. Intussusception VE techniques, however, have been adopted by many urologic microsurgeons, and these techniques seem to have simplified the performance of VE.

References

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Sperm Acquisition in Nonobstructive Azoospermia: What Are the Options?
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Department of Urology, Medical College of Wisconsin, 9200 West Wisconsin Ave., Milwaukee, WI 53226, USA

Approximately 10% of male factor infertility is caused by azoospermia, and nearly two thirds of these patients have nonobstructive azoospermia (NOA) [1]. Before intracytoplasmic sperm injection (ICSI), donor insemination was the only viable option available for these men. With the advent and improvement in ICSI, these men have the opportunity of using in vitro fertilization (IVF). As experience has been gained, increasing numbers of men who have NOA are having sperm retrieved from their testes and used for ICSI/IVF [2]. Although various degrees of NOA, from Sertoli cell–only syndrome to hypospermatogenesis, can be treated in this fashion, it seems that the more advanced the spermatogenesis on diagnostic biopsy, the greater is the chance of recovering mature sperm [2,3]. This article reviews the various sperm retrieval techniques, discussing the advantages and disadvantages and the outcomes of each. Predictive factors for sperm retrieval are presented, as are some of the controversies that exist regarding sperm acquisition in NOA.

Testing
A diagnosis of true azoospermia requires that the semen specimen be centrifuged, the pellet examined with high-power microscopy, and the complete absence of sperm confirmed in two specimens. The next determination made is whether the azoospermia is obstructive or nonobstructive, based on history, physical examination, and seminal fluid analysis. A thorough history should be taken, assessing factors such as cryptorchidism at birth, previous scrotal or inguinal surgery, and exposure to gonadotoxins (including chemotherapy and radiation therapy). A detailed physical examination also should be performed, with special attention paid to the inguinal region looking for old incisions as well as for the presence or absence, consistency, and symmetry of the testes. The presence or absence of the vas deferens should be established. The presence or absence of epididymal distension with or without nodularity is important in distinguishing obstructive azoospermia (OA) from NOA. Seminal factors such as volume, pH, and presence or absence of fructose aid in distinguishing OA from NOA.

Once a presumptive diagnosis of NOA is made based on history and physical examination, hormonal testing including follicle-stimulating hormone (FSH) and testosterone levels should be undertaken to examine the hypothalamic-pituitary-gonadal axis. In addition, genetic testing, including Y-chromosome microdeletion analysis and karyotype, should be performed. These tests can reinforce the presumptive diagnosis of NOA and can provide useful prognostic information, as discussed later in this article. Once the diagnosis of NOA has been confirmed, the couple needs to be informed that to conceive they will need to undergo an attempted sperm acquisition technique and, if sperm are obtained, IVF. If the couple wishes to proceed, any genetic or karyotypic abnormality should be addressed by a genetic counselor. The couple then should be counseled regarding options, including the use of donor sperm for backup should no sperm be retrieved at the time of the sperm acquisition, the timing of...
the sperm acquisition, and cryopreservation of any identified sperm.

**Predictive factors**

When counseling men regarding the probability of finding sperm via any technique, multiple factors have been assessed to attempt to predict the success of sperm retrieval. Easily observed physical examination findings, such as testicular volumes, and frequently obtained laboratory values, including testosterone and FSH levels, have not demonstrated reliability in predicting success [4–6]. There has been some interest in inhibin B, a glycoprotein produced by the Sertoli cells, but results using this marker also have been mixed. Some studies found elevated inhibin B levels correlated poorly with success at retrieval [7,8], but others found that inhibin B levels higher than 40 pg/mL had 90% sensitivity and 100% specificity for a successful retrieval using testicular sperm extraction (TESE) with a diagnostic accuracy of 94.1% [9]. In addition to using each value alone, some investigators have combined clinical parameters of FSH, total testosterone, and inhibin B levels in a prognostic equation with a sensitivity of 71% and a specificity of 71.4% for successful retrieval [10].

More reliable predictors of successful sperm acquisition include testicular histology and azoospermia factor (AZF) deletions. Based on histology, success rates range from 80% to 90% (in men who have hypospermatogenesis) to 15% to 20% (in men who have Sertoli cell–only syndrome), with intermediate success rates for men who have various degrees of maturation arrest [11,12]. Table 1 [11–13] compares the success rates for sperm retrieval based on testicular histology. It seems that the more advanced the level of spermatogenesis, the more likely is the successful retrieval of mature sperm, although even finding sperm on diagnostic biopsy does not guarantee a successful outcome. Furthermore, one of the studies [11] used histology from the sperm retrieval procedures, rather than a diagnostic biopsy. Finally, most studies that discuss sperm retrieval rates did not correlate outcomes with histology, so the studies in Table 1 may not be comparable to all other studies. The ability to retrieve mature sperm increases as the level of spermatogenesis increases. The drawback of the use of histology is that it requires a testis biopsy for this information to be of use; nonetheless, it is available to assess further chance of success in men who have undergone prior sperm retrieval procedures or even prior diagnostic biopsies. In addition to the histologic information, the success or failure of prior biopsies offers insight into the success of subsequent additional procedures. Ramsamy [14] reported that retrieval rates with microscopic TESE (microTESE) were lower (23%) in patients who had had three or four negative biopsies than in patients who had had no prior biopsies (56%) or one or two to prior negative biopsies (51%).

DNA material is obtained more easily, and the assessment of karyotype abnormalities or gene deletions has predictive value. It has been reported

<table>
<thead>
<tr>
<th>Sperm retrieval rates by histologya</th>
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<tbody>
<tr>
<td><strong>Histology</strong></td>
</tr>
<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Hypospermatogenesis</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Maturation arrest—early</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Maturation arrest—late</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Sertoli cell–only syndrome (pure)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*a Testicular histology based on the most advanced level of spermatogenesis in all three articles. Su and Sousa used diagnostic biopsies, whereas Seo used tissue recovered from biopsies taken at the time of microdissection.
that no sperm is recovered from men who have complete deletions in AZFa and/or AZFb, whereas, recovery rates in men who have AZFc deletions are similar to those in patients without deletions [15]. This finding holds true for men who have Klinefelter’s syndrome as well [16,17]. Other less well-recognized factors have been assessed also. Protamine-2 is a gene expressed postmeiotically by round spermatids. Song and colleagues [18] found spermatozoa in 85.7% (18/21) of men found to have protamine-2 transcripts on reverse-transcription polymerase chain reaction from testicular tissue.

Methods

The two general methods of sperm acquisition are percutaneous acquisition and open biopsy. Percutaneous sperm acquisition has been well described in conjunction with OA, but the literature is conflicting regarding its utility for NOA. Most authors describe sperm acquisition in NOA using some type of open testicular biopsy. The obvious advantages of percutaneous acquisition are the minimally invasive nature of this method and the ability to sample multiple sites within the testes with minimal potential of harm to the testis. The obvious disadvantage is that the area of tissue sampled is decreased markedly compared with the open biopsy. Although most studies favor open testicular sperm extraction for men who have NOA, some studies have demonstrated the utility of testicular sperm aspiration (TESA) [19]. Table 2 [20–30] compares the likelihood of sperm retrieval using various methods. In many of these studies, however, patient factors determined the retrieval method, and success rates were not broken down by histology (see Table 1).

Percutaneous aspiration

Whether identified as percutaneous testicular sperm aspiration (PTSA), testicular fine-needle aspiration (FNA), or TESA, sperm is aspirated with a 19- or 21-gauge butterfly needle that is inserted percutaneously into the testis using a local anesthetic. Using a Cameco piston syringe to generate suction, the needle is manipulated in an in-and-out fashion to release a series of testicular tubules. These tubules are grasped at the skin and collected into media for analysis and, if viable sperm are identified, storage [31].

Mercan and colleagues [21] report their experience with this procedure in 452 men who had NOA. Their overall retrieval rate was 64.4% (291/452). Approximately 15% of the study group had a successful PTSA procedure; the remaining 228 went on to have success with TESE. Men who had successful PTSA retrievals were more likely to have hypospermatogenesis and were less

Table 2

<table>
<thead>
<tr>
<th>Method</th>
<th>Year</th>
<th>Author</th>
<th>Case (n)</th>
<th>Sperm retrieval rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspiration</td>
<td>2001</td>
<td>Vicari et al. [20]</td>
<td>55</td>
<td>47.3</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>Mercan et al. [21]</td>
<td>452</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1998</td>
<td>Ezeh et al. [22]</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>Friedler et al. [23]</td>
<td>37</td>
<td>11</td>
</tr>
<tr>
<td>Mapping</td>
<td>1999</td>
<td>Turek et al. [24]</td>
<td>57</td>
<td>47</td>
</tr>
<tr>
<td>Testicular sperm extraction</td>
<td>2006</td>
<td>Vernaeve et al. [25]</td>
<td>628</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>Okada et al. [26]</td>
<td>24</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>Tsujimura et al. [27]</td>
<td>37</td>
<td>35.1</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>Amer et al. [28]</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>Vicari et al. [20]</td>
<td>55</td>
<td>46.3</td>
</tr>
<tr>
<td></td>
<td>1998</td>
<td>Ezeh et al. [22]</td>
<td>35</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>Schlegel and Su [29]</td>
<td>16</td>
<td>62</td>
</tr>
<tr>
<td>Microdissection testicular sperm extraction</td>
<td>2002</td>
<td>Okada et al. [26]</td>
<td>74</td>
<td>44.6</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>Tsujimura et al. [27]</td>
<td>56</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>Amer et al. [28]</td>
<td>100</td>
<td>47</td>
</tr>
</tbody>
</table>

a In this comparison study, the difference in retrieval rates is not statistically significant.
likely to have germ cell aplasia or maturation arrest. In addition, couples with a successful PTSA had a significantly higher clinical pregnancy rate than those who underwent random biopsy (46% versus 29%), but this finding may have been influenced by the higher number of embryos transferred (4.38 versus 3.90). Findings reported by Vicari and colleagues [20] in a sample of 55 men who had NOA and who underwent either PTSA or TESE were slightly better than the 11% to 14% success rate with PTSA reported by other authors [18, 22,23]. In Vicari’s [20] population, 47.3% of all PTSA attempts were successful. Further stratification revealed that sperm retrieval with PTSA was successful in 100% of men who had diagnostic biopsies demonstrating hypospermatogenesis or maturation arrest with focal spermatogenesis, but the success rates with this technique were lower in complete maturation arrest (42.3%), Sertoli cell–only syndrome (14.3%), and Sertoli cell–only syndrome with focal spermatogenesis (0%) [20].

The main advantage of this technique is its minimally invasive nature, which may help limit the rate of complications. In a study of 32 men who had OA undergoing TESA with ultrasound follow-up at 1.5, 3, and 6 months, 6.3% of patients developed hypoechoic lesions consistent with an intratesticular hematoma. All had resolved by 3 months [32]. Similar data exist in the NOA population; Carpi [33] monitored 54 patients with ultrasound following a combination of FNA and large-needle aspiration biopsy and found hypoechoic lesions in 11.1% of patients undergoing biopsy up to 63 days following biopsy. The long-term or functional significance of these lesions has not been ascertained.

The relatively small samples of tissue obtained with TESA limit the amount of sperm that can be recovered, particularly in men who have severe spermatogenic failure. Therefore success rates are not as high as with open sperm retrieval in all populations. This method tends to work better in men who have more advanced degrees of spermatogenesis. As a result, most authors conclude that most patients who have NOA require open testicular extraction to obtain sperm adequate for IVF [21,23].

Open sperm extraction

Open TESE can be accomplished using various methods, including multiple random biopsies, multiple directed biopsies, and microTESE. Numerous studies have demonstrated the success of open TESE in the acquisition of sperm in men who have NOA. These techniques vary in their invasiveness, the amount of tissue removed, and the success rate in retrieving usable sperm.

Random biopsies

Random biopsies have been used to sample multiple areas within the testis. These biopsies typically have yielded large amounts of tissue, and the success rates have varied. Avascular regions near the midportion of the medial, lateral, or anterior surface of the tunica are incised, avoiding the capsular and testicular vessels. The amount of tissue taken varies from 150 mg [25] to 400 to 500 mg [29]. One early series noted a 62% successful retrieval rate, with a live delivery rate of 25% [29]. In a later series of 628 men who had NOA undergoing 784 TESE procedures, the overall retrieval rate was 48%; 41.6% were successful on the first attempt [25]. Most practitioners agree that multiple random biopsies are likely to yield poorer results than the more directed biopsy (as discussed in the following section) and remove more tissue [34]. In addition, the risk of side effects may be amplified after random biopsies. Using ultrasound, Harrington [35] demonstrated a 29% rate of intratesticular hematoma, which can lead to significant loss of testicular parenchyma secondary to interruption of the blood supply or even to atrophy. In another study, 64% of men developed hypoechoic areas consistent with linear scars after open biopsy [36]. Because of the increased risks of complications and decreased rate of sperm acquisition, random biopsies are not the first choice for retrieval in patients who have NOA, although some patients who have hypospermatogenesis may have adequate outcomes.

Multiple directed biopsies

Various techniques have been investigated in attempts to improve efficiency and minimize the theoretic risks of multiple random biopsies, which include testicular hematoma, further reduction in spermatogenesis, hypogonadism, or even complete devascularization. Multiple directed biopsies using various imaging modalities such as power Doppler have been described as a way to take advantage of architectural differences in sperm-producing areas of the testis [37,38]. These imaging modalities detect a difference in blood flow, with areas of increased perfusion being the most likely to contain more advanced spermatogenesis, thereby directing
the surgeon to the most likely sites of sperm production. Other studies investigated whether specific areas within the testis (eg, near the hilum) are more or less likely to contain sperm and found that there is no area that is more or less likely to yield mature sperm [34,39].

**Testicular mapping**

To identify sites of successful sperm acquisition and to minimize the emotional and financial costs of cancelled ICSI cycles, testicular mapping has been developed to guide the surgeon intraoperatively to areas most likely to yield mature sperm. The technique of testicular mapping is based on the concept that spermatogenesis often is sporadic and focal throughout the testis [40]. FNA systematically assesses the testicular quadrants for the presence or absence of retrievable sperm. Initial analysis revealed that FNA mapping was more sensitive than testis biopsy and was as specific for detecting sperm [39]. In follow-up studies, Turek [24] found that FNA mapping identified sperm in 47% of men who had NOA (27/57) and that sufficient sperm was obtained for all oocytes in 95% of cycles (20/21); the clinical pregnancy rate was 48%. This less invasive approach can be done in the office with a cord block, but it does require the patient to undergo two procedures. It also requires expertise in cytologic technique, because it is important that the specimens be fixed and read correctly for optimal outcomes: a prospective blinded review of 113 testis biopsies found a concordance rate of only 54% between the first pathologic diagnosis and the second. More importantly, in the cases in which differences in histology between the reviewers were detected, the differences in 30 cases had the potential to change management (ie, from pure Sertoli cell–only syndrome to a mixed pattern that would raise the potential for sperm retrieval) [41]. Because this study looked at intraobserver concordance in biopsies, not cytologies, it is pertinent to note that there does seem to be better correlation between FNA cytology and biopsy cytology; reported concordance rates range from 86% to 97% [42]. The possibility that a misreading of histology might alter the treatment course is one risk specific to mapping. The remaining procedural risks include those noted with other percutaneous testicular biopsy procedures.

**Microdissection testicular sperm extraction**

Expanding on the concept of directed biopsy, microTESE has been described by several authors and is thought by some to be the most accurate method of obtaining sperm in NOA [30,43]. This technique involves delivery and bivalving of the testis. The operating microscope then is used to examine individual seminiferous tubules. It has been reported that tubules containing germ cells are significantly larger and more opaque than those without germ cells. These areas are then sampled and examined by embryologists in the operating room, who confirm the presence or absence of mature sperm. This technique allows greater accuracy in finding sperm with significantly less tissue sampled. In one comparison study, microTESE retrieved 160,000 spermatozoa per 9.4 mg of testicular tissue, whereas conventional TESE obtained 64,000 spermatozoa per 720 mg of tissue obtained [30].

Numerous authors have reported fairly consistent sperm retrieval rates of approximately 50% in unselected patients. In patients who have had previous diagnostic biopsies, however, the positive identification of sperm on microdissection correlates with the most advanced degree of spermatogenesis on those biopsies [11,13]. Complications are rare but include the possibility of intratesticular hematomas (12% at 1 month; 2.5% at 6 months) [26], decreased testicular volume (2.5%) [26], temporary declines in serum testosterone values [44], and, in theory, the development of long-term hormonal insufficiency. These are inherent potential complications of all sperm acquisition techniques.

**Controversies**

**When to biopsy?**

Because the underlying histology influences the sperm retrieval rate, when is it appropriate to biopsy the male partner before proceeding with sperm retrieval? One author’s recommendations for biopsy include a high risk of carcinoma in situ (ie, men who have prior history of testicular cancer, unilateral cryptorchidism, or unexplained unilateral testicular atrophy), an unclear etiology of azoospermia, or results that will affect the couple’s decision-making process regarding proceeding with IVF-ICSI (ie, couples unwilling to use donor sperm who have a low chance of sperm retrieval) [45]. Otherwise, many authors do not recommend routine testicular biopsy in NOA.

**Fresh versus frozen sperm**

Given the inability to predict with certainty the likelihood of retrieving sperm, a decision must be
made whether to time the sperm retrieval in-cycle or to use frozen sperm. Coordinating in-cycle retrieval can be complex as well as stressful for the couple should a cycle be lost because of the inability to retrieve viable sperm. Before making the decision to perform an in-cycle retrieval or to use frozen sperm, the couple’s preferences regarding the use of donor sperm should be understood. Some believe that a couple’s refusal to use donor sperm for backup makes advanced sperm retrieval a better option. Studies using frozen sperm have noted fertilization rates of 44% to 48% [46,47], and a comparative study found the fertilization rate with frozen sperm not to be significantly different from that obtained with fresh sperm. This same study demonstrated equivalent clinical pregnancy rates, although ongoing pregnancy or delivery rates demonstrated a trend toward favoring fresh spermatozoa [46]. One caveat when using frozen sperm is that if retrieval numbers are very low, loss of viability secondary to the freeze-thaw process, which was as high as 50% in one study [48], could make a frozen cycle a less desirable option. Nonetheless, using frozen sperm can allow multiple IVF-ICSI attempts while minimizing the number of sperm extraction procedures required and the attendant risks to the male partner.

Multiple biopsies versus microdissection testicular sperm extraction

Studies comparing microTESE and conventional TESE typically demonstrate improvement in sperm retrieval when employing the microdissection technique. Schlegel’s [29] initial study of 27 patients noted a 45% retrieval rate with conventional TESE compared with a 63% retrieval rate with microTESE. A larger series of 460 patients reported retrieval rates of 32% with conventional TESE and 57% for microTESE [44]. This trend also was demonstrated in a prospective comparison study that assessed sperm retrieval rates of the two techniques performed on the opposite testes in 100 men; the retrieval rate was 30% for TESE and 47% for microTESE [28].

The complication rates of the two procedures also have been compared. In a series of 435 men who had NOA undergoing either TESE or microTESE, there was a significant decline in serum testosterone levels (average, 20%). In this study average testosterone values declined from 316 to 251 ng/dL in the conventional TESE group and from 303 to 248 ng/dL in the microTESE group. By 18 months, serum testosterone levels returned to 95% of the pre-TESE level in the majority of patients, and this return was independent of the type of TESE performed. No significant difference was noted in luteinizing hormone levels. After sperm retrieval the mean FSH level rose significantly, from 22 to 30, in 102 patients in this series, but the type of retrieval was not specified in the subset analysis of luteinizing hormone or FSH [44]. In a separate study, one patient experienced significant hypogonadism caused by bilateral testicular atrophy requiring hormonal supplementation following conventional TESE, but this single case was not statistically significant [26].

Postoperative hematomas can occur and may be seen on ultrasound as hypoechoic lesions or as diffuse echogenicity of the testicular parenchyma. It is feared that these lesions may cause pressure atrophy of the surrounding testicular tubules, and there are rare reports of complete devascularization [29]. Ultrasonographic follow-up studies also have generally favored microTESE. Postoperative hematomas were identified by a 1-month follow-up ultrasound in 51% of patients undergoing TESE versus 12% of patients undergoing microTESE; by 6 months identifiable hematomas had resolved in all but 7.5% and 2.5% of patients, respectively [26]. In Ramasamy and colleagues’ study [44] the rates were much higher, with acute ultrasonographic findings in 44% of patients undergoing microTESE versus 82% in the conventional TESE group at 3 months. At 6 months persistent changes were seen in 6% and 25% of patients, respectively, but most hematomas had resolved, leaving linear scars or calcifications.

Testicular size also has been shown to decrease significantly more often after conventional TESE (in 25% of men) than after microTESE (in 2.5% of men) [26]. This change may be explained partially by the findings of Tash [49], who found that the ratio of tubular volume to interstitial space decreased after one TESE.

Repeat procedures were more likely to obtain spermatozoa if performed more than 6 months (80%) than if performed sooner than 6 months after the first procedure (25%) [29]. This finding should be kept in mind when counseling couples who require a second procedure.

Summary

Sperm acquisition in the setting of NOA often is difficult, but as techniques have evolved, the success rates have improved. Review of the literature demonstrates that open biopsy is better than
FNA, even in the setting of hypospermatogenesis. Although multiple biopsies yield better results than a single random biopsy, directed biopsies may improve the yield and sacrifice less testicular tissue. As in OA, there is no difference in outcome with the use of fresh versus frozen testicular sperm; in some cases, however, the cryopreservation process may not allow adequate recovery of viable sperm. Finally, there is no definitive evidence showing a difference between multiple directed biopsies and microTESE. Although many studies demonstrate higher success rates with microTESE, these patients are more likely to have less favorable histology (ie, Sertoli cell–only pattern). In these cases, microTESE is clearly a better choice. Patients who have hypospermatogenesis or greater levels of spermatogenesis may do just as well with multiple directed biopsies. The choice of procedure depends on multiple factors, including surgeon and laboratory experience and preference, predictive factors such as the cause of azoospermia (eg, chemotherapy, cryptorchidism, Klinefelter’s syndrome), genetic factors (Y-chromosome microdeletions, karyotypic abnormalities), and perhaps hormonal results. As knowledge of the spermatogenic process improves, the ability to determine who has retrievable sperm, and where it resides, will improve as well.

References


Our understanding about genetics is rapidly changing. The goal of this article is to provide an overview of the basics of and new developments in medical genetics—a Genetics 101 primer for 2008. In this article we review the structure and function of the genes, how genes are packaged, gene replication, gene mutations, and the different modes of inheritance.

Basic gene structure

The basic building blocks for genes, the genetic encyclopedia, is “written” using four deoxyribonucleotides (A, G, T, C: adenylic acid, guanylic acid, thymidylic acid, and cytidylic acid). A single strand of DNA is composed of a string of these deoxyribonucleotides linked together on a sugar phosphate backbone (Fig. 1) [1,2]. The 3' carbon of one of the sugars is attached to a phosphate, which is in turn linked to the 5' carbon on the next nucleotide. The complete DNA is formed when two strands of DNA are joined together to look like a ladder twisted in the form of a double helix.

The link between the two strands that form the complete DNA is caused by hydrogen bonding between the nucleotides on each of the opposite strands of DNA. This binding is specific: A on one strand always binds to T on the complementary strand with two bonds, and G always binds to C on its complementary strand with three bonds. This combination of A-T and G-C is referred to as a base pair. The order of these base pairs on the genes provides the precise blueprint for the entire structure of the organism. There are approximately 3 billion of these base pairs in the human genome. Each human cell contains 6.6 billion bases (A, C, G, or T) or approximately 5 ft of DNA. The adult body contains approximately 10 trillion cells, so with 5 ft in each of 10 trillion cells, there is enough DNA to reach from Earth to the sun 90 times.

Most genetic research has focused on genes that code for protein products. Most of the known gene sequences in humans that code for proteins are discontinuous: coding regions (areas that actually code for the proteins) called exons, which are usually split by noncoding regions called intervening sequences (introns) (Fig. 2). Although it was originally thought that there were 50 to 100,000 protein coding genes in the human genome, based on the Human Genome Project it seems that only 25 to 30,000 genes encode for proteins [1,2]. It is currently thought that only 1% to 2% of the human genome codes for proteins.

DNA packaging

Because each cell contains all of the genetic material, the DNA must be packaged to fit into the nucleus of each cell, be protected against damage from external factors (eg, radiation), be available for replication and transcription, and be accessible for repairs. The DNA in the nucleus is set in a histone backbone and then spooled (like thread) before being stacked in groups of eight (Fig. 3) [1,2]. These stacks of histone-rich spooled DNA are subsequently compacted further to form...
each of the 46 chromatids. Each of the chromatids is composed of two asymmetric arms (the short arm is called the p arm; the longer is the q arm) joined at the centromere. A chromosome is composed of two chromatids. It is important to understand that one of the chromatids on each chromosome is of maternal origin, whereas the opposite is of paternal origin. The diploid genome consists of 22 pairs of autosomes numbered from largest to smallest and one pair of sex chromosomes (X/Y or XX). (Although chromosome number 22 is larger than number 21, it was decided to keep it this way because geneticists were used to calling Down syndrome trisomy 21.) A karyotype is composed of these 23 pairs of chromatids arranged in order (Fig. 4).

Fig. 1. Basic building blocks of DNA and RNA. The basic building blocks of DNA are the deoxyribonucleotides (A, G, T, C: adenylic acid, guanylic acid, thymidylic acid, and cytidylic acid). These deoxyribonucleotides are set in a sugar phosphate backbone and then linked to the opposite strand of DNA by hydrogen bonds to form a double helix. A always links to T and C to G. RNA is single stranded and is formed from A, G, U, C (uracil [U] replaces the T found in DNA). (Courtesy of The National Human Genome Research Institute, National Institute of Health, Bethesda, MD.)
Gene expression

Each gene encodes a particular protein that has a specific cellular function. Initially, in the nucleus, the DNA is copied in a process called transcription into a single strand of messenger RNA (mRNA) (Fig. 5). The mRNA is similar to DNA with four nucleotide bases, with the exception of uracil (U), which replaces T in the sequence. The mRNA is immediately modified to excise the introns or noncoding sequences and cap the 3’ and 5’ ends. This mRNA then translocates into the cytoplasm of the cell to contact the ribosome (protein producing mechanism). The ribosome then reads the mRNA sequence and, in a process called translation, produces a chain of amino acids that precisely mirrors the information found in the mRNA. The ribosome begins protein translation at the initiation codon (AUG) and then reads packages of three nucleotides from the mRNA at a time (Fig. 6). Each of these triplets, called a codon, translates into a specific amino acid. There is a total of 20 different amino acids, which are added in sequence to the growing polypeptide chain according to the codons (Fig. 7). The code is “degenerate” because in most cases there are several different codons for each amino acid (eg, phenylalaline is encoded by TTT or TTC). Finally, a terminator or stop codon (UAG, UAA, or UGA) cannot be decoded into an amino acid, so protein synthesis terminates.

DNA replication: mitosis and meiosis

The process of mitosis occurs in all cells and serves to precisely replicate the DNA to produce two genetically identical daughter cells from each mother cell. Initially, DNA strands are copied to produce two pairs of chromatids with identical DNA structure as the parent cell. The cell then divides and distributes one copy of the chromatids to each of the daughter cells. Each of the daughter cells has a full diploid complement of DNA.

In contrast, meiosis occurs only in the germ cells and involves a process of replication, pairing, recombination, and reduction in the chromosome number. Eventually only one half of the diploid DNA is transmitted to the spermatozoas or oocytes. In the premeiotic phase, most of the DNA is replicated to create pairs of sister chromatids (the original and the replicated chromatid) (Fig. 8) [3]. These pairs of sister chromatids then bind to the homologous chromosomal pair by unique structures called synaptonemal complexes. These complexes, formed by the protein core of the chromatids, link and align the two homologous chromosomes pairs together during meiosis (Fig. 9). This close alignment allows genetic material to cross over between the pairs of homologous chromosomes. Up to this point, one side of the chromosomal pair had DNA strictly derived from the maternal DNA and the complementary side was strictly paternal. With recombination caused by crossing over, maternally and paternally derived genetic material appears on the same chromatid.

The pairs of homologous chromosomes then align along the spindle apparatus, after which two reductive divisions reduce the DNA to a haploid content for oocytes and spermatozoa (Fig. 10). A total of four functional spermatozoa is produced from one germ cell undergoing meiosis, whereas one oocyte and three polar bodies are produced with meiosis for the oocyte. Each spermatozoa normally has either an X or a Y chromosome,
whereas the oocyte normally has a single X. When the spermatozoa and the oocyte fuse, the diploid state is restored, with either a 46,XY (male) or a 46,XX (female) zygote being produced.

**DNA alterations: polymorphisms**

Polymorphisms are variations in the DNA found in at least 1% of the population [1,4]. In general, DNA polymorphisms do not cause disease, although they may alter the severity of diseases. As an example, in intron VIII in the gene responsible for cystic fibrosis (called cystic fibrosis transmembrane conductance regulator gene), three different lengths of the poly T tract (5T, 7T, or 9T) are found in the population [5,6]. Although none of these variations causes cystic fibrosis, if the 5T variant is found in association with cystic fibrosis gene mutations, a mild form of cystic fibrosis may be converted into a more severe form of cystic fibrosis [7]. There are several types of polymorphisms:

1. Single nucleotide polymorphisms (a change in one nucleotide). Single nucleotide changes in the DNA are the most common polymorphism and may occur in up to 1 in 100 base
pairs for specific genes. Most single nucleotide polymorphisms do not cause disease.

2. Tandem repeat polymorphisms. This is a polymorphism with repeated di- or tri-nucleotides within one gene. Although this variable repeat length usually does not cause disease, there are a few exceptions (see later discussion).

3. Restriction fragment length polymorphisms. A variation, usually the result of a single nucleotide substitution, may be detected if a restriction endonuclease site is affected. If the restriction site is affected by the polymorphism, then restriction enzymes acting on the endonuclease site may be unable to cleave the nucleotide resulting in altered restriction fragment lengths.

**DNA alterations: mutations**

A mutation is an alteration in the DNA that may be passed from parent to daughter cells [1,4]. Somatic mutations occur in cells other than germ line cells. These particular mutations are not passed to the next generation, but they are passed on to the daughter cells. These types of mutations may be silent or may play a central role in disease pathogenesis. An example of a somatic mutation causing a disease is in segmental neurofibromatosis type I, in which a somatic mutation in the NF1 gene occurred after conception and caused a segmental NF1.

Germ line mutations are passed on to the next generation. The rate of mutations is approximately one per ten loci per generation [6]. Most of these mutations are in noncoding regions and may not cause any phenotypic alterations. Mutations in coding regions may cause alterations in the protein’s function and lead to diseases. Some de novo (not inherited from an affected parent) mutations that cause autosomal dominant conditions, such as achondroplasia, thanatophoric dysplasia, and Marfan syndrome, are known to be associated with advanced paternal age. Single base changes in coding regions may lead to (1)
silent mutations, in which the change in the nucleotide results in a change in the codon that still codes for the same amino acid, (2) missense mutations, in which nucleotide changes result in altered amino acid sequences, and (3) nonsense mutations, in which nucleotide changes change the codon to a terminator or stop codon.

Mutations that result in a change in the length of the gene are called insertions or deletions. An insertion or deletion of nucleotides that is not a multiple of three results in a frame shift mutation, in which the reading frame of the DNA is altered. This may result in premature termination of the translation process and altered or absent protein or protein function. Germ line mutations also may involve larger fragments of DNA. Millions of base pairs could be altered with deletions, insertions, duplications, or
translocations. These types of alterations could potentially be seen on chromosomal analysis, fluorescent in-situ hybridization (FISH) analysis, or microarray analysis. Finally, sequence alterations in the noncoding regions of the DNA might affect mRNA splicing, transcription, or regulated tissue expression.

Genetic germline disorders

Genetic defects have been divided into three major categories: (1) single gene disorders (mendelian disorders), (2) chromosomal disorders (which can be microscopic or submicroscopic), and (3) non-mendelian genetic disorders.

Single gene disorders (mendelian disorders). These disorders are caused by a mutant allele or pair of mutant alleles at one genetic locus. They may be inherited from one of the parents, or they may result from new mutations in the sperm or egg that conceived the baby. The mode of inheritance can be autosomal dominant (eg, autosomal dominant polycystic kidney disease, autosomal recessive (eg, cystic fibrosis), X-linked dominant (eg, hypophosphatemic rickets), or X-linked recessive (eg, Kallman’s syndrome). These single gene disorders are cataloged in McKusick’s Mendelian Inheritance in Man at http://www.ncbi.nlm.nih.gov/Omim/ [8]. Although only 1500 entries were in the first edition, as of August 30, 2007, the online version had 18,037 entries, most of which are autosomal (16,914), with a lesser number being X-linked (1004), Y-linked (56), or mitochondrial (63).

Autosomal dominant disorders. Autosomal dominant mutations are expressed with the inheritance of a single mutant allele. Each of the offspring of an affected parent has 50% risk for inheriting the mutated gene [9,10]. Many of these mutations cause late-onset conditions, such as autosomal dominant polycystic kidney disease and achondroplasia. These conditions also have intra- and interfamilial variability in the clinical manifestations. In some persons the penetrance is not complete (ie, not all the carriers of the gene mutations show clinical manifestations). An example of an autosomal dominant disease is autosomal dominant polycystic kidney disease, which is characterized by numerous cysts in the kidney. Cysts also may be found in the liver, spleen, pancreas, lung, ovary, testis, epididymis, and seminal vesicle. Some men may present with infertility caused by cystic dilation of the seminal vesicles and the epididymis. Most patients are asymptomatic for the first three or four decades of life. The severity of the disease phenotype varies between and even within families. A small percentage of patients have onset prenatally or early in infancy or childhood. The incidence of autosomal dominant polycystic kidney disease is between 1 in 400 to 1 in 1000 persons. Spontaneous mutations occur in 1 in 1 million gene loci, and the frequency of these de novo mutations increases with increasing paternal age.

Autosomal recessive disorders. Autosomal recessive mutations are only expressed if a disease
that causes mutation is present on both alleles of a gene. An example of an autosomal recessive condition is cystic fibrosis. Clinical hallmarks of cystic fibrosis include recurrent pneumonias and chronic pulmonary obstruction, exocrine pancreatic insufficiency, neonatal meconium ileus, elevated sweat electrolytes, and male infertility [6,10]. Male infertility is caused by absence or abnormalities of the structures derived from the Wolffian duct. The body and tail of the epididymis, vas deferens, seminal vesicles, and ejaculatory ducts are atrophic, fibrotic, or completely absent. Approximately 1 in 25 persons of the Northern European population carries the gene mutation for cystic fibrosis, with a disease frequency of approximately 1 in 2500 [6,10]. If two parents carry one mutation each, then 3 things can occur:

1. 25% of their children have two mutations (likely to be affected by cystic fibrosis). These mutations may be identical (homozygous: eg, ΔF508: ΔF508) or at different sites in the gene (compound heterozygous: eg, ΔF508: R75Q).
2. 50% of the children are carriers for the mutation without any evident disease.
3. 25% are not carriers.

Unlike the phenotype typically seen in patients with autosomal dominant mutations, if patients have autosomal recessive mutations, then the disease usually is manifested early in life.

X-linked disorders. X-linked mutations cause disease in men (46,XY) with the mutation and in women who inherit two copies of the X-linked mutation. Unlike the autosomal dominant or autosomal recessive mutations, which are autosomal and affect men and women, these conditions affect more men than women. The disease is passed from an affected man through his unaffected daughters to the grandsons. An example of an X-linked disorder is Kallmann’s syndrome [10]. Male patients with Kallmann’s syndrome have hypogonadism secondary to deficiency of hypothalamic gonadotropin-releasing hormone. Clinical features of Kallmann’s syndrome include anosmia, craniofacial asymmetry, cleft palate and lip, color blindness, congenital deafness, cryptorchidism, infertility, and renal anomalies. The disease occurs with an incidence of 1 in 10,000 to 1 in 60,000 persons.

Chromosomal abnormalities. Chromosomal disorders are caused by the loss, gain, or abnormal arrangement of 1 or more of the 46 chromosomes [1,4,10]. Most chromosomal disorders are de novo events that result from major mutations in the parent germ cells. When inherited, chromosomal disorders often show modified patterns of
mendelian transmission. Chromosomal disorders are typically referred to as microscopic or submicroscopic and numerical or structural. Numerical chromosomal abnormalities fall into two categories: (1) Polyploidy: a chromosomal number that is a multiple of 23. For example, triploidy consists of 69 and tetraploidy consists of 92 chromosomes. Both conditions are essentially incompatible with life. (2) Aneuploidy: a gain or loss of one or more chromosomes is the more common type of numerical chromosomal abnormality. Mosaicism may occur, with individuals whose tissues consist of a mixture of cell lineages with different chromosomal complements.

An example of a numerical chromosomal abnormality is Kleinfelter’s syndrome with a karyotype of 47,XXY [10,11]. This is a common aneuploidy in men (frequency of 1/500 men). Patients who have classic Klinefelter’s syndrome (47,XXY) have small firm testes and azoospermia, among other manifestations. Almost all cases of Klinefelter’s syndrome result from meiotic nondisjunction of the X chromosome of the gametes from either parent. During meiosis, the gametes normally have a haploid complement of DNA (23,X or 23,Y for sperm and 23,X for oocytes). With meiotic non-disjunction, the DNA complement becomes 24,XY from the man or 24,XX from the woman. These gametes then combine with the oocytes or sperm with a normal haploid DNA content to become a 47,XXY zygote.

The mosaic form of Klinefelter’s syndrome is caused by mitotic nondisjunction of the X chromosome after fertilization and development of the zygote. The zygote is formed with a normal diploid complement; during mitosis an unequal distribution of DNA leaves one of the daughter cells with a 47,XXY DNA complement, whereas
the other cells maintain the normal diploid DNA pattern. This process produces a mosaic Klinefelter’s syndrome, with a variable percentage of the cells having the 47,XXY pattern (depending of the timing of the mitotic nondisjunction). The mosaic variant of Klinefelter’s syndrome (46,XY/47,XXY) is usually not as severe as the classic form.

Structural chromosomal abnormalities or genomic disorders result from a loss of material (deletion), gain of material (duplication), or rearrangement of material (inversion and translocations) (Fig. 11) [1,4,10]. Translocation of segments from one chromosome to another and inversion of a segment in one chromosome—if not associated with deletion or duplication of genetic material—may not result in clinical manifestations. There seems to be a common mechanism for these genomic disorders. Blocks of DNA, so-called “low copy repeats,” which are 10 to 400 kb in length and have almost identical sequences, are dispersed throughout the chromosome [1]. These low copy repeats account for 5% of the human genome. Some are located on the same chromosome, whereas others are found on more than one chromosome. The proximity, size, and sequence similarity promotes recombination, which can lead to duplication, deletion, inversion, or translocation depending on the chromosome and the configuration.

An example of a structural chromosomal abnormality caused by a deletion is the well-known deletion of parts of the Y chromosome leading to male infertility. Originally, in 1976, Tiepolo and Zuffardi [12] identified a deletion on the q arm of the Y chromosome related to infertility and postulated that this region of the chromosomes was important for spermatogenesis. Although this particular group used light microscopy to recognize a deletion (several million base pair deletions would be recognized in this fashion), a smaller deletion (submicroscopic deletions) would not be detected using chromosomal analysis and requires techniques such as DNA analysis, FISH analysis, and microarray. Advances in molecular biology subsequently allowed several other researchers to map the pattern of Y microdeletions found in men with infertility [13–15]. We currently recognize that Y microdeletions are the most common genetic alterations found in men with testicular failure and severe oligospermia [15]. Inheritance is Y
linked: all of the male offspring of men with a Y microdeletion inherit the deletion and should have the same infertility phenotype as the fathers. Evidence exists, however, that the sperm of men with a Y microdeletion have higher rates of sex chromosomal aneuploidy, which indicates that the children of these men might have higher rates of a sex chromosomal aneuploidy [16].

How does the Y microdeletion occur? It seems that the original defect can be traced back to the father of the man with the Y microdeletion. The fathers of the men had no Y microdeletions noted in the somatic cells, yet in studies on the sperm of the fathers of these men, there was a mixture of sperm with and without Y microdeletions, a condition called gonadal mosaicism. This finding indicates that the original defect is caused by a tissue-specific mosaic pattern of Y microdeletions in the germ cells. This explains why the frequency of Y microdeletions in the brothers of men with a Y microdeletion is low.

Translocations of material from one chromosome to another may be in the form of a reciprocal translocation, with the exchange of material between two chromosomes. This is in general...
a balanced translocation, with no gain or loss of material and no alteration to the phenotype [1,4,16]. Although there is no loss of DNA in the individual, once meiosis occurs, the resulting haploid sperm or egg may have a gain or loss of DNA. As an example, if there is a reciprocal translocation between chromosomes 13q and 14q, the individual would have no loss of DNA, but after meiosis, some of the now haploid gametes would be unbalanced (haploid gametes could have a gain or deletion of parts of chromosomes 13 and 14). These patients have higher rates of pregnancy losses [17]. Evidence also indicates that some reciprocal translocations result in altered spermatogenesis [16].

Finally, a Robertsonian translocation is caused by the fusion of two acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22) [1,4,16]. The long arms remain and form a single chromatid, whereas the short arms may be lost. The individual then ends up with 45 chromosomes. Although a Robertsonian translocation is in almost all cases balanced and causes no phenotypic changes, it may result in spermatogenic alterations when carried by men and higher rates of pregnancy losses caused by an unbalanced gamete [16,17].

**Nontraditional inheritance**

**Multifactorial disorders**

Most disorders are neither the results of a chromosomal abnormality nor the result of a single gene disorders. They are multifactorial [1,4]. Multifactorial disorders include many of the common diseases of adulthood (eg, neural tube defects, schizophrenia, insulin-dependent diabetes mellitus, and essential hypertension) and most congenital malformations (eg, cleft lip or club foot). Multifactorial disorders are believed to involve interactions between genes and environmental factors, although the number and nature of genes and environmental factors are poorly understood.

**Conditions caused by expansion of trinucleotide repeats**

An expansion of a trinucleotide repeat sequence has been associated with more than 12 different neurologic diseases [1]. Although the best studied is the Fragile X syndrome, the example used for this article is the CAG repeat length in the gene coding for the androgen receptor causing Kennedy disease. The gene coding for the androgen receptor is found on the X chromosome, and within this gene is a CAG trinucleotide repeat. The CAG repeats code for a polyglutamine tract. The normal repeat length varies in different series but is generally around 20 to 22 repeats [18–23]. A longer polyglutamine tract causes the protein to form insoluble nuclear aggregates [18–23]. In several studies, CAG repeat length of between 38 and 62 leads to Kennedy disease (a neurodegenerative disorder) and male infertility. In some studies, a more moderate expansion of the CAG repeat length has been associated with male infertility without Kennedy disease [18,24–30]. This disorder is inherited in an autosomal dominant fashion, but the severity of the phenotype may worsen with subsequent generations (anticipation) with a further expansion of the CAG repeat length.

**Mosaicism**

Mosaicism, or a different genotype in different cells in the same individual, may occur in somatic or germline cells [1,4]. For somatic mosaicism, an event occurs at the postzygotic stage leading to a population of affected and unaffected cells in the individual [1]. The percentage of cells affected depends on the stage when the event occurs. Klinefelter’s syndrome mosaic, described previously, is an example of this type of mosaicism. The clinical manifestations noted in persons with a somatic mosaic pattern depend on the percentage of cells affected in each body organ and the body organs affected. A germline mosaicism refers to two populations of cells with different genotypes in the gonads. The fathers of men with infertility caused by a Y microdeletion are likely to have a gonadal mosaicism for Y microdeletion. The fathers have normal somatic cell Y chromosomes but have a population of sperm with a Y microdeletion. The percentage varies.

**Mitochondrial inheritance**

The mitochondrial genome is a relatively small and carries only 37 genes [31,32]. These genes are involved in different stages of oxidative phosphorylation, or the process of ATP production. Inheritance of a mitochondrial mutation is by a unique pattern: because only the oocytes—and not sperm—mitochondrial DNA is passed to the next generation, the mitochondrial mutations have a maternal mode of inheritance. The mutation is passed by women to all their offspring. Men are unable to pass the condition on, so transmission ends with
a son. Mitochondrial mutations affect body organs that require high energy, such as the muscles, pancreas, bone marrow, and brain. Various degenerative conditions are associated with mitochondrial defects, including dystonia, late-onset Alzheimer’s disease, Parkinson disease, diabetes mellitus, and the process of aging [31]. Recently, Nakada and colleagues [32] showed in a murine model that spermatogenesis is affected by mitochondrial mutations.

**Genomic imprinting**

In some genes, one of the maternal or paternal gene copies is silenced. This is in contrast to what happens for most genes, in which the maternal and paternal copies of the gene are expressed [1,4]. This is a poorly understood process. Several diseases are associated with imprinting disorders: Prader-Willi syndrome, characterized by short stature, hypogonadism, hypotonia, and failure to thrive in infancy as a result of feeding difficulties, and obesity that starts in childhood, is caused by the deletion/impairment of the paternal copies of the imprinted SNRPN gene and neclin gene on chromosome 15 located in the region 15q11-13 [33]. Conversely, in most cases, Angelman syndrome is caused by an alteration of the maternally derived gene in the same chromosomal region [31]. Prader-Willi syndrome and Angelman syndrome represent the first reported instances of imprinting disorders in humans [31].

**Uniparental disomy**

Uniparental disomy refers to the inheritance of two copies of a genetic locus or an entire chromosome from one parent and none from the other. This is a rare occurrence and can occur as a random event during the formation of egg or sperm cells or may happen in early fetal development. This latter event is thought to occur when one allele of an embryonic cell with trisomy is eliminated, leaving two copies of one allele from one parent.

**Summary**

Genetic alterations have a profound impact on the formation and function of the genitourinary system. Some of these genetic alterations have obvious effects early in life (chromosomal alterations such as Kleinfelter’s syndrome or trisomy 21), some affect patients later in life (some of the single gene defects, such as autosomal dominant polycystic kidney disease or the androgen insensitivity syndrome), some may have an isolated effect on fertility (such as a Y microdeletion), and still others may be multifactorial and involve an interaction between genetic and environmental causes (eg, many cases of prostate cancer or vesicoureteral reflux). The tremendous advances in the area of genetics have allowed us to understand more about the contribution that genetic alterations make to urologic diseases. As urologists, we are increasingly expected to be experts about the genetic basis of the diseases we treat and be knowledgeable about genetic counseling. Knowing the genetic cause is the first step in finding ways to cure and prevent abnormalities.

**References**


Darwinian evolution is most concerned with adaptation and change of morphology or phenotype (ie, survival of the fittest). The lineage of almost all current species can be traced back to ancestral forms by similarity in form and function and also by molecular signatures. We can outline our human roots back millions of years by these methods and know that we have attained our present form through some incredibly remarkable changes, most notably brain development. Evolution and adaptational modification continue unabated as the environment changes; stressors are altered and new niches open up, whereas others close down. But all along, nature and evolution have been, and still are, paying close attention to another function: reproduction [1]. Transformation of the genome, at the genetic or epigenetic level, is what underlies the process of evolution. This transformation may take place in a slow and steady fashion or in quantum leaps, relatively speaking. If an advantageous variation in the genome occurs that, for example, allows the proband organism to survive better in a particular environment but completely impairs reproductive capability, that variation will not be transmitted to the next generation and that beneficial genetic “mishap” will be lost. If that same advantageous variation did not alter reproductive capacity, it will indeed be passed along to benefiting recipient offspring who will also have an improved survival, allowing them to procreate more successfully and affecting the future offspring of that lineage. If nature is all about the transmitting of DNA, then survival and reproduction are equally important and, therefore, it would be distinctly unusual for any surviving organism from any species to have an impaired reproductive ability. Survival and evolution depend on procreation. One should look at human reproductive failure the same way; unless we can prove otherwise, we should assume impaired fertility has a genetic basis and we should inform couples about this. Our therapies to treat severe male factor infertility have far outstripped our knowledge of the reasons behind that failure. Intracytoplasmic sperm injection (ICSI) is a process whereby a single sperm is directly injected into a single oocyte, allowing the use of sperm from the testis, even in the most impaired situations in which only a handful of sperm are produced secondary to near-total spermatogenic failure. Is the use of this sperm safe? What are the short- and long-term consequences? What will be the reproductive capability of the offspring? It is a large human experiment, the subjects of which are not the couples we are helping, but the children we are creating. We are bypassing the natural selection process that forms the basis of evolutionary selection.

In addition, the male reproductive axis of every species is designed to be quantitatively and qualitatively maximized; reserve sperm production potential is not needed. Finally, meiosis occurs in only two places, oogenesis and spermatogenesis, and for each, a cadre of genes has evolved just for this purpose; these genes may be dysfunctional with no somatic effects, just defective egg or sperm production. These last two points fully describe many men with severe spermatogenic inadequacy; they are phenotypically healthy in all respects, except for reduced or absent sperm production [2]. In this article, the author concentrates on some of the known causes of nonobstructive azoospermia (NOA) (spermatogenic failure) and obstructive azoospermia (OA) with a well-established genetic cause such as
congenital bilateral absence of the vas deferens (CBAVD). These, in combination, represent most of what will be seen in an infertility practice. Finally, mention will be made of the syndromes that may be rarely encountered that affect sperm form (globozoospermia and fibrous sheath dysplasia) and sperm function (immotile cilia syndrome).

**Nonobstructive azoospermia: Y chromosomal microdeletions**

The basic molecular geography of the Y chromosome is important to appreciate before delving into the clinical consequences of Y chromosomal microdeletions. The Y chromosome is approximately 60 million base pairs in length, equally divided between the euchromatic and heterochromatic regions [3]. At the ends of both the short (Yp) and long (Yq) arms are “pseudoautosomal regions” that pair and recombine with like regions on the X chromosome [4,5]. One of the most important genes in the cascade that determines the fate of the bipotential gonad, sex-determining region Y (SRY), is located on Yp [6]. Between the two pseudoautosomal regions at the ends of the Y is unique chromosomal material not represented elsewhere in the genome, which is termed the “male-specific Y” (MSY), composing approximately 95% of the Y chromosome (Fig. 1) [7]. As such, it is nonrecombining and was previously known as nonrecombining Y (NRY). Within MSY, multiple genes are sprinkled throughout, most involved with spermatogenesis yet still poorly characterized at this point [8]. Examples of these genes include USP9Y, DBY, DAZ, RBMY1, and BPY2. Within the euchromatic portion of the long arm of Y are interspersed eight palindromic sequences, labeled in order, P8 to P1, beginning closest to the centromere. Each palindrome is of a different length but all have a central short base pair core from which mirror-image, identical stretches spread outwards, reading exactly the same but, of course, in opposite directions. Certain palindromes are made up of subsegments or amplicons, of which at least two copies exist (one on each arm) but occasionally of which more than two copies are present (spatially separate in a different palindrome and reading in either the same orientation [direct] or in the opposite direction [inverted]) [9]. It is thought that this repetitive-style molecular organization of the MSY has evolved as a mechanism for maintaining the presence and fidelity of the MSY because the genome does not have a counterpart where correction and renewal take place during mitotic pairing and meiotic recombination. However, this same molecular arrangement may also rarely lead to ectopic homologous recombination during Y chromosomal replication, a process whereby two spatially distanced subsegments “stick” together, with resultant loss of all chromosomal material in the intervening portion [10]. Such losses are referred to as microdeletions because they are not visible by cytogenetic analysis. If these microdeletions occur, then the resident gene population is also lost. In summary, most of the Y chromosome is unique, the organization is unusual, genes involved in the spermatogenic process reside in MSY, long segments of the Y may be lost if ectopic homologous recombination occurs, and, if this recombination occurs, any genes that live in these regions will also disappear.

The description of the molecular anatomy of the Y is important to understand vis-à-vis clinical male reproduction because several genes sprinkled along the length of proximal Yq are either necessary or helpful for optimal spermatogenesis. It was recognized in 1976 that cytogenetically recognizable gross deletions and anomalies of the Y chromosome could be found in men with spermatogenic failure. However, as the molecular anatomy of the Y chromosome was being elucidated in the mid-1990s and beyond, specific microdeletions of the Y chromosome were being discovered in men with sperm production deficiency. The original acronym nomenclature arose at this time when it was thought that only three of these spatially and topographically distinct microdeleted regions existed, AZF (azoospermia factor)

![Fig. 1. Yp, Yq, MSY, palindromes P8 to P1, sites of recognized microdeletion.](image-url)
a, AZFb, and AZFc. A Y chromosomal microdeletion assay is a blood test, readily available, that can determine whether one of the clinically important microdeletions is present and it should be obtained in all NOA or severely oligospermic men, before either using ejaculated sperm in conjunction with ICSI or surgically attempting to harvest testis sperm.

The AZFa region is located in proximal Yq, is 792 kilobases (kb) in length, and contains DDX3Y (also known as DBY) and USP9Y, two genes felt to be important in the spermatogenic process [11–13]. For example, DDX3Y is 16.3 kb in length, generates an ATP-dependent RNA helicase that shuttles between the nucleus and cytoplasm, and may play a part in the later stages of spermatogenesis [14,15]. USP9Y may play less of a quantitative role in spermatogenesis than DDX3Y; mutations in USP9Y have been described in men with some spermatogenic potential [16,17]. However, flanking the genomic material where DDX3Y and USP9Y call home are two 10-kb endogenous retroviral elements, HERV15yq1 and HERV15yq2 [18,19]. Ectopic homologous recombination may occur between these two sequences, with resultant loss of the intervening material, including DDX3Y and USP9Y. An AZFa microdeletion is found to be the proximate cause in approximately 1% of NOA men. Spermatogenic failure is the ultimate clinical consequence and the literature suggests that sperm will not be found in the tissue on testis sperm extraction (TESE). Therefore, detection of an AZFa microdeletion dictates that TESE not be performed because the prognosis for sperm retrieval is grave [20,21].

The AZFb and AZFc regions are located further along Yq in the P5 through P1 genomic span. In actuality, multiple possible sites of microdeletion may arise in this stretch. Known by various names, however, such as AZFb and AZFc, they are simply different microdeletions of different lengths, occurring at different frequencies, and with different proximal and distal end points, but all within the boundaries mentioned earlier and all a consequence of ectopic homologous recombination. AZFb and AZFc appeared to be distinct and nonoverlapping on Yq in the early days of sequencing of the Y chromosome. Precise definition of the P5 to P1 interval has shown, however, that the AZFb and AZFc regions are indeed overlapping and simply represent different sites of ectopic homologous recombination within this expanse [10].

AZFc is the most common microdeleted region found, happening de novo in approximately 1:4000 men overall, and found in 13% of azoospermic and 6% of severely oligospermic men [22,23]. The AZFc region spans 3.5 megabases (Mb), beginning in the distal aspect of the P3 palindrome and extending into the P1 palindrome [9]. The AZFc region microdeletion is also known as b2/b4 because two of the four blue amplicons (as colorized in the Kuroda-Kawaguchi article, 229 kb in length) flank this 3.5-Mb expanse and are the sequences that undergo ectopic homologous recombination; the resulting loss of intervening genomic material is what we define as an AZFc microdeletion. An AZFc microdeletion event removes several genes that inhabit this region, perhaps most importantly, the four copies of DAZ that exist here [24,25]. DAZ encodes an RNA-binding protein expressed primarily in spermatogonia, possibly activating silent mRNAs during meiosis [26,27]. The genes within the AZFc region are not critical for meiotic recombination but “in the absence of the AZF region, the transient zygotene stage is extended, and chromosome condensation is reduced” [28].

Oates and colleagues [29] have provided the most relevant clinical correlations to AZFc microdeletions, as follows. An AZFc microdeletion quantitatively impairs spermatogenesis. Infertility and sterility are the rule, although natural paternity has been reported [30]. It is extremely rare to find sperm density greater than 5 ×106/mL. In their sample of 42 men, 38% were severely oligospermic and 62% were azoospermic. Of the azoospermic men, 67% had some level of spermatogenesis noted on testis biopsy or TESE. Critical to remember, however, is that 19% of the overall group did not have sperm available from either the ejaculate or testis tissue. AZFc-microdeleted men do not appear to have any other health or testis-specific consequences; the genes lost appear to be important only in spermatogenesis. Nearly all AZFc microdeletions are de novo (the father is not microdeleted himself, but the Y chromosome in the sperm that he produced and that fertilized the egg that became the patient had suffered a microdeletion event). Spermatogenic potential, at whatever low level it might be, appears to be stable over time. No historical or physical findings, or hormonal predictive factors, can forecast whether sperm will be found in the ejaculate, in the testis tissue only, or not at all. When spermatozoa from an AZFc-microdeleted man are used in conjunction with
intracytoplasmic sperm injection, they appear to work well; quality is preserved [31]. The children of \textit{AZFc}-microdeleted men are somatically healthy but all male offspring will harbor an \textit{AZFc} microdeletion and the spectrum of reproductive impairments is similar to that found in de novo cases, with their level of ultimate spermatogenesis not necessarily the same as their fathers’ (Fig. 2) [32]. If a Y chromosomal microdeletion assay defines an \textit{AZFc} microdeletion, the couple may choose not to use his sperm, may choose to use it for ICSI, or may choose to use preimplantation genetic screening such that only female embryos will be transferred, thereby eliminating the propagation of an \textit{AZFc} microdeletion and its consequent infertility/sterility [33].

The \textit{AZFb} region is not distinct from the \textit{AZFc} region but simply represents another possible site of ectopic homologous recombination within that expanse that extends from the P5 palindrome to the P1 palindrom. As Repping and colleagues [10] have clearly described, \textit{AZFb} is a 6.2-Mb microdeletion, beginning in the P5 palindrome and ending in the proximal portion of the P1 palindrome (Fig. 3). The \textit{AZFb/AZFc} microdeletion is an even longer one, 7.7 Mb, with its origins in P5 (as for \textit{AZFb}) and its termination in distal P1. Of utmost clinical importance is the fact that with both of these microdeletions, little, if any, hope exists of sperm retrieval by TESE and this fact should be clearly communicated to non-obstructive azoospermic patients before any surgical attempts at testicular sperm retrieval [21]. Once again, a Y chromosomal microdeletion assay, unfortunately, will be prognostic for these men. Combined, these two microdeletions may be found in approximately 1% to 3% of the NOA population.

**Nonobstructive azoospermia: chromosomal abnormalities**

\textit{47,XXY} Klinefelter syndrome

Numeric and structural chromosomal aberrations may be identified by karyotype in approximately 14% of NOA or severely oligospermic men [34]. Klinefelter syndrome (KS) occurs in 1:500 to 1:1000 live male births and in up to 10% of NOA men [35–38]. Most cases are of the nonmosaic form, 47,XXY. The presence of the extra X chromosome sets in motion several undefined events leading to spermatogenic and androgenic failure or compromise, gynecomastia, and learning difficulties. The presence of male children with \textit{AZFc} microdeletions should prompt a search for their father’s karyotype; if an \textit{AZFc} microdeletion is found in the father, the couple should be counseled about the risk of transmitting another \textit{AZF}\textit{bc} microdeletion to the male offspring. The exact genetic and phenotypic spectrum of the male babies born with \textit{AZF}\textit{bc} microdeletions remains to be determined, but we do know that these men are at risk for developmental delays and learning difficulties.

**Fig. 2. Vertical transmission of \textit{AZFc} microdeletion (spectrum of spermatogenic deficiency in sons).** Transmission of \textit{AZFc} deletion to male offspring: If sperm are found in the ejaculate or testis tissue and used in conjunction with ICSI, any male offspring will inherit the microdeleted Y chromosome. The male offspring would then be predicted to display the same phenotypic variability of the men we diagnose currently: severe oligospermia treatable with ICSI, azoospermia with sperm present in the testis tissue and treatable with ICSI, or azoospermia but without sperm in the testis tissue. The exact phenotypic spectrum of the male babies born will only be known in many years when these boys become of reproductive age. The fact that their father had sperm to be used for ICSI does not imply that the son will also have sperm in the future; he may have no useable sperm.
difficulties, particularly in expressive language [38]. The origin of the supernumerary X chromosome may be either maternal or paternal. Fathers of KS boys have higher levels of 24,XY sperm. “Compared with fathers in their 20s (who had an average frequency of 7.5 XY sperm per 10,000), the frequencies of XY sperm were 10% higher among fathers in their 20s, 31% higher among those in their 40s, and 160% higher among those in their 50s” [39,40]. KS exhibits a wide clinical spectrum [37]. On the severest end are boys identified as having KS when they fail to undergo puberty and virilization as a result of near complete androgenic malfunction. These boys have a eunuchoid appearance and may be taller than their predicted height and require testosterone replacement to mature. Little hope would exist of their testes harboring any normal seminiferous tubules because testicular histology would show extensive fibrosis and sclerosis. On the opposite end of the phenotypic spectrum are those adequately androgenized men who are found to have KS during the evaluation of their NOA. They had enough testosterone output during the teenage years to pass through puberty normally. They generally have a normal libido and erectile function and their testosterone level ranges from slightly below normal to normal [41]. It is important that the clinician be aware that KS may predispose men to a higher mortality from breast cancer and non-Hodgkin’s lymphoma (standardized mortality ratios of 57.8 and 3.5, respectively), while protecting against prostate cancer [42]. Young men with extragonadal mediastinal germ cell tumors may have KS and need to be karyotyped [43]. Follicle-stimulating hormone is elevated, reflecting spermatogenic compromise. Regardless of the testosterone output, luteinizing hormone is elevated in a compensatory fashion because the Leydig cells are maximally stimulated with little reserve capacity [44]. What is common to all in the KS spectrum, however, is the small testis size, generally about 8 to 10 cm³. Some teenage boys are suspected of having KS because of their testis volume as found on physical examination, even though they may be experiencing Tanner stage progression on schedule (vide infra).

Successful sperm retrieval is reported in up to 69% of KS men [45–47]. No patient characteristics have been widely agreed on as predicting sperm presence, although Madgar and colleagues [48] believe that testis volume and testosterone levels may be helpful. Okada and colleagues feel the chances are better before age 35 than after, and recommend sperm retrieval before this age [49,50]. Sperm retrieved from individuals with KS yields high fertilization rates. Embryo development is adequate and live births have been reported [51–53]. TESE is typically performed coincident with oocyte retrieval, but cryopreserved spermatozoa (obtained at a time before an ICSI cycle) have been used successfully [54]. One fetus in a triplet gestation was found to be 47,XXY and terminated [55]. All other offspring have been 46,XX or 46,XY. Theoretic concerns exist about the possible genotype of any conceptus, based on the knowledge that sex chromosomal and autosomal disomy occurs in 0.9% to 7.5% of individual sperm cells analyzed [39,56]. Using the real-life data presented earlier about the karyotypic fate of those children actually born, this concern would not seem to be major, but some investigators still recommend preimplantation genetic diagnosis be used [57,58]. It is unclear whether completion of spermatogenesis can only be accomplished from a miniscule number of cryptic 46,XY spermatagonia that reside scattered about a few healthy seminiferous tubules (low-level gonadal mosaicism) or that a rare 47,XXY spermatogonium may, on occasion, undergo proper meiosis with loss of the extra X and production of only 23,X or 23,Y spermatozoa [59]. Okada and colleagues [60] have documented the potential decline in testosterone levels after

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Fig. 3. AZFb, AZFb/c, and AZFc microdeletions in the P5 to P1 span.

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either conventional or microdissection TESE, a fact that the patient has to be aware of before undergoing the procedure. Most likely, the remaining Leydig cells are simply not able to compensate with an increased output and the biologic levels of testosterone adequate for that patient may not be reached. However, the initial drop to 80% of pre-TESE levels that occurs at 3 to 6 months following surgery may rise back up to 95% after 18 months and therefore, native testosterone levels need be followed longitudinally [61].

Some young men are diagnosed at a time when they may not be actively engaged in attempting to achieve pregnancy. It is unclear whether TESE with cryopreservation of any retrieved spermatozoa should be undertaken at that time, as reported by Damani and colleagues [62], or whether it would be wiser to wait until later in life when biologic paternity is desired. Sperm production decline may be ongoing and progressive, beginning in early infancy [63]. These young men do not necessarily require testosterone therapy, and the disadvantages of early surgery (precipitation of need for testosterone replacement, failure of cryopreserved sperm to be functionally competent in the future, scarring of testes) may outweigh the possible theoretic advantages relating to improved chances of finding sperm the younger the individual is on whom TESE is performed [64,65]. The phenotypic spectrum is wide and KS should be suspected in any NOA patient, especially with coexistent impairment of the androgenic axis. A karyotype should be performed before any attempt for operative sperm retrieval in a patient who has presumptive NOA.

46,XX male syndrome

Found in approximately 1:20,000 newborn male babies, 46,XX testicular disorder of sex development (also known as 46,XX male syndrome) is found rarely in an infertile, phenotypic man [66]. Ninety percent will have SRY (within a small distal portion of Yp) translocated to either the tip of the X chromosome (mostly an abnormal XY interchange between PRKX and PRKY) or to an autosome [67]. The remaining SRY-negative 46, XX men are presumed to have abnormalities somewhere along the genetic cascade, leading to gonadal differentiation [68]. If SRY is present in the genome, the bipotential gonads become testes, which secrete testosterone. Testosterone induces the mesonephric duct to form the distal two thirds of the epididymis, the vas deferens, and the seminal vesicles and, after conversion to dihydrotestosterone, induces the external genitalia to virilize. Mullerian-inhibiting substance, a product of Sertoli cells, induces regression of the paramesonephric duct [69]. Therefore, 46,XX men are phenotypic men in terms of internal and external anatomy, but the testes will be small, their height may be less than the average man, and the incidence of gynecomastia and cryptorchidism may be elevated [70]. However, the AZFa, AZFb, and AZFc regions are not present and spermatogenesis does not occur. In this disorder, the karyotype is prognostic and the patient does not need either testis biopsy or an attempt at TESE.

Other structural abnormalities can be found on karyotypes that are important to recognize in the infertile man [71]. Y chromosomal abnormalities specifically may include ring Y, truncated Y, isodicentric Y, and various mosaic states involving loss of the Y chromosome in a percentage of cells [34]. A ring Y results from loss of chromosomal material and circularization of the remaining material. Ring Y chromosomes are models for other structural abnormalities of the Y in that a Y chromosomal microdeletion assay must be obtained as a complementary test to determine if any of the AZF regions are present or if the replicative mishap was extensive enough that these regions are missing [72]. If all are absent, TESE will not be successful and surgery does not need to be performed. Robertsonian and reciprocal translocations occur more often in the severely oligospermic population than in the azoospermic man [73]. In these circumstances, genetic counseling is mandatory and in those in whom it is appropriate, preimplantation genetic diagnosis may increase the chances of a healthy live birth [74].

In summary, a Y chromosomal microdeletion assay and karyotype should be obtained in all NOA and severely oligospermic men before use of any ejaculated sperm or TESE. These results must be interpreted based on available knowledge to maximize the chances for, and the health of, offspring.

Obstructive azoospermia: congenital bilateral absence of the vas deferens

CBAVD occurs in 6% of cases of OA and in 1% of infertile men [75]. Of the two genetic causes, one is postulated and one definitive [76,77]. On physical examination, testis size and
consistency are normal, and the caput of the epididymis is full and firm because the efferent ducts that compose it are distended with sperm. The distal two thirds of the epididymis may or may not be present and the vasa are absent. Otherwise, the patient has normal male external genitalia and pulmonary and pancreatic function are not compromised [78,79]. The seminal vesicles are atrophic, dysfunctional, or absent. Without seminal vesicle fluid, the ejaculate consists only of the small amount (0.6 mL) of acidic (pH 6.5) prostatic secretion. These seminal vesicle anomalies can be defined with transrectal ultrasonography [80].

Cystic fibrosis (CF) afflicts 1:1600 people of northern European descent [81]. It is less common in those with other ethnic or geographic backgrounds. Therefore, the panel used to detect CF mutations should be somewhat population specific, selected based on the patient’s ethnic and racial origins [82]. Obstructive pulmonary disease secondary to thickened respiratory epithelial secretions is the hallmark of clinical CF [83]. Pancreatic exocrine failure may also be present, again secondary to thickened and occlusive ductal secretions. Absence of the vas deferens occurs in male CF patients [84]. Mutations must be present in maternal and paternal CF genes for the disease to be manifest. This gene encodes the cystic fibrosis transmembrane conductance regulator (CFTR), a protein crucial for the maintenance of proper sodium/chloride balance in epithelial secretions necessary to optimize their viscosity and fluidity. If only one CF allele is mutated, the patient will not suffer clinical consequences because the total normal pool of CFTR is enough (at least 50%) that pulmonary and pancreatic function are unaffected. This state is the carrier state. However, when both alleles harbor a mutation or abnormality, the amount of competent CFTR will be diminished, the degree to which depends on the nature of the two CF gene anomalies. If dysfunctional enough, pulmonary and pancreatic secretions become tenacious and thick, with consequent obstructive sequelae. The vasa will also be absent. If the CFTR anomalies are less severe, pulmonary and pancreatic function may be maintained and vasal agenesis may be the only recognizable effect. This presentation is the mildest presentation of CF mutation-related disease and is the clinical picture seen in CBAVD [85]. It is the nature of the mutations that determines the disease presentation; the more “severe” the effect on CFTR, the more devastating the phenotypic outcome. As with all genetic disease, a wide clinical spectrum exists; one of the “mildest” presentations of CFTR dysfunction is CBAVD.

A three-base pair deletion, termed deltaF508, is the most common mutation found in the northern European CF and CBAVD population [86]. The resultant aberrant CFTR protein product is severely handicapped and is dysfunctional. When deltaF508 is found on both alleles, the homozygous state, the clinical picture will be pulmonary and pancreatic disease with vasal agenesis. If the two mutations present have less of a profound effect on CFTR quantity or quality (eg, deltaF508 and 5T [the most common combination in CBAVD men]), the pancreatic and pulmonary systems may be unaffected and CBAVD may be the only clinical manifestation [87]. Sinusitis, bronchitis, and pneumonia, along with CBAVD, may be an intermediate clinical expression, based on intermediate mutation “severity.” In men with CBAVD, approximately 80% will have at least one detectable CF mutation (the second one presumed present but not defined) [88]. The vas, seminal vesicles, and distal epididymis become atretic during the latter stages of embryogenesis and early fetal development, implying that the mesonephric ducts are embryologically normal. This finding is further supported by the fact that men with CFTR-related disease have normal renal anatomy and function. As per the American Urological Association Best Practice Policy Committee’s Report on the Evaluation of the Azoospermic Male, “At a minimum, genetic testing for CFTR mutations in the female partner should be offered before proceeding with treatments that use the sperm of a man with CBAVD. If the female partner tests positive for a CFTR mutation, the male should be also be tested. If the female partner has a negative test for CFTR mutations, testing of the male is optional” [89]. However, the CBAVD man may be the first in his family to have confirmation of CF mutation spectrum disease, if tested, and his siblings may benefit from delineation of their own mutation status. In addition, a patient’s heretofore unexplained conditions such as recurrent sinusitis or bronchitis may now be defined as a CF mutation spectrum pathology.

Sperm aspiration procedures, either microsurgical or percutaneous, can be successfully used to harvest fully functional spermatozoa from the epididymal remnant, the efferent ducts, or the testis, for use in conjunction with ICSI [90–93]. Frozen-thawed sperm work as well as those freshly obtained [94]. If the patient’s partner
harbors a CFTR mutation, preimplantation genetic screening can be used to eliminate those embryos destined to develop CFTR mutation spectrum disease and allow transfer of those that will not. In this circumstance, a 25% chance exists that an offspring will inherit both maternal mutation and paternal abnormal alleles, with clinical CF the possible result (Fig. 4). This possibility should be delineated well before aspiration and ICSI are performed. The birth of a child with clinical CF would be a tragedy and proper genetic assessment and counseling should always be obtained before any surgical intervention.

A putative second CBAVD genetic cause involves failure of appropriate mesonephric duct differentiation [76]. Any abnormality before week 7 of gestation may affect division or morphogenesis of the mesonephric duct into its two derivatives: (1) the ureteral bud, which induces the metanephric blastema to form the kidney, collecting system, and ureter and (2) the reproductive ductal portion, which gives rise to the ejaculatory duct, the seminal vesicle, the vas deferens, and the corpus and cauda epididymis [95]. Any insult to the nascent mesonephric ducts before week 7 may impair urinary and reproductive ductal structure formation. Renal agenesis or ectopia and vasal agenesis may be the consequence of such an abnormality, whereas any aberrant event that occurs after week 7 may damage either system individually.

This possible genetic and phenotypic scenario may be realized in its most severe fashion as bilateral renal agenesis with Potter’s syndrome and early neonatal death. CBAVD, with associated unilateral renal agenesis, may be a slightly less pronounced phenotypic outcome. As such, CFTR mutations are not the underlying genetic reason and do not occur at any higher frequency than in the general population. The couple needs to be warned that the genetic basis is unknown, the transmission pattern is undefined, and the likelihood that the fetus will have bilateral renal agenesis in undetermined, although it has been reported. Early prenatal ultrasound is recommended. Renal ultrasound should be performed in CBAVD men to identify this entity.

A few men with low-volume, low-pH, azoospermic semen analyses and unilateral vasal agenesis will have the same mutation CFTR spectrum as CBAVD men [96]. The vas that is palpable in the scrotum is either nonpatent throughout its course or becomes so in the inguinal or pelvic area (in actuality, bilateral vasal disease). Partner testing is advised, as for CBAVD.

**Genetic defects affecting sperm form and function**

**Globozoospermia**

Globozoospermia is a rare condition afflicting less than 1% of the infertile male population [97]. Absence of the acrosomal cap leads to a perfectly circular or pear-shaped head [98]. The sperm are functionally impotent and unable to penetrate the outer investments of the oocyte. The nuclear chromatin and membrane, the sperm midpiece, and the mitochondrial sheath may also all be dysmorphic [99]. A higher rate of sex chromosome disomy and diploidy has been documented, raising the specter of aneuploid embryo creation with ICSI [100]. However, ICSI has met with success, with and without calcium ionophore oocyte activation, and the offspring have been phenotypically normal [98,101–103]. When oocyte activation was used, Dirican and colleagues [101]...
reported a fertilization rate of 33%, and without activation, a 9% rate, possibly because of a lack of a sperm-derived oocyte activation factor in some cases [104]. Although the genetic basis has long been a mystery, familial cases strongly suggest a genetic origin. Investigation of homologous human genes in cases of similar sperm pathology (such as the murine casein kinase II alpha catalytic subunit gene, which is mutated in mice with a globozoospermia phenotype) has not shown similar mutations or defects [105]. Recently, however, Dam and colleagues [106] described a homozygous mutation in SPATA16 in three affected brothers from a consanguineous family. SPATA16 is a spermatogenesis-specific gene. Perhaps the genetic cause is close at hand. At this time, couples can only be informed that the children born have been healthy and that the genetic basis of all types of globozoospermia has not been elucidated.

**Primary ciliary dyskinesia**

Primary ciliary dyskinesia (PCD) comprises various autosomal recessive syndromes resulting from a defect in the motor apparatus, the axoneme, of ciliated cells in the respiratory tract or in the tail of spermatozoa, with a prevalence of 1:20,000 to 1:60,000 [107]. The axoneme is based on a 9 + 2 microtubular arrangement, with nine doublets of tubules circumferentially surrounding a central doublet. The dynein complex is responsible for the generation of movement through its ATPase function. Numerous structural defects have been cataloged by electron microscopy, including absence of the outer or inner dynein arms, the radial spokes, or the central doublet [108]. Kartagener’s syndrome describes the triad of chronic sinusitis and bronchiectasis, dextrocardia, and infertility, all clinical consequences of ciliary inadequacy and accounting for 50% of cases of PCD [109]. Most cases of PCD are diagnosed in childhood with confirmatory electron microscopy on nasal biopsy cells [110]. Occasionally, the diagnosis is not made until adulthood, when complete sperm immobility provides the necessary clue [111]. Spermatogenesis is normal and the reproductive ductal system is patent. Semen analysis shows adequate ejaculate volume and appropriate sperm density, but absent sperm motility. The semen analysis may, incorrectly, be reported as demonstrating necrospermia, which is incorrect because most of these sperm are perfectly viable. Probably no single genetic defect underlies all cases of PCD because numerous substructures are part of the construction of the axoneme [112]. However, recent data show that 10% of patients who have PCD harbor mutations in DNAI1, a gene encoding an axonemal outer dynein arm protein and located on 9p21 [113]. In cases of PCD with normal axonemal substructure, mutations have been found in DNALH11, a gene encoding a dynein heavy chain and located on 7p21 [114]. Finally, mutations have also been found in DNAH5, another gene encoding a heavy dynein chain protein and located on 5p15 [115,116]. Routine genetic analysis of suspected patients is not yet available. ICSI can be used to generate pregnancies and the offspring have been phenotypically normal [117].

**Fibrous sheath dysplasia**

Fibrous sheath dysplasia is a distinct syndrome affecting the development of the sperm tail fibrous sheath. The fibrous sheath maintains the structural integrity of the spermatozoal tail. Interaction with the axoneme is necessary to generate movement [118]. Also known as Stump-tail syndrome, partial and complete forms have been described. By using ICSI, fertilization, embryo development, and live birth can be accomplished [119]. Worrisome, however, are data showing higher rates of diploidy and sex chromosomal disomy in sperm affected by fibrous sheath dysplasia [120,121]. According to Moretti and Collodel [121], “These data contribute to the growing body of evidence that genetic sperm defects of sperm flagella are generally correlated with meiotic segregation derangement.” The underlying genetic basis has not been elucidated, although mutations in three genes that encode for different fibrous sheath proteins (AKAP4, AKAP3, and AKAP82) have not always been found [122].

**Summary**

In summary, our evolving therapies have allowed the use of sperm from men with spermatogenic compromise, OA, and sperm functional deficiency, enabling these men to procreate when unable to do so naturally. The genetic basis of only a portion of these conditions is known and we must be vigilant and continue to pursue research into the genetic underpinnings of those that have not yet been delineated. Education and provision of information to our patients is the responsibility of all involved in the care of these men with reproductive failure. The consequences
of their decisions to try to achieve biologic paternity and the outcomes for their children cannot be underestimated when no, or only partial, data are available about the genetic cause of their reproductive compromise.

References


[113] Zariwala MA, Leigh MW, Ceppa F, et al. Muta-


It has been estimated in developed countries that as many as one in four couples has difficulty conceiving a child [1]. Because infertility can be caused by male factors, female factors, or combined factors, much research and innovation have been used to determine the best treatment modality for each cohort of patients. One of the oldest reproductive technologies, which was described more than 200 years ago and is still currently in use, is artificial insemination [2]. Since its emergence, many refinements have been made with respect to its clinical indications and use. Although significantly cheaper and technically easier than many other reproductive technologies, it is an effective tool in the armamentarium of reproductive specialists. In this article we define male subfertility, review clinical indications for the use of intrauterine insemination, evaluate insemination preparation and administration technique, and ultimately advocate for its use as a first-line therapy in the treatment of infertile couples with male subfertility.

Definition of male subfertility

When defining male subfertility, many authors use nomenclature such as normozoospermia, oligozoospermia, asthenozoospermia, teratozoospermia, and oligoasthenoteratozoospermia. Grimes and Lopez [3] recently highlighted that these terms are actually vague because their definition and use seem to be author dependent. As a result, it is inherently difficult to construct an authoritative working definition of male subfertility and discuss its prevalence when reviewing the current body of literature. Keeping in mind the inherent challenges in such terminology and in an attempt to generate a comprehensive clinically relevant definition, we advocate adopting an approach that is based on previously published definitions stating that male subfertility is the presence of one or more subnormal sperm parameters—sperm concentration less than 20 × 10⁶/mL, motility less than 40%, or normal morphology less than 5%—in two consecutive semen analysis and in the absence of antisperm antibodies [4–8]. Although we realize that no definition is perfect and that many fertile men may be diagnosed as subfertile with the aforementioned motility and morphology thresholds, leading to them being potentially “overtreated,” we believe that by stating clear parameters and avoiding confusing terminology, it is much easier to interpret the available data and integrate these findings into clinical practice. In their study population of 495 couples with semen analyses that revealed more than 2 million motile postwash sperm on day of in vitro fertilization, Keegan et al [5] found that 55% had less than 5% normal sperm morphology, which lends credence to the idea that a large part of the population may be defined as subfertile.

Clinical indications for intrauterine insemination

Abnormal semen analysis

According to the World Health Organization guidelines for the standardized diagnosis, investigation, and management of infertile men, all couples who are unable to conceive after 1 year of unprotected intercourse warrant further investigation into the causes of their infertility [9]. As part of this initial evaluation, semen analyses should be conducted ideally after 2 to 3 days of sexual abstinence. Standardized procedures should be
followed by patients submitting semen for analysis. Research has shown that there may be variability for samples collected via masturbation versus those collected via a silastic condom [10]. Regardless of mode of collection, we believe that it is important to conduct at least two, preferably four, separate identically collected semen samples because of the inherent variability of semen parameters in men [4]. If the semen analyses are abnormal (as defined previously) but do not demonstrate azoospermia, the couple is a candidate for intrauterine insemination using the sperm preparation techniques discussed later.

**Ejaculatory abnormalities**

There are different forms of ejaculatory dysfunction, which should be defined and approached as discussed by Ohl (see the article elsewhere in this issue). More than 50 years ago, one of the earliest descriptions of successfully treated ejaculatory failure was the artificial insemination of a 24-year-old otherwise healthy woman with sperm collected from her husband’s postcoital urine [11]. Although this patient’s retrograde ejaculation was surmised to be secondary to an inflamed ectopic right ureteral orifice opening into the prostatic urethra, there are many causes of ejaculatory failure other than anatomic aberrations [2,11]. As discussed in the article by Ohl elsewhere in this issue, when ejaculatory failure is considered, it is important to formulate a wide differential, such as—but not limited to—neurologic impairments, iatrogenic causes (eg, certain surgical procedures and medications), psychologic impairment, and anatomic abnormalities [12–15]. Although many of these causes may be obvious from the history, it may be necessary to conduct further diagnostic evaluation using ultrasonography. Transrectal ultrasonography may be used to evaluate for the presence of ejaculatory duct obstruction [16,17]. Intrauterine insemination may be used if sufficient sperm are present in the ejaculate or in postejaculatory urine. It also may be used if sperm are obtained using ejaculation induction techniques, as described in the article by Ohl elsewhere in this issue.

**Immunologic**

Much controversy exists over the presence and clinical relevance of sperm autoantibodies [18–21]. Unfortunately, despite the mounting body of literature that characterizes their presence, no study has characterized the type and titer that cause infertility. Although many antibodies have been found in seminal fluid and antigen/antibody complexes have been seen on sperm, the direct effects of these antigen/antibody interactions are still not known [19,20]. In a recent article, the authors recommended initiating an immunologic evaluation when (1) the semen analysis displays aggregates of sperm, (2) there is low motility with other normal parameters, (3) there is a risk of autoimmune infertility (as in the case of torsion, testis injury, or vasal reconstruction), or (4) there is unexplained infertility with a normal routine semen analysis [22]. Vasectomy has been demonstrated to induce antisperm antibodies in animal models [23–26]. Patients who have undergone successful vasal reconstruction (vasectomy reversal) may experience infertility secondary to autoantibodies induced by the vasectomy.

**Unexplained factors**

A large volume of literature debates the use of intrauterine insemination in couples in whom there is no clearly defined cause of infertility. The true benefit of intrauterine insemination in this circumstance actually may be caused by the close monitoring of the female partner’s cycle, use of ovarian stimulating agents, or even sperm preparation [2,6,7,27–30]. With that being said, intrauterine insemination is significantly less expensive and less invasive than in vitro fertilization and is an appropriate tool in the treatment of infertile couples.

**Methodology**

**Sperm preparation**

Over the last 20 years as in vitro fertilization has become increasingly more popular, improvements have been made in sperm preparation techniques such that motile viable sperm can be separated easily from the seminal plasma and dead, suboptimal spermatozoa, leukocytes, and bacteria. It is important to segregate the sperm from these potential detrimental elements and inseminate with the most concentrated sample possible. Leukocytes, dead sperm, and bacteria can produce oxygen radicals and other metabolic byproducts, which can lead to a suboptimal environment for fertilization to take place [31–34]. The seminal plasma contains a high concentration of seminal prostaglandins, which can induce uterine cramps and make it uncomfortable for the patient.

Although these principles are widely held, much research has been done to investigate the
optimal sperm preparation technique. After evaluating five randomized controlled trials (262 couples), a recently published Cochrane review of semen preparation techniques for intrauterine insemination found that there is a dearth of significant evidence to support gradient, swim-up, wash, or centrifugation as a superior technique over any other [35]. There was no difference in the miscarriage rate in two studies that compared swim-up versus a gradient technique [35]. With respect to the concentration of sperm in inseminate, Martinez et al [29] found that pregnancies were only obtained when more than $2 \times 10^6$ sperm were inseminated, with the mean number of sperm in their conceptional cycles being 2.4 to $55 \times 10^6$. The mean number of sperm in their nonconceptional cycles was 0.2 to $240 \times 10^6$. We can surmise that if a high concentration of morphologically normal motile sperm can be collected, the choice of the preparation modality is up to the practitioner.

Ovarian stimulation

Previous authors have demonstrated that intrauterine insemination has a definite advantage over timed intercourse [29]. Although many researchers would agree with these findings, because the sperm has been prepared and refined as stated previously, much controversy still exists over the use of ovarian stimulation in conjunction with intrauterine insemination in treating couples with male subfertility. For example, Cohlen and colleagues [27] found that when a moderate to severe semen deficit is noted, there is no advantage to using controlled ovarian hyperstimulation; however, when there is a mild semen defect ($> 10 \times 10^6$ spermatozoa), controlled ovarian hyperstimulation can improve the chances of conception. In a systematic review of 5214 cycles reported in 22 trials, researchers found that when stratifying the results of a couple with 2 years of unexplained secondary infertility, their untreated cycle fecundity would be approximately 4%, when treated with follicle stimulating hormone it would be 8%, and when used in conjunction with intrauterine insemination it could be as high as 20% [36]. A more recent study demonstrated that intrauterine insemination did not improve pregnancy rates in couples with unexplained or mild male factor subfertility. In the mildly hyperstimulated group (follicle-stimulating hormone) there was a significantly higher rate of multifollicular development and subsequent multiple pregnancies [28]. Taken together, the literature shows that the use of controlled ovarian hyperstimulation is of added value when used in an appropriately selected patient population.

We advocate a step-wise approach to intrauterine insemination starting without an ovulation-inducing agent, using clomiphene citrate, which is lower in potency than injectable gonadotropins, progressing to low-dose gonadotropins, and ultimately using higher dose injectable medications over progressive cycles. As discussed later, as with any procedure, ovulation induction is not without risk. It requires close monitoring and a practitioner who is committed to canceling a cycle if the patient is at any potential risk for multiple pregnancy, ovarian hyperstimulation syndrome, or other known complications.

Timing and number of inseminations per cycle

In patients undergoing natural cycles with insemination, it is our practice to monitor a patient’s serum luteinizing hormone levels as a marker of ovulation and perform serial sonograms to look for correlating follicles of an ideal size of 18 to 20 mm. In patients undergoing a natural cycle, a patient is inseminated approximately 24 hours after the luteinizing hormone surge is noted. With patients undergoing ovarian stimulation, human chorionic gonadotropin is administered to induce ovulation once the luteinizing hormone surge is detected; a patient is subsequently inseminated approximately 24 hours later.

The number of inseminations per cycles also may influence pregnancy rates after intrauterine insemination. In a review of 18 randomized and prospective trials that compared two inseminations versus one insemination per cycle (1156 intrauterine insemination cycles), researchers found that although the pregnancy rate per cycle was higher in the group that received two insemination per cycle (14.9% versus 11.4%), there was no significant difference [37]. The authors noted that there was considerable heterogeneity regarding the ovarian management and timing of the insemination, but more research is needed to further elucidate the use of multiple inseminations per cycle. In our practice we only administer a single, appropriately timed insemination per cycle.

Technique of insemination

As opposed to in vitro fertilization, in which extensive research has been done on the success rates of different catheters at the time of embryo
transfer, fewer studies have compared the catheters used during intrauterine insemination [38].
With respect to those used for in vitro fertilization, soft catheters, regardless of manufacturer, were found to be superior to rigid catheters [38]. Although speculative, the prevailing thought is that endometrial trauma at the time of transfer may induce uterine contractions, which could lead to a suboptimal environment for implantation [38,39]. In a systematic review and meta-analysis of intrauterine insemination catheters, researchers found that catheter choice does not influence outcome. The authors hypothesized that because fertilization takes place in the fallopian tube, even if uterine trauma occurred, it would be potentially healed by the time of implantation days later [40]. More studies are needed to elucidate any potential differences between rigid and soft catheters, however.

Another area of controversy regarding intrauterine insemination techniques relates to insemination delivery technique. A randomized cross-over study demonstrated that the pregnancy rate was three times higher after slow-release intrauterine insemination compared with the bolus technique [41]. Unfortunately, the aforementioned study was limited to patients diagnosed with cervical hostility, and the applicability to the patient subpopulation of subfertile men has yet to be determined. The authors described their slow-release method as requiring a patient to lie in bed with a continuous infusion of spermatozoa (3 x 10^6/hr) for 3 hours. Although we find this method of insemination to be of academic value, we believe that it is less than practical because it requires a patient to endure a significantly longer period of discomfort during the insemination process and lends itself to an increased risk of introducing pathogens into the uterine canal.

Risks of intrauterine insemination

In a review of 38 reported series of intrauterine insemination, it was found that 5 of the 3129 couples studied experienced an infectious complication [42]. The prevalence of infectious complications was estimated to be 1.83 per 1000 women undergoing intrauterine insemination, and this rate was not altered by semen washing with antibiotics or by the administration of prophylactic antibiotics to the women.

Beyond the risk of infection in the setting of an undiagnosed infection, there is a risk for multiple pregnancies. When ovulation-inducing agents are administered, the risk of multiple pregnancies increases significantly, as seen in Table 1, which was adapted from an analysis of 17 studies evaluating the outcomes of more or less aggressive stimulation protocols [7]. Finally, in a recent Cochrane review of intrauterine insemination for male subfertility, the authors stated that there was insufficient data available to statistically evaluate adverse outcomes, such as miscarriages, ovarian hyperstimulation syndrome, and ectopic pregnancies, which are all potentially life-threatening conditions [43]. When counseling patients about their specific treatment plan, it is important to note that intrauterine insemination is not without risk and that with increasing potency of stimulating agents there is a well-recognized risk of multiple pregnancies and other untoward outcomes.

Summary

Despite a considerable amount of research regarding the use and methodology of intrauterine insemination in the treatment of infertile couples with male subfertility, more research is necessary. More than 20 years ago research showed that when the motile sperm count is more than 1 million/mL, intrauterine insemination may provide for near-normal fecundity if there is no contributing female factor [44]. Researchers recently showed that the total motile sperm count actually has the highest predictive value of success because it is a measure of not only sperm concentration and motility but also the effects of the sperm preparation [45]. As we have discussed, however, semen parameters alone are not a predictor of a successful outcome.

<table>
<thead>
<tr>
<th>Type of stimulation</th>
<th># of cycles</th>
<th>% multiple pregnancies</th>
<th>% triplets or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC/HMG^a</td>
<td>593</td>
<td>5</td>
<td>0.0</td>
</tr>
<tr>
<td>75–250 IU HMG^b</td>
<td>2560</td>
<td>6.2</td>
<td>1.4</td>
</tr>
<tr>
<td>150 IU HMG^a</td>
<td>1528</td>
<td>19</td>
<td>3.2</td>
</tr>
<tr>
<td>150–225 IU HMG^a</td>
<td>1500</td>
<td>21</td>
<td>4.5</td>
</tr>
<tr>
<td>Analog/HMG^a</td>
<td>259</td>
<td>31</td>
<td>8.5</td>
</tr>
</tbody>
</table>

When evaluating the cost effectiveness of intrauterine insemination versus in vitro fertilization, couples in the in vitro fertilization group were found to be more likely than couples in the intrauterine insemination group to stop therapy before six attempts. Overall, intrauterine insemination was found to be more cost effective [8]. Ultimately, the aforementioned authors found that a woman's age was the only factor that influenced success in their population of couples with male subfertility.

It is important to recognize that a couple's fertility is based on a spectrum of variables, including the age of the partners, medical history, and other contributing factors. When treating the subfertile male population as defined by semen parameters, we advocate for the use of intrauterine insemination as a suitable reproductive technology when conducted at fertility centers that are able to provide appropriate sperm preparation and a stepwise approach to ovulation-inducing agents with close monitoring.

References


Intracytoplasmic Sperm Injection (ICSI) – What are the Risks?

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Over the past 30 years, the treatment of infertility has seen the development of revolutionary new assisted reproductive technologies (ARTs), first in 1978 with the birth of baby Louise Brown, who was conceived by in vitro fertilization (IVF) [1], then with microassisted reproduction using techniques such as intracytoplasmic sperm injection (ICSI) [2], and most recently with the development of preimplantation genetic diagnosis as a technique [3–6]. These highly complex technologies are used with increasing frequency in the treatment of couples around the world; more than 1 million babies worldwide have been conceived in this manner [7]. In 2003 nearly 3\% of children born in Scandinavia and 1.7\% of children born in France were conceived using ICSI. During this time, almost 500,000 cycles were performed in Europe (122,872 cycles in the United States), resulting in the birth of almost 300,000 infants [8,9]. The number of IVF cycles in the United States has increased twofold since 1996, when Society for Assisted Reproductive Technology (SART) began monitoring the IVF programs [10].

Unlike most therapeutic procedures used in medicine, ARTs never underwent rigorous safety testing before clinical use. Treatments for infertility overcome natural barriers that prevent fertilization. Because these technologies are used to overcome infertility phenotypes that may have a genetic basis, the possibility exists that unwanted genetic traits may be transmitted to offspring. Although researchers believe that perhaps 75\% or more cases of all infertility have a contributing genetic basis, our ability to diagnose these defects remains limited. For untreated couples, their infertility represents “lethality” within the gene pool, because their condition essentially blocks the transmission of undesirable genetic traits to any offspring. Put simply, large numbers of couples undergo fertility treatments without a complete understanding of the basis of their infertility or the potential long-term risks for their offspring.

No better example of this phenomenon exists than that of IVF/ICSI as a treatment for severe male factor infertility. Before the development of IVF/ICSI, these men simply could not have reproduced by any means. Currently, a growing percentage of the population of countries in the Western world is comprised of the offspring of these men. What health risks do these people face? What burden will they create from a public health standpoint? Questions regarding the safety of ART—and IVF/ICSI in particular—become even more important.

Retrospective data suggest that IVF and IVF/ICSI are safe. Health risks to mother and offspring that are significantly increased with assisted reproduction include multiple gestation, preterm delivery (even in singleton pregnancy), and congenital abnormalities in the offspring [11–13].
Most IVF pregnancies proceed uneventfully and result in the birth of healthy babies; however, studies consistently identify an increased absolute risk of problems in IVF and IVF/ICSI pregnancies and deliveries [14].

It is challenging to establish the nature of these risks and dissect out whether they are related to the technology itself or the genetic defects of the parents. Other factors cloud this analysis. For example, in the United States, multiple embryos are routinely transferred to increase the ultimate likelihood of a live birth (while also enhancing the chances of a multiple gestation). Whether the altered endocrine milieu that results from hormonal induction of ovulation by ovarian hyperstimulation contributes further to this risk is unclear. Hormonal induction profoundly changes the normal uterine environment and body homeostasis. Because the eldest child conceived by IVF/ICSI is only approximately 15 years old, long-term studies of multiple cohorts of offspring conceived by IVF/ICSI from conception through to adulthood have not been possible. Incomplete follow-up caused by couples seeking treatment at large fertility clinics far from their homes is a problem. After a pregnancy is achieved, families are again followed by their community physicians. Because we are a mobile society, couples are frequently lost to follow-up for other reasons, which makes long-term follow-up of children conceived by ART difficult.

Unfortunately, epidemiologic studies regarding the safety of IVF and IVF/ICSI in general are faced with additional challenges, both in retrospective and prospective studies. These challenges include incomplete reporting and inconsistent definition of congenital abnormalities and other adverse outcomes. Importantly, the assessment of offspring conceived by IVF/ICSI is commonly performed by pediatricians as part of a routine neonatal health examination, yet a medical geneticist may have different criteria for disease. Alternatively, the physician may examine these children more closely than naturally conceived children, and inevitably the closer one looks, the greater the likelihood of finding an abnormality.

The ideal study design to answer the question of whether any ART is safe is nearly impossible to achieve. Fertility cannot be compared directly using standard statistics, because the combined fertility potential of the couple ultimately determines whether a couple is fertile or infertile. The identification of an appropriate control population for infertile couples can present a nearly impossible quest. Selection criteria may bias findings because variability between the two groups exists at baseline. Birth defects and adverse ART events are relatively rare compared with the overall total number of pregnancies and live births each year. The design and implementation of adequately powered research studies are difficult.

Despite these significant limitations, numerous investigations of IVF/ICSI safety have been performed and are reviewed in this article. Although ARTs include various methods to process oocytes and sperm in vitro to enhance the likelihood of fertilization and pregnancy, this article focuses specifically on IVF with ICSI regardless of whether the sperm are retrieved from an ejaculate, the epididymis, or the testis. Importantly, no discussion of the risks associated with IVF/ICSI can be conducted outside of the context of the existing IVF safety data. As such, the safety of IVF and IVF/ICSI are considered here.

**Congenital disorders and hormonal abnormalities in children conceived by in vitro fertilization/intracytoplasmic sperm injection**

Multiple large studies (Table 1) consistently show a higher risk of genitourinary, cardiovascular, musculoskeletal, and gastrointestinal defects in offspring conceived by IVF and IVF/ICSI [15–30]. Other older studies with less methodologic consistency also report similar findings [15,18,19,26,31]. Comparison of the birth defect studies is confounded by differences in the definitions used to classify the birth defects, reporting of congenital abnormalities, and methods used for statistical analysis. These caveats present challenges for interpretation of the data. Given these issues, it may be difficult to conclude that the increased risk of birth defects seen in the IVF and IVF/ICSI cohorts results from ART.

In this regard, several studies are noteworthy. Hansen and colleagues [16,30] reported a higher likelihood of congenital abnormalities in children conceived by IVF and IVF/ICSI based on a study of the birth registry of Western Australia, which focused on 1138 children conceived using ART (301 ICSI children, 837 standard IVF children). This report was followed by a meta-analysis of 25 existing studies of birth defects in children conceived by ART [30]. There was a significantly increased incidence of birth defects in children conceived by ART, including cardiovascular, urogenital, musculoskeletal, and chromosomal abnormalities in the IVF cohort. This trend was
not statistically significant in the IVF/ICSI cohort, with the exception of musculoskeletal and chromosomal abnormalities, perhaps because of inadequate sample size in the IVF/ICSI cohort. Overall, this meta-analysis demonstrated a higher likelihood of birth defects in children conceived by IVF and IVF/ICSI.

A cohort of offspring conceived by IVF/ICSI has been followed over time by the Bonduelle group [28]. This group of 8-year-old children conceived by ICSI was compared with a group of naturally conceived children [32]. Although the children conceived by IVF/ICSI were generally healthy and had no higher likelihood of requiring surgery, hospitalization, or rehabilitation, there was an increased likelihood of congenital malformation in the IVF/ICSI cohort. Approximately 10% of these children had major birth defects, compared with 3.3% of the control group. Minor birth defects were similar in the children conceived by IVF/ICSI and the control group. Using the Western Australian birth registry system referenced in the Hansen studies, 6 of 150 children conceived by IVF/ICSI had a major malformation compared with 1 of 147 of the control children.

Again, some controversy remains. Analysis of the Danish National Birth Registry suggested no greater likelihood of congenital abnormalities for children born to women with significant delay to natural conception (> 12 months) compared with children conceived by IVF/ICSI, with one exception [20]. The incidence of genitourinary tract abnormalities is statistically significantly increased in children conceived by IVF/ICSI. These findings are consistent with other studies, which suggest that patients with subfertility are at higher risk of having a child born with congenital abnormalities. This finding argues that the ART procedures in and of themselves do not contribute to this risk.

Throughout the world, the criteria used to define birth defects differ, which presents unique challenges to analysis of ART safety data. This is best illustrated in a study from Kurinczuk and Bower [33], who analyzed an earlier study published by the Bonduelle group. Four hundred twenty-three children were followed prospectively after IVF/ICSI in the Bonduelle study [34] with no evidence of increased major congenital malformations. Kurinczuk correctly observed that the control population used in this series included spontaneous conceptions pooled from worldwide registries, including the Western Australian birth registry. Again, the different definitions of birth defects in Australia and Belgium (the location of the Bonduelle group) were not considered in the study; when Kurinczuk recategorized the IVF/ICSI cohort based on Western Australian birth registry standards, approximately a twofold higher risk of major congenital malformation in the IVF/ICSI cohort was observed. Clearly, standardization of reporting of birth defects should be mandatory for any study of ART safety given the fact that these two analyses reached different conclusions, albeit with the same data.

An increased incidence of genitourinary tract abnormalities (specifically hypospadias in boys) is consistently found in offspring conceived by ART. Although impaired or abnormal hormonal function is one possible cause of hypospadias, the causes of these defects in some offspring conceived by IVF/ICSI remain unknown. Genital tract abnormalities in some male parents of offspring conceived by IVF/ICSI (eg, hypogonadism or poor testis function) raise the question of whether the congenital genitourinary defect in offspring conceived by IVF/ICSI is the consequence of a genetic abnormality inherited from their fathers. A recent Danish series did observe statistically significantly lower testosterone levels in 125 male offspring conceived by ICSI when compared with testosterone levels in age-matched boys who were naturally conceived [35].

Detrimental effects of oocyte handling and in vitro maturation of immature oocytes were considered possible contributors to the hormonal and developmental effects found in offspring [36]. Studies to date show no risk with in vitro maturation; however, this area remains one of active investigation.

Multiple gestations, preterm labor, and other perinatal complications are more common in pregnancies that result from in vitro fertilization/intracytoplasmic sperm injection

In the United States, most pregnancies conceived by ART result in a multiple-birth delivery. In contrast, only 1.5% of naturally conceived pregnancies result in a multiple birth [10]. Multiple gestation is associated with an increased risk of preterm delivery, low birth weight, and increased perinatal mortality. Theoretically, multiple gestation could even account for higher risks of seemingly unrelated conditions in children conceived by IVF/ICSI, such as the risk of cerebral palsy. As such, it is important to consider the reported differences observed in IVF and IVF/ICSI cohorts [11].
<table>
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<tr>
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<th>Year</th>
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<th>Control</th>
<th>Outcomes</th>
<th>Sample size</th>
<th>Findings</th>
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<tr>
<td>Hansen [16]</td>
<td>Australia</td>
<td>1993–1997</td>
<td>Registry</td>
<td>Yes</td>
<td>Congenital malformations</td>
<td>1138 ART children (301 IVF/ICSI, 837 IVF alone, versus 4000 SC children)</td>
<td>Significantly higher likelihoods of birth defects with IVF and ICSI even after correction (OR 2.0 for both)</td>
</tr>
<tr>
<td>Westergaard [17]</td>
<td>Denmark</td>
<td>1994–97</td>
<td>Registry</td>
<td>Yes</td>
<td>Congenital malformations, pregnancy outcomes</td>
<td>2245 ART children versus 2245 naturally conceived children (cohorts matched for maternal age, parity, and multiplicity)</td>
<td>No difference in risk of congenital malformation (poorer pregnancy outcomes observed in ART cohort)</td>
</tr>
<tr>
<td>Loft [18]</td>
<td>Denmark</td>
<td>1994–1997</td>
<td>Registry/questionnaire</td>
<td>No</td>
<td>Congenital malformations, genetic abnormality, and pregnancy outcome in ICSI conceptions</td>
<td>665 questionnaires returned</td>
<td>No statistically significant differences identified, &gt;90% responder rate to questionnaire</td>
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<td>Zhu [20]</td>
<td>Denmark</td>
<td>1997–2003</td>
<td>Registry/questionnaire</td>
<td>Yes</td>
<td>Time to pregnancy, treatments for infertility, congenital malformations</td>
<td>64,405 children (50,897 singletons and 1366 twins from fertile couples, 5764 singletons and 100 twins naturally conceived by subfertile couples, 4588 singletons and 1690 twins by ART)</td>
<td>A higher likelihood of congenital malformation in ART group and subfertile group with delay to spontaneous conception (&gt;12 mo); as delay increased so did likelihood of malformation</td>
</tr>
<tr>
<td>Koivurova [15]</td>
<td>Finland</td>
<td>1990–1995</td>
<td>Registry</td>
<td>Yes</td>
<td>Congenital malformations and pregnancy outcomes</td>
<td>304 IVF children versus 569 SC children</td>
<td>All adverse pregnancy outcomes were significantly higher in the IVF group but corrected largely after consideration of multiplicity; cardiac malformations higher in IVF cohort regardless of multiplicity</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Year Period</td>
<td>Study Design</td>
<td>Follow-up</td>
<td>Outcomes</td>
<td>Study Population</td>
<td>Key Findings</td>
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<tr>
<td>Ludwig [21]</td>
<td>Germany</td>
<td>1998–2002</td>
<td>Registry</td>
<td>Yes</td>
<td>Congenital malformations</td>
<td>3372 IVF/ICSI children versus 30,940 spontaneous conceptions</td>
<td>RR 1.25 (95% CI 1.11–1.40); used separate population (Mainz Birth Registry) for control cohort</td>
</tr>
<tr>
<td>Katalinic [22]</td>
<td>Germany</td>
<td>1998–2002</td>
<td>Registry</td>
<td>Yes</td>
<td>Congenital malformations and pregnancy outcomes</td>
<td>3372 IVF/ICSI children versus 8016 naturally conceived children</td>
<td>Adjusted OR 1.24 (95% CI 1.02–1.50), higher incidence of birth defects in ICSI cohort after correcting for multiplicity, true control cohort</td>
</tr>
<tr>
<td>Zádori [19]</td>
<td>Hungary</td>
<td>1995–2002</td>
<td>Registry</td>
<td>Yes</td>
<td>Pregnancy outcomes, neonatal complications (including birth defects)</td>
<td>221 ART pregnancies (185 singleton and 36 twin versus identical SC cohort)</td>
<td>A minimal difference between cohorts, except for a significantly higher prematurity rate in IVF singletons; IVF triplets had much higher risks of adverse outcomes in a separate analysis</td>
</tr>
<tr>
<td>Anthony [23]</td>
<td>The Netherlands</td>
<td>1995–1996</td>
<td>Registry</td>
<td>Yes</td>
<td>Congenital malformations</td>
<td>4224 ART children versus 314,605 SC children</td>
<td>Risk of any congenital malformation only slightly higher, corrects after accounting for confounders [Crude OR 1.20 (95% CI 1.01–1.43), corrected OR 1.03 (95% CI 0.86–1.23)]; cardiovascular malformation higher in ART cohort regardless</td>
</tr>
<tr>
<td>Wennerholm [24]</td>
<td>Sweden</td>
<td>1993–1998</td>
<td>Registry</td>
<td>Yes</td>
<td>Congenital malformations</td>
<td>1008 IVF/ICSI children versus all SC in Sweden over comparable time period (no number given)</td>
<td>Adjusted OR (after correcting for multiplicity) 1.19 (95% CI 0.79–1.81); only hypospadias higher in ICSI cohort (RR 3.0)</td>
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<table>
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<tr>
<th>Author</th>
<th>Country</th>
<th>Year</th>
<th>Study type</th>
<th>Control</th>
<th>Outcomes</th>
<th>Sample size</th>
<th>Findings</th>
</tr>
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<tbody>
<tr>
<td>Ericson [25]</td>
<td>Sweden</td>
<td>1982–1997</td>
<td>Registry</td>
<td>Yes</td>
<td>Congenital malformations</td>
<td>9111 IVF children versus 1,690,577 SC children</td>
<td>Unadjusted OR 1.47, adjusted 0.89; higher risk of alimentary atresia, neural tube defects, and hypospadias in ICSI cohort even after correction</td>
</tr>
<tr>
<td>Sutcliffe [26]</td>
<td>UK</td>
<td>1989–1994</td>
<td>Prospective</td>
<td>Yes</td>
<td>Congenital malformation and neurologic development in cryopreserved embryos</td>
<td>91 ART children (cryopreserved embryos) versus 83 SC children</td>
<td>Incidences were higher in the ART cohort, although no numbers reached statistical significance</td>
</tr>
<tr>
<td>Olson [27]</td>
<td>US</td>
<td>1989–2002</td>
<td>Registry</td>
<td>Yes</td>
<td>Congenital malformation and mortality</td>
<td>1462 ART children versus 8422 SC controls</td>
<td>Adjusted OR 1.30 (95% CI 1.00–1.67), higher incidence of birth defects in IVF cohort even after correcting for multiplicity</td>
</tr>
<tr>
<td>Rimm [28]</td>
<td>NA</td>
<td>2004</td>
<td>Meta-analysis</td>
<td>Yes</td>
<td>Compiled 16 IVF studies, 7 ICSI studies</td>
<td>28,524 IVF versus 2,520,988 SC children; 7234 IVF/ICSI children versus 978,078 SC children</td>
<td>Pooled OR of 1.29 (95% CI 1.01–1.67) (statistically significant); all studies examined had design flaws</td>
</tr>
<tr>
<td>Lie [29]</td>
<td>NA</td>
<td>2005</td>
<td>Meta-analysis</td>
<td>Yes</td>
<td>Compiled 4 prospective, well-designed studies (out of 22)</td>
<td>5395 IVF/ICSI children versus 13,086 IVF children (pooled from all four studies)</td>
<td>Pooled RR 1.12 (95% CI 0.97–1.28), no significant increase for any single category of defect with ICSI</td>
</tr>
<tr>
<td>Hansen [30]</td>
<td>NA</td>
<td>2005</td>
<td>Meta-analysis</td>
<td>Yes</td>
<td>Compiled 25 studies (only 7 well-designed per author’s criteria)</td>
<td>28,638 ART children</td>
<td>On both analyses (all 25 studies or limited just to the 7 appropriate studies) pooled OR of malformation was significantly higher; 1.40 (95% CI 1.28–1.53) on analysis of 7 well-designed studies</td>
</tr>
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</table>

**Abbreviations:** CI, confidence interval; OR, odds ratio; RR, relative risk.
When considering this line of reasoning, at first glance it is difficult to account for the fact that several meta-analyses demonstrate an increase in perinatal complications even in singleton pregnancies that result from IVF [28,37–39]. The IVF/ICSI cohorts in these series, regardless of multiplicity, had higher likelihoods of low birth weight, preterm labor (< 36 weeks), and usage of hospital resources (ie, the need for surgery or neonatal intensive care unit admission).

The Danish National Patient Registry provided a large body of IVF/ICSI data; in this population, a higher likelihood of adverse outcomes was observed in multiple gestational pregnancies. Importantly, no such trend existed in IVF/ICSI singleton pregnancies [11,40]. These authors addressed the inconsistencies between their own findings and the meta-analyses and hypothesized that vanishing twin syndrome in these pregnancies plays a causal role in the poorer outcome observed in IVF/ICSI singleton pregnancies [41]. This risk relates to the number of embryos transferred. Importantly, a multicenter, prospective, randomized controlled trial compared outcomes after single-embryo transfer (SET) with transfer of multiple embryos. Pregnancy rates were not substantially lower in the SET cohort, but the number of multiple births was dramatically reduced [42].

SET represents an important intervention because it allows for an adequate success rate to be maintained with IVF and IVF/ICSI while it significantly decreases the risks in offspring that derive from multiple gestation [42]. Remarkably, SET is not routinely performed as standard of care in the United States, and it is still only offered to few women in Europe [8]. The idea that routine performance of SET can mitigate risks such as preterm labor in offspring conceived by IVF/ICSI might seem obvious to most readers, but additional benefits such as a decreased rate of cerebral palsy in offspring conceived by IVF/ICSI would help to minimize the risk of conditions, such as neurologic and developmental delay, in offspring conceived by ICSI cohorts. Prospective research with the goal of confirming this hypothesis is vitally important.

Developmental delay and neurologic impairment concerns for children conceived by intracytoplasmic sperm injection/in vitro fertilization may be unfounded

Studies suggest that developmental delay and impaired neurologic status occur in children conceived using IVF and IVF/ICSI, although these findings are controversial. Two prospective analyses of small numbers of children conceived by IVF/ICSI at early age (1–2 years or higher) did not identify a higher likelihood of developmental delay [43–48]. Similarly, Bonduelle and colleagues [49] found no difference in the likelihood of developmental delay in children conceived by IVF and IVF/ICSI compared with natural conceptions. A statistically significant trend toward higher IQ and verbal performance in children conceived by IVF/ICSI compared with controls was observed [32,50]. This finding may reflect differences in the level of maternal education in the IVF/ICSI cohort versus the spontaneous conceptions cohort.

Again, there is disagreement in the literature. A retrospective study of 5680 Swedish offspring conceived by IVF found statistically higher likelihoods of developmental delay and neurologic impairment requiring rehabilitation [51]. Singleton pregnancies were associated with a lower risk. Children conceived by IVF were at greater risk of these conditions, however, even after considering the risks of multiple gestation. Cerebral palsy was the major neurologic impairment reported in the Swedish children conceived by IVF; the children from multiple gestation pregnancies conceived by IVF displayed a statistically significantly increased likelihood of cerebral palsy, whereas a similar but not statistically significant trend was observed in singletons conceived by IVF. A higher risk of cerebral palsy also was found in a second, more recent study of children conceived by IVF regardless of single versus multiple gestational status [52]. Regression analysis suggests that the complications associated with preterm versus term delivery and multiple gestational status in combination contribute to this risk. These observations give credence to the ongoing efforts of some groups to mandate the transfer of fewer embryos [42].

A retrospective analysis of the Danish National Birth Registry revealed that children conceived by ICSI/IVF have a higher likelihood of cerebral palsy regardless of multiplicity [40]. The vanishing twin syndrome, which occurs in approximately 10% of singleton births conceived by IVF [11] and in naturally conceived multiple gestations, is associated with cerebral palsy. There is no reason to believe that this association would not be present in ART pregnancies. It is argued that this observation supports the practice of SET [41].
The risk of genetic disorders is increased in children conceived by intracytoplasmic sperm injection/in vitro fertilization

Many patients with idiopathic male infertility are thought to have a contributing but as yet unidentifiable genetic cause. As such, there exists a risk of transmission of these causes to any offspring. The model offered by the transmission of well-understood genetic causes of male infertility illustrates this risk. For example, congenital bilateral absence of the vas deferens is a genital form of cystic fibrosis that results from a loss of function mutation in the cystic fibrosis transmembrane regulator gene. Men with congenital bilateral absence of the vas deferens transmit their mutation to their offspring. Cystic fibrosis transmembrane regulator mutation screening of the most common disease associated variants is mandatory for female partners of men with congenital bilateral absence of the vas deferens. Likewise, Y chromosome microdeletions are transmitted to male offspring by ICSI. Because microdeletions of Y chromosomal AZF-a, b, or c regions is associated with male infertility, any male offspring that result from IVF/ICSI using sperm from these patients also are affected by these deletions and are expected to be infertile like their fathers.

There is an increased risk of imprinting disorders in offspring conceived by IVF/ICSI [53–56]. Imprinting influences gene expression and transmission of phenotypic syndromes or traits in a non-mendelian manner. These imprinting syndromes, such as Angelman and Beckwith-Wiedemann syndromes, are rare disorders that occur after abnormal gene methylation of relevant genes. The methylation pattern of these genes, defined during parental gametogenesis, regulates gene expression in an epigenetic manner in the offspring. Although the absolute risk of these disorders is low, researchers agree that there is an increased incidence of imprinting disorders in children conceived by IVF/ICSI [53–56]. Defective imprinting of the gamete DNA and the ART procedures have been implicated as potential causes of these defects.

Childhood malignancies can result from gene defects, some of which are caused by failures of DNA mismatch repair. Maduro and colleagues [57] reported evidence of DNA mismatch repair defects in men with nonobstructive azoospermia, and mouse models and available human studies have found that defective DNA repair is associated with male infertility. Do offspring conceived by ART have a higher incidence of malignancy caused by DNA repair defects? Two large retrospective studies did not report evidence of an increased risk of childhood malignancy in offspring conceived by IVF and IVF/ICSI [58,59]. In one report, a significant increase in the incidence of retinoblastoma in children conceived by IVF/ICSI compared with the general population and a control cohort was described; five cases of retinoblastoma were found in the cohort of offspring conceived by IVF/ICSI [60]. Although the possibility that DNA mismatch repair defects in offspring conceived by IVF and IVF/ICSI may put them at higher risk of childhood malignancy, further research must be performed before this relationship can be defined clearly.

Finally, offspring conceived by IVF/ICSI are at higher risk of autosomal and gonosomal aneuploidy [61,62]. Infertile men have a tenfold higher incidence of sperm aneuploidy when compared with fertile men. These aneuploidies likely result from meiotic errors during synopsis and homologous recombination in the testis. Regardless of the cause, the clinical implications of abnormal sperm aneuploidy are incompletely understood, and well-designed outcomes studies are required. Simpson and Lamb [63] listed possible additional explanations for chromosome aneuploidy in children conceived by ICSI/IVF, including (1) in vitro versus in vivo selective mechanisms for sperm, (2) transmission of pleiotropic genes that cause somatic abnormalities in offspring, whereas these genes had caused oligo- or azoospermia in the male parent, (3) physical damage that occurs during ICSI, (4) the in vitro hormonal milieu, and (5) point mutations that result from physical and chemical stressors during the in vitro process.

Barriers to further research on the safety and efficacy of in vitro fertilization/intracytoplasmic sperm injection

A consistent theme throughout this article is the difficulty faced by investigators in accurately determining the risks of any ART, including ICSI. These challenges include the lack of standardized reporting of congenital abnormalities throughout the world, the lack of standardized methodologies for ovarian hyperstimulation and ART procedures, the lack of comparable study patient cohorts because of the unique and combined characteristics of each couple, and the inability to design and execute prospective, blinded, and well-controlled
trials because of obvious ethical constraints. The use of retrospective trials that focus on questions of ART safety remains controversial. These retrospective trials continue to generate outcomes that alarm patients, despite low absolute risks and poor study methodology.

Reporting of congenital abnormalities is not consistent and sometimes inaccurate. It is difficult to determine whether these children are healthy. Simpson and Lamb [63] outlined the shortcomings of IVF outcomes research and focused in part on the baseline control incidence of congenital abnormalities in the overall population of 1% to 2%. These numbers are derived from the report of “congenital abnormalities” identified in the neonatal nursery and stop when the child leaves the hospital. Thus, significant underreporting of existing conditions in the spontaneous conception cohort may occur [63]. Using the Western Australian birth registry criteria consistently estimates a higher incidence of birth defects in the ART population than is self-reported by ART practitioners, leading Hansen and colleagues [64] to conclude that practitioner reporting of birth defects is inaccurate. The question of whether birth defects can be defined and recorded accurately is of utmost importance to ART safety research. Other defects also may become evident with aging, such as those that manifest themselves only in adulthood (ie, congenital malformation of the kidney). Because of the challenges in accurately reporting defects in the ART cohort and the spontaneous conception cohort, the conclusions that are reached using these data become difficult to interpret.

Buck Louis and colleagues [65] suggested that standardization of clinical and developmental endpoints is necessary for meaningful data to be generated. Prospective study design with standardization of study protocols would reduce the confounding effects, although the data remain complex for additional reasons. One of the most basic problems with ART research is that the ideal study design for answering whether we can differentiate ART treatment effects from underlying fecundity impairments requires a randomized clinical trial. In everyday clinical practice this approach is impossible because it would require administering ART to fertile couples.

In conclusion, despite these unique and perhaps insurmountable challenges, research on the safety of IVF and IVF/ICSI remains a high priority from a public health perspective. The ever-increasing use of these techniques mandates that we continue research on ART safety, regardless of its limitations. ART research can generate meaningful conclusions, the best example of which is the trend toward SET. Outcomes research showed that SET limits multiple gestation pregnancies with the associated advantage of lowering the risk of poor perinatal outcomes while maintaining an acceptable pregnancy rate. This single and powerful example proves that ART safety research can generate clinically essential data.

Summary

Research on the safety of IVF/ICSI is crucial, especially given the increasing popularity of this treatment and the vast numbers of IVF/ICSI procedures performed each year throughout the world. In general, IVF and IVF/ICSI are associated with multiple gestations and an increased risk of congenital abnormalities (including hypospadias). IVF/ICSI, in particular, carries an increased risk of endocrine abnormalities and epigenetic imprinting effects. Conversely, SET seems to diminish at least some of the risks. Regardless, the absolute risk of any of these conditions remains low.

Patients are only able to make informed decisions about these procedures after proper education about the risks. Infertile couples should receive this information before they embark on the financially costly and emotionally burdensome process of ART. The health care profession has a responsibility to counsel couples about the health risks to the mother and any future offspring. Patients should be reminded that although the absolute risks of any of the conditions described herein are still negligible, there can be no guarantees of a perfectly healthy baby. No such guarantee exists, of course, for fertile couples either. Further research is required, not only to define IVF/ICSI outcomes but also to better understand the molecular basis of the infertility phenotypes that necessitate these treatments. Efforts also must include standardized reporting of birth defects, proper study design, and responsible analysis of the results. Complete information on defective reproductive processes and outcomes-based research data will allow physicians to determine the real risk of IVF/ICSI.

References


Bonduelle M, Van Assche E, Joris H, et al. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation...


Reassessing Reconstruction in the Management of Obstructive Azoospermia: Reconstruction or Sperm Acquisition?

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\textsuperscript{b}James Buchanan Brady Foundation, Department of Urology and Cornell Institute for Reproductive Medicine, Weill Medical College of Cornell University, 525 E. 68th Street, Starr 900, New York, NY 10021, USA
\textsuperscript{c}Population Council, Center for Biomedical Research, 1230 York Ave., New York, NY 10021, USA

Infertility currently affects approximately 15\% of all couples, with an increase anticipated over the next 20 years [1,2]. Approximately 50\% of cases of infertility may be attributed to male factors. Male reproductive medicine has undergone significant changes in recent years, and the advent of assisted reproductive technology (ART) has substantially improved our ability to successfully manage male factor infertility. Specifically, improved techniques in microsurgical reconstruction and refinement in techniques for sperm retrieval combined with in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have materially altered our ability to treat obstructive azoospermia.

Selecting the optimal therapy for couples with obstructive azoospermia can be challenging. In this article, we limit our discussion to patients with reconstructable obstruction, such as the common situation of men desiring fertility after vasectomy. Sperm retrieval with IVF/ICSI offers the allure of early achievement of a relatively high live delivery rate, although its use consigns the female partner to the greater costs and complications of an IVF cycle and potential health problems in the resulting offspring. In contrast, surgical reconstruction does not require treatment of the female partner, and pregnancy usually occurs naturally after sexual intercourse. However, reconstruction may not always be successful, and the time to achieve a pregnancy is longer, especially in patients who have endured long durations of obstruction. We examine the various therapeutic options available for surgical reconstruction and sperm retrieval, specifically their rates of success and attendant costs in an effort to define the optimal treatment for couples with obstructive azoospermia.

Methods of surgical reconstruction

\textit{Vasovasostomy}

The first-line method for surgical reconstruction of obstructive azoospermia secondary to vasectomy consists of vasovasostomy, in which the obstructed length of vas deferens is excised and the cut ends are reanastomosed [3,4]. Microsurgical reconstruction seems to be superior to macrosurgical reconstruction and currently represents the standard of care. Variations of the microsurgical technique exist, including multilayer vasovasostomy versus a modified single-layer adaptation.

\textit{Vasoepididymostomy}

Some patients with obstructive azoospermia require a vasoepididymostomy instead of...
a vasovasostomy if their epididymis is found to be obstructed. Vasoepididymostomy consists of anastomosing a patent epididymal tubule directly to the vas deferens, thus bypassing any obstruction in the epididymis distal to the tubule. Multiple techniques have been described, although three variations are currently used: direct end-to-end, direct end-to-side, and end-to-side intussusception [5]. Epididymal obstruction and the need for vasoepididymostomy seem to be related to the duration of deferential obstruction [6–8]. Fuchs and Burt [6], for example, reported that 62% of patients who had undergone vasectomy at least 15 years before reversal required vasoepididymostomy. Significantly lower patency and pregnancy rates have been reported after vasoepididymostomy compared with vasovasostomy [5].

**Methods of sperm acquisition**

One should note that all methods of sperm acquisition consign the female partner to IVF/ICSI for successful fertilization and delivery.

**Microsurgical epididymal sperm aspiration**

Microsurgical epididymal sperm aspiration (MESA) was introduced in the 1980s by Temple-Smith and colleagues and Silber and colleagues [9,10] originally to enable sperm retrieval in the setting of congenital bilateral absence of the vas deferens. It consists of microsurgically exposing the epididymis, incising the epididymal tunic, and then aspirating sperm-filled epididymal fluid. MESA enables the collection of large quantities of motile sperm for cryopreservation.

**Percutaneous epididymal sperm aspiration**

Percutaneous epididymal sperm aspiration (PESA) was introduced in 1994 by Craft and Shrivastav [11,12] as a simpler, less invasive alternative to MESA for patients with obstructive azoospermia who were unable to undergo or who decided against surgical reconstruction. A needle is introduced through the skin into the epididymis and is then aspirated. Three pregnancies (43%) were obtained in seven couples, and one set of twins was delivered in the original description [12]. Criticisms of this technique include frequently unreliable sperm retrieval [5]. Our data analysis focuses on the more reliable microsurgical approach.

Some surgeons prefer MESA or PESA as the source of sperm in obstructive azoospermia, because epididymal sperm tend to be more mature and are obtainable in higher, bankable numbers relative to that obtained from the testis [13]. In 1998, Sheynkin and colleagues [14] compared percutaneous and microsurgical sperm retrieval in men with obstructive azoospermia. Nine men underwent simultaneous MESA, testicular fine needle aspiration, and PercBiopsy. As expected, the mean number of sperm retrieved via MESA (15 × 10⁶) was higher than that retrieved percutaneously (testicular fine needle aspiration = 0.014 × 10⁶ and PercBiopsy = 0.116 × 10⁶). Overall, testicular sperm aspiration pregnancy rates have been reported to be as high as 31%, with a calculated live delivery rate of 27% if one assumes a miscarriage rate of 11.6% rate after ICSI [15,16]. Similarly, PESA pregnancy rates have been reported to be as high as 43%, with a calculated live delivery rate of 38% if one makes a similar assumption regarding ICSI miscarriage rate [12,16,17].

**Open testis biopsy**

The original description of sperm retrieval for assisted reproduction by open testicular biopsy was proposed by Silber and colleagues [18] in 1995. Multiple pieces of testicular tissue from the same incision are taken for use in IVF/ICSI.

**Microsurgical testicular sperm extraction**

Microsurgical testicular sperm extraction (TESE), as described by Schlegel and colleagues [19] in 1999, uses the operating microscope to identify larger caliber, sperm-containing seminiferous tubules. Microsurgical TESE is traditionally used in the setting of nonobstructive azoospermia and offers the advantages of less bleeding and greater sperm extraction per gram of testicular tissue extracted. It plays little role in the setting of obstructive azoospermia.

**Percutaneous testicular sperm extraction**

Like PESA, percutaneous TESE offers a less costly and invasive alternative to its microsurgical counterpart. A needle is introduced percutaneously into the testis and is then aspirated; the tissue obtained is then processed for use in IVF. Percutaneous TESE also represents a less invasive choice compared with open testis biopsy, although less tissue is generally obtained with the percutaneous technique. Belker and colleagues [15] described a 100% sperm retrieval rate when used in obstructed patients. Fine-needle mapping
as described by Turek and colleagues [20] is designed for use in nonobstructive azoospermia and plays little role in this particular analysis.

Outcome metrics

This article focuses on three main metrics to assess outcomes: (1) cost, (2) effectiveness, and (3) various analytic methods to combine the information embodied in cost and effectiveness data.

Costs

Costs may be broken down into two main components: direct and indirect [21–23]. Direct costs encompass expenditures for medical products or services, including office examination fees, surgeon fees for microsurgical reconstruction or sperm retrieval, associated anesthesia and operating room or facility fees, recovery room fees, the cost of diagnostic imaging tests, the cost of blood tests, the cost of gonadotropins if IVF/ICSI is used, and finally the cost of the IVF cycle, including all technical and professional fees if sperm retrieval is chosen. Indirect costs represent the economic impact that occurs from morbidity, mortality, or loss of livelihood secondary to a procedure. In this analysis, indirect costs would represent the economic impact of procedure-associated complications, lost productivity because of time away from work, and the impact from multiple gestation pregnancies that may ensue.

This analysis uses complication and multiple gestation rate data that have appeared in the peer-reviewed literature. Male infertility procedure-related complications include bleeding, infection, and testicular atrophy and occur at a rate of 0.3% to 2% [5,24,25]. Maternal complications caused by IVF are estimated to occur in 3% to 6% of all cases and include ovarian hyperstimulation syndrome, pelvic hemorrhage, infection, stroke, myocardial infarction, and possibly ovarian cancer [26–29]. The impact of multiple gestation pregnancies has been well studied. Such pregnancies are associated with higher rates of neonatal complications and longer intensive care unit stays compared with singleton infants [27,30]. Much of the increase in costs associated with higher order gestations can be traced to greater neonatal lengths of stay in addition to greater direct use of medical resources.

It is important to note that the true cost of care is best represented by the amount resources consumed in providing that care. Because the true economic burden of providing services is usually difficult to measure, charges are instead used as a proxy [31]. Charges are set by the marketplace and may not accurately reflect the true burden of providing care, although they do represent the best available metric for cost-effectiveness evaluations.

Effectiveness

Multiple definitions of success are possible when treating obstructive azoospermia. Patency, as signified by the return of sperm to the ejaculate, may be used as one measure of success with surgical reconstruction. Successful fertilization and pregnancy after reconstruction or sperm retrieval may constitute a separate metric. Finally, delivery of at least one or more live children after either treatment may represent yet another measure of success. It is the opinion of the authors that live delivery represents the most relevant and appropriate metric to consider: the outcome of most value to couples is the delivery of at least one live child. All other markers of success are of secondary value.

Analysis and evaluation methods

Economic analyses weave the dual components of cost and effectiveness into a rational framework for decision making. Because choices must be made between alternative uses of scarce or limited health care resources, economic analyses are able to consider cost and outcome to arrive at an optimal allocation decision [21–23]. Different types of economic analyses include cost-identification analysis, cost-effectiveness analysis, and cost-benefit analysis [31,32].

Cost-identification analysis consists of ascertaining the economic resources involved in providing a product or service or that involved in disease burden. Cost-identification studies do not consider the benefits derived from the expenditure of economic resources. In contrast, cost-effectiveness analysis considers the cost of providing a service in addition to the benefit or outcome that arises from that service; the metric given in this type of analysis usually refers to cost per unit of outcome. This evaluation allows a comparison of the relative value of different treatment approaches. Cost-benefit analyses attempt to determine if a given outcome is worth its requisite cost to an individual. Clinical outcomes are translated into monetary terms via willingness-to-pay...
approaches and the outcomes compared with the benefits on a direct monetary basis.

Like most infertility-related peer-reviewed literature, this article focuses primarily on cost-effectiveness analysis as a method of identifying optimal treatment for obstructive azoospermia. First, the effectiveness and then more importantly the cost effectiveness of IVF treatments in general are examined because they constitute a major component of treatment by sperm retrieval. The analysis then focuses on examining male factor infertility treatments for obstructive azoospermia in similar fashion.

In vitro fertilization studies

Effectiveness of in vitro fertilization for male factor infertility

The most complete set of data regarding the effectiveness of IVF for male factor infertility is found within the Society of Assisted Reproductive Technology (SART) database, published by the Centers for Disease Control and Prevention under the 1992 Fertility Clinic Success Rate and Certification Act [33]. A summary of SART data from 1995, the first available year, to 2004, the latest available year, is shown in Table 1. Although the number of total IVF cycles has risen from approximately 46,000 to 89,500 cycles over the intervening years, the percentage of cycles undertaken for male factor infertility alone has declined from a peak of 32% to the current level of 17%.

Table 1
Summary SART statistics for 1995 to 2004 for couples undergoing assisted reproductive technology treatment

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<tr>
<td>Total cycles (fresh embryo,nondonor eggs)</td>
<td>45,906</td>
<td>49,584</td>
<td>55,002</td>
<td>61,650</td>
<td>63,303</td>
<td>71,556</td>
<td>77,102</td>
<td>81,888</td>
<td>86,753</td>
<td>89,533</td>
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<tr>
<td>% cycles for male factor infertility by diagnosis</td>
<td>32.0</td>
<td>23.0</td>
<td>16.0</td>
<td>24.0</td>
<td>18.0</td>
<td>17.0</td>
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<td>17.0</td>
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<tr>
<td>Pregnancies per cycle (%)</td>
<td>29.7</td>
<td>27.5</td>
<td>29.4</td>
<td>30.5</td>
<td>31.6</td>
<td>31.8</td>
<td>34.0</td>
<td>35.5</td>
<td>35.7</td>
<td>35.2</td>
</tr>
<tr>
<td>Live deliveries per cycle (%)</td>
<td>25.3</td>
<td>22.6</td>
<td>24.0</td>
<td>24.9</td>
<td>26.1</td>
<td>26.5</td>
<td>28.1</td>
<td>29.5</td>
<td>29.5</td>
<td>28.9</td>
</tr>
<tr>
<td>Live delivery rate for male factor infertility (%)</td>
<td>21.0</td>
<td>24.3</td>
<td>25.5</td>
<td>27.1</td>
<td>28.9</td>
<td>29.3</td>
<td>32.0</td>
<td>33.6</td>
<td>33.8</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Multiple gestation live births

| Single (%) | 63.0 | 52.0 | 50.1 | 62.0 | 63.4 | 65.0 | 64.2 | 64.6 | 65.8 | 67.5 |
| Twin (%) | 31.1 | 39.3 | 41.4 | 32.0 | 31.7 | 30.7 | 32.0 | 31.6 | 31.0 | 29.9 |
| Triplet or more (%) | 5.9 | 8.7 | 8.5 | 6.0 | 4.9 | 4.3 | 3.8 | 3.8 | 3.2 | 2.6 |

Similarly, the percentage of total ICSI cases used for male infertility cases has declined from 57.8% in 2001 to 51.4% in 2004. The live delivery rate for male factor infertility IVF cases in contrast has improved from 21% to 33% over the same time period.

One should note that although the SART summary data offer an impression of the effectiveness of IVF-driven treatments for male factor infertility, they do not offer fine enough resolution to distinguish IVF treatments undertaken for obstructive versus nonobstructive azoospermia cases. SART data reflect a mixture of the two. Theoretically, however, IVF treatments undertaken solely for obstructive azoospermia should be even more effective than the outcomes reported by SART, as the nonobstructive azoospermia cases reported by SART would be expected to generally yield lower live delivery rates compared with their obstructive counterparts.

Cost effectiveness

Neumann and colleagues [27] were the first to study the cost of a successful live delivery with an IVF pregnancy. Direct and indirect costs were considered in this analysis. The cost per live delivery ranged from $66,667 in 1992 dollars with one cycle of IVF to $114,286 by the sixth cycle in the study. A subgroup analysis that examined couples with advanced maternal age (ie, > 40 years) and male-factor subfertility (ie, sperm concentration < 20 million/mL or motility...
< 40%) was conducted. The cost per live delivery increased to $160,000 for the first cycle to $800,000 by the sixth cycle.

Since the study conducted by Neumann and colleagues, various other groups have examined the costs of IVF. Chambers and colleagues [34] performed a population-based costing study of resources consumed during ART in Australia using a decision analytic model that drew upon data from the Australian and New Zealand Assisted Reproduction Database. Direct costs were queried from various fertility centers and rebates through the Medicare or Pharmaceutical Benefit Scheme. The cost per live delivery was calculated to be $32,903 in 2005 Australian dollars, although this cost increased to $182,794 for women older than 42 years. The most complete survey of IVF costs was perhaps undertaken in a review by Collins [35] in 2002. The use of IVF was studied in 48 countries, where direct and some indirect costs were considered. The mean cost per live delivery in the United States was estimated to be $58,394 in 2002 dollars per live birth, compared with $22,048 in non-US countries. As in previous studies, multiple gestation pregnancies were shown to pose a significant economic burden, costing 36% more than regular IVF singleton pregnancies. Price elasticity estimates indicate that a 10% decrease in IVF/ICSI costs would result in a 30% increase in overall ART use. Of note, the study emphasized that most IVF-related economic studies in the peer-reviewed literature possessed no outcomes assessment or comparison with alternative policies.

The costs of multiple gestation pregnancies have been well studied. The landmark study by Callahan and colleagues [30] demonstrated that predicted charges for an IVF singleton pregnancy were $9845 in 1991 dollars, compared with $37,947 for twins and $107,965 for triplets. Low birthweight and gestational age were found to represent the major contributors to the increased use of health care resources with IVF-related multiple gestation pregnancies [36]. Subsequent studies have confirmed the major contribution of multiple gestation pregnancies toward overall IVF cost. Lukassen and colleagues [37] retrospectively compared the relative cost of twin versus singleton IVF pregnancies in a single institutional study in the Netherlands from 1995 to 2001. They calculated the cost of twin pregnancies to be €13,469 in 2002 euros, more than five times higher than the €2,550 of a singleton pregnancy, because of longer maternal and neonatal admissions. Ledger and colleagues [38] modeled the cost impact to the British National Health System of IVF-related multiple births and concluded that multiple gestation pregnancies represented 56% of the cost of all IVF pregnancies, although they represented less than one third of the total number of maternities in the United Kingdom. Singletons cost £3313 in £ year 2002 sterling, whereas twins cost £9122, and triplets cost £32,354. Wolner-Hanssen and Rydhstroom [39] modeled the use of single-embryo transfer, compared with actual standard two-embryo transfer protocols, and concluded that although more cycles would be needed to achieve a single live delivery with single-embryo transfer, the strategy would still be more cost efficient than the standard two-embryo transfer protocol because of the lower rate of twin pregnancies.

The highest quality studies to examine the cost effectiveness of IVF consist of three randomized controlled trials (Table 2). As the earliest, the Ontario trial compared one stimulated treatment cycle without embryo freezing versus a 6-month period of untreated observation or elective conventional therapy, including ovulation induction and intrauterine insemination (IUI), in the 1980s [40]. The live delivery rate was 10% in the former group versus 6% in the latter. The marginal cost of live delivery was calculated as $89,427 in 1992 Canadian dollars. A major

<table>
<thead>
<tr>
<th>Trial</th>
<th>Reference</th>
<th>Intervention</th>
<th>Marginal cost of delivery (in trial year currency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ontario</td>
<td>Soliman et al [40]</td>
<td>IVF cycle versus 6 mo of observation or IUI with ovulation induction</td>
<td>$89,427</td>
</tr>
<tr>
<td>Illinois</td>
<td>Karande et al [41]</td>
<td>IVF cycle versus 6 mo of clomiphene and gonadotropin cycles</td>
<td>$21,627</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>Goverde et al [42]</td>
<td>IVF versus IUI versus IUI/ovarian hyperstimulation</td>
<td>26,779 NLG</td>
</tr>
</tbody>
</table>
weakness of the trials was that it occurred in the 1980s; ostensibly, the effectiveness of ART treatments has since improved considerably. A second trial in Illinois compared 46 couples undergoing IVF to 50 couples randomized to 6 months of standard therapy that consisted of three clomiphene cycles and three gonadotropin cycles followed by four IVF cycles [41]. The former group achieved a 35% pregnancy rate, whereas the latter group achieved a 56% pregnancy rate. As with the Ontario trial, only direct costs were considered. The marginal cost of an additional live delivery was calculated to be $21,627 in 1999 dollars (ie, IVF was deemed to be not only more expensive but also to offer less benefit).

The final trial occurred in the Netherlands [42]. Eighty-six couples with idiopathic subfertility or male subfertility were assigned to six cycles of IUI alone, 85 to six cycles of IUI with ovarian hyperstimulation, and 87 to six cycles of IVF. After 3.5 years, the live birth rates were 7.4%, 8.7%, and 12.2%, respectively. Couples in the IVF arm were more likely to discontinue treatment before the maximum of six attempts. IUI (10,406 NLG per live delivery for male subfertility in 1995 NLG) and IUI with ovarian hyperstimulation (15,448 NLG per live delivery) were found to be more cost effective than IVF (37,185 NLG per live delivery), even at higher maternal ages, when the effectiveness of IUI declines. Questions regarding the generalizability of the Netherlands trial arise because few couples undergo more than three IVF cycles, whereas the trial tested up to six cycles. Overall, these three trials differed in terms of patient population, treatments offered, and country-specific health economic systems, thus potentially accounting for the differences in results seen. It was unclear whether these studies included patients with obstructive azoospermia undergoing sperm acquisition and IVF.

On a broader basis, one should note that the improved live delivery rates and decreased multiple gestation rates with IVF reported by more recent SART data might materially affect the outcomes of the cost-effectiveness analyses mentioned. Direct comparison of the IVF studies is also limited by the heterogeneity in the definition of costs used. Some studies examined only direct costs, whereas others included direct and indirect costs. Finally, some studies assumed costs to be equal to charges, whereas others considered the two to be separate. Several well-written reviews regarding the cost impact and cost effectiveness of IVF treatments have been published [31,32,35,43,44].

Male factor infertility studies

Effectiveness of surgical techniques

The peer-reviewed literature was queried for articles pertaining to microsurgical vasectomy reversal and sperm retrieval; specifically, a Medline search using the terms “vasectomy reversal,” “vasovasostomy,” “vasoepididymostomy,” “sperm retrieval,” “sperm aspiration,” “sperm extraction,” “TESE,” “TESA [testicular sperm aspiration],” “PESA,” “MESA,” and “testis biopsy” was conducted. All relevant studies were identified, and only articles that presented original primary data sufficient to calculate patency, when relevant, and live delivery rates were included for analysis. All data from the studies were pooled and are presented in Tables 3–5.

The overall patency rate for vasectomy reversal is approximately 86% in the peer-reviewed literature (see Table 3). The corresponding live delivery rate in these studies is 58%. One should note that the results of one study were substantially different from the remainder of published literature; the rationale for this difference and the generalizability of the study could not be determined [45]. In this study, the overall reported patency rate was 90% for the 3378 (86.5%) patients with data available. Live delivery rates were 84% for the 1738 (44.5%) patients with data available. If data from this particular study were separated from the others, the overall patency rate for microsurgical vasectomy reversal would decrease to 81%, and the live delivery rate would decrease to 44%.

More than the rate of successful sperm retrieval, the live delivery rate with sperm retrieval techniques represents the critical metric of success if a couple chooses retrieval as treatment for obstructive azoospermia. According to the peer-reviewed literature, the overall live delivery rate for couples undergoing MESA is 44%. One should note that Tables 3 and 4 present all vasectomy reversal and MESA studies identified by Medline and contain multiple studies from same clinical groups. Some of these studies may represent subgroup analyses of an identical larger patient population. Because improvements in surgical technique may have occurred over time, however, all studies have been presented in toto.

Data were gathered for the 1999 and 2005 years for TESE procedures from the SART
<table>
<thead>
<tr>
<th>Vasectomy reversal studies</th>
<th>Patency rate (%)</th>
<th>Live delivery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuchs EF, Burt R. Vasectomy reversal performed 15 years or more after vasectomy: correlation of pregnancy outcome with partner age and with pregnancy results of in vitro fertilization with intracytoplasmic sperm injection. Fertil Steril 2002;77:516–9</td>
<td>147/173 (85)</td>
<td>66/173 (38)</td>
</tr>
<tr>
<td>Kolettis PN, Sabanegh ES, D’amico AM, et al. Outcomes for vasectomy reversal performed after obstructive intervals of at least 10 years. Urology 2002;60(5):885–8</td>
<td>57/74 (77)</td>
<td>26/74 (35)</td>
</tr>
<tr>
<td>Kolettis PN, Sabanegh ES, Nalesnik JG, D’Amico AM, Box LC, Burns JR. Pregnancy outcomes after vasectomy reversal for female partners 35 years old or older. J Urol 2003;169(6):2250–2</td>
<td>37/46 (81)</td>
<td>15/46 (33)</td>
</tr>
<tr>
<td>Kolettis PN, Woo L, Sandlow JI. Outcomes of vasectomy reversal performed for men with the same female partners. Urology 2003;61:1221–3</td>
<td>30/32 (93)</td>
<td>18/32 (56)</td>
</tr>
<tr>
<td>Schlegel PN, Goldstein M. Microsurgical vasoepididymostomy: refinements and results. J Urol 1993;150:1165–8</td>
<td>77/110 (70)</td>
<td>43/110 (39)</td>
</tr>
<tr>
<td>Silber SJ. Results of microsurgical vasoepididymostomy: role of epididymis in sperm maturation. Hum Reprod 1989;4:298–303</td>
<td>NA/190 (NA)</td>
<td>81/190 (42)</td>
</tr>
<tr>
<td>Silber SJ, Grotjahn HE. Microscopic vasectomy reversal 30 years later: a summary of 4010 cases by the same surgeon. J Androl 2004;25:845–9</td>
<td>3040/3378 (90)</td>
<td>1460/1738 (84)</td>
</tr>
<tr>
<td>Total</td>
<td>5386/6266 (86)</td>
<td>2808/4816 (58)</td>
</tr>
</tbody>
</table>
database, the former representing the earliest year for which complete data were readily available and the latter the latest year for which robust SART data existed for TESE (see Table 5). Only 1.6% of cycles undergone for male factor infertility used TESE with IVF/ICSI to treat male factor infertility in 1999 (1029 cycles); this percentage remained unchanged for 2005 (1425 cycles). The live delivery rate for TESE cycles increased from 28.3% to 33.6% (*P* = .042) in couples in whom sperm was successfully retrieved. Although the multiple gestation pregnancy rate decreased from 37% to 31.9% for all IVF cycles, it did not do so for TESE cycles (29.6% to 28.0%, *P* = .737). The percentage of cycles resulting in triplet or more infants in the latter group did decline from 5.8% to 2.1%, however (*P* = .008). One should note that the “TESE” designation within the SART database does not differentiate between sperm obtained via percutaneous TESE versus microsurgical TESE versus open testicular biopsy, nor does the database distinguish between

<table>
<thead>
<tr>
<th>MESA studies</th>
<th>Live delivery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anger JT, Wang GJ, Boorjian SA, et al. Sperm cryopreservation and in vitro</td>
<td>21/30 (70)</td>
</tr>
<tr>
<td>intracytoplasmic sperm injection with frozen-thawed epididymal spermatozoa.</td>
<td></td>
</tr>
<tr>
<td>Hum Reprod 1995;10:903–6</td>
<td></td>
</tr>
<tr>
<td>Heidenreich A, Altmann P, Engelmann UH. Microsurgical vasovasostomy versus</td>
<td>19/69 (28)</td>
</tr>
<tr>
<td>microsurgical epididymal sperm aspiration/testicular extraction of sperm</td>
<td></td>
</tr>
<tr>
<td>Janzen N, Goldstein M, Schlegel PN, et al. Use of electively cryopreserved</td>
<td>82/141 (58)</td>
</tr>
<tr>
<td>microsurgically aspirated epididymal sperm with IVF and intracytoplasmic</td>
<td></td>
</tr>
<tr>
<td>injection using intentionally cryopreserved epididymal spermatozoa. Hum Repro</td>
<td></td>
</tr>
<tr>
<td>d 1996;11:133–8</td>
<td></td>
</tr>
<tr>
<td>epididymal sperm with in vitro fertilization: importance of in vitro</td>
<td></td>
</tr>
<tr>
<td>micromanipulation techniques. Urology 1995;46:238–41</td>
<td></td>
</tr>
<tr>
<td>Schroeder-Printzen I, Zumble J, Bspink L, et al. Microsurgical epididymal</td>
<td>35/93 (38)</td>
</tr>
<tr>
<td>Sharma RK, Padron OF, Thomas AJ Jr, et al. Factors associated with the quality</td>
<td>64/131 (49)</td>
</tr>
<tr>
<td>before freezing and after thawing of sperm obtained by microsurgical epididymal aspiration. Fertil Steril 1997;68(4):626–31</td>
<td></td>
</tr>
<tr>
<td>frozen-thawed epididymal spermatozoa and the outcome of intracytoplasmic</td>
<td></td>
</tr>
<tr>
<td>intracytoplasmic sperm injection for patients requiring microsurgical sperm</td>
<td></td>
</tr>
<tr>
<td>and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. Fertil Steril 1994;61:1045–51</td>
<td></td>
</tr>
<tr>
<td>Tournaye H, Merdad T, Silber S, et al. No differences in outcome after</td>
<td>48/176 (27)</td>
</tr>
<tr>
<td>intracytoplasmic sperm injection with fresh or with frozen-thawed epididymal spermatozoa. Hum Reprod 1999;14(1):90–5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>372/843 (44)</td>
</tr>
</tbody>
</table>
Obstructive versus nonobstructive azoospermia for "male factor infertility." The live delivery rates cited by the database likely underestimate the success rates that would be obtained by patients with obstructive azoospermia.

Cost effectiveness: vasectomy reversal versus sperm retrieval studies

Prior groups have compared different treatments for obstructive azoospermia. Pavlovich and Schlegel [25] studied the use of vasectomy reversal via vasovasostomy or vasoepididymostomy versus sperm retrieval via MESA and percutaneous and testicular sperm retrieval with ICSI in men with postvasectomy infertility and female partners 39 years old or younger. Direct costs for all procedures were considered and surveyed from multiple US centers reporting results for ICSI and vasectomy reversal, as were the indirect costs of complications, lost productivity, and multiple gestation pregnancies. Vasectomy reversal was calculated to cost $25,475 per live delivery (95% confidence interval: $19,609–$31,339) in 1994 dollars. Sperm retrieval and IVF, in contrast, were calculated to cost $72,521 per live delivery (95% confidence interval: $51,024–$93,099). These figures also considered the impact of direct and indirect costs. Donovan and colleagues [47] compared MESA versus repeat surgical reconstruction in postvasectomy patients in the University of Iowa experience. A patency rate of 78% was achieved in the latter group. Only direct costs were considered. The cost per live delivery for MESA was calculated to be $35,570 in 1998 dollars compared with $14,892 for repeat vasectomy reversal.

Deck and Berger [48] described the University of Washington experience with vasectomy reversal compared with IVF/ICSI. The clinical course of 29 patients undergoing vasectomy reversal with ovulating partners older than 37 years was retrospectively studied. With a patency rate of 75%, the live birth rate achieved was 17%. The cost per live delivery was calculated to be $28,530 in 2000 dollars compared with $103,940 for testicular sperm aspiration/IVF/ICSI. These figures only accounted for direct procedural costs and did not consider the impact of indirect costs.

Meng and colleagues [49] also examined the issue of vasectomy reversal through either microsurgical vasovasostomy or vasopipidymostomy versus sperm retrieval via unspecified means by use of a decision analytic model. In contrast to the Pavlovich and Schlegel analysis, although direct procedural costs were considered, the impact of indirect costs was not; all costing data came from a single institution. The results of the analysis by Meng and colleagues favored vasectomy reversal as the more cost-effective treatment for postvasectomy related obstructive azoospermia, as long as postreconstruction patency rates could be maintained more than 79%. Their analysis calculated that the cost per live delivery for vasectomy reversal in the base case scenario was $38,983 in 2004 dollars, whereas that for sperm
retrieval/ICSI was $39,506. Although their sensitivity analysis suggested that the cost effectiveness of vasectomy reversal depends on male—not female—fertility factors, it is important to note that the effects of age on maternal fecundity were not considered. It was also suggested that sperm retrieval/ICSI would be more cost effective in situations where the need for uni- or bilateral vasoepididymostomy arose (ie, where the expected patency rate of reconstruction would be lower).

Lee and colleagues (unpublished data) most recently compared vasectomy reversal versus MESA versus percutaneous TESE for the treatment of obstructive azoospermia via use of a decision analytic model that accounts for direct and indirect costs. Costing and IVF outcomes were taken from population-based databases for maximum generalizability of results. The cost-effectiveness performance of all three therapies was also examined over time. In this study, the cost per live delivery for vasectomy reversal was $20,019 in 1999, compared with $43,886 for percutaneous TESE and $46,133 for MESA. In 2005, vasectomy reversal ($21,304) remained the most cost-effective treatment over TESE ($53,356) and MESA ($55,317). The cost effectiveness of all treatments during this time period improved over projections by inflation. Unlike the analysis by Meng and colleagues, however, sensitivity analysis suggested that cost effectiveness of vasectomy reversal was superior to MESA and TESE under all conditions, implying that the additional cost per pregnancy generated by the lower patency rates in patients requiring uni- or bilateral vasoepididymostomy is still outweighed by the cost of IVF in MESA and TESE. The duration of obstruction becomes an insignificant factor in deciding which therapy to recommend. Conversely, the improved cost effectiveness from an enhanced ability to achieve successful delivery with IVF is still outweighed by the indirect costs of the therapy. The magnitude of IVF-related indirect costs seemed to significantly alter the outcome of this decision model compared with prior studies. For instance, the probability- and inflation-adjusted cost of multiple gestation pregnancies alone ($31,637 in 1999 and $35,105 in 2005) outweighed the procedural cost of an IVF cycle in the base ($9765) and latter ($12,507) years of the study.

It should be emphasized that all cost-effectiveness studies reflect locoregional costs, which can vary dramatically. As mentioned elsewhere in the surgical literature, the results of the most experienced or successful surgeons form the basis of this analysis, which creates further bias in the results.

Summary

A detailed examination of the data regarding surgical reconstruction versus sperm retrieval with IVF/ICSI for the treatment of obstructive azoospermia reveals several key points. First, multiple techniques for surgical reconstruction and sperm retrieval exist. Vasovasostomy represents the first-line modality for surgical reconstruction and is clearly preferable to vasoepididymostomy. MESA is more effective than PESA in terms of quantity and quality of sperm retrieved with consequent impact on live delivery rates. Excellent sperm retrieval and pregnancy rates can be achieved with epididymal or testicular sperm obtained by each of these techniques. All modalities of sperm retrieval consign the female partner of a couple to undergo the greater costs and complications of an IVF cycle and expose the resulting offspring to potential health problems. It seems that reconstructive procedures should be offered as a first-line therapy to couples who seek conception after vasectomy. The treatment eventually chosen by an individual couple, however, should be an informed one based on the data available.

Cost-effectiveness analysis reveals multiple implications. First, although the direct cost of undergoing IVF can be tremendous and may vary greatly between countries, the indirect costs are even more significant, most specifically because of the impact of multiple gestation pregnancies. Randomized controlled trials have failed to consistently demonstrate the cost effectiveness of IVF over conventional, less invasive fertility treatments, such as IUI. When these data are taken into consideration for sperm retrieval in the treatment of obstructive azoospermia, it is clear why multiple studies have demonstrated the superior cost effectiveness of surgical reconstruction over sperm retrieval in linear and decision analytic models: the cost of the IVF that must be coupled to sperm retrieval is so great that it becomes a less cost-effective therapy compared with surgical reconstruction.

Limitations

It is important to note that limitations exist with cost-effectiveness analysis. Cost-effectiveness
analysis by its nature involves implicit assumptions and judgments. First, such analysis operates on the premise of maximizing health care benefits across a target population given a limited amount of economic resources (ie, it encompasses a societal perspective). Individual outcomes to specific patients are not considered, because the analysis instead considers net gains and benefits to all individuals in the population equally. Issues such as equity in individual access to health care services, the internal validity and comparability of cost-effectiveness studies, and the external validity of applying generalized cost-effectiveness models to specific locoregional conditions all remain unanswered.

Many of the studies assumed costs to be equal to charges to best evaluate the overall impact of ART on society. The impact on individual patients and individual patient willingness to undergo ART varies depending on the extent of specific health insurance coverage. None of the studies considered the downstream costs of raising children conceived by ART; higher rates of chromosomal anomalies, prematurity, and low birthweight are found in ART children, which would lead to greater downstream costs in children born via sperm retrieval IVF [50–52]. Petrou and colleagues, for example, studied the cumulative cost impact of preterm birth infants and found longer duration of hospital admissions, significantly greater inpatient service costs, and a persistent cost difference of £11,958 in £ 1998 sterling up to £14,614 over the first 5 years of life depending on gestational age. Few studies also considered the issue of maternal age impact on fecundity. For example, vasectomy reversal would represent a suboptimal therapy with greater maternal age; couples would be more likely to choose sperm retrieval in the interests of expediting the time to pregnancy and delivery, yet few of the studies considered this trend. How would the decision process be altered if a couple desired more than one child? Finally, some of the broader effects of ART were also not considered. The economic impact of multiple births, for instance, on downstream social welfare and public health programs may become significant in the future. What proportion of public resources should optimally be used to help pay for ART versus other potentially life-saving or extending technologies? What is the most efficient allocation of these resources, and how does one determine who should be the recipients? These are critical and possibly the most important questions that remain unanswered in the limited scope of cost-effectiveness analysis.

References


Endocrine Manipulation in Male Infertility
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The Population Council, Center for Biomedical Research, Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

Enthusiasm for hormonal therapy for male infertility has waned in recent years, especially with the advent of assisted reproductive techniques (ART). Given the important role of the hypothalamic-pituitary-gonadal (HPG) axis and its associated hormones in the growing economy of assisted reproduction with ovarian hyperstimulation, one cannot miss the irony of the marginal role that hormones play in the treatment of infertile men. Current clinical practice reflects two obvious realities. Endocrine therapy for male infertility has been disappointing despite many years of experimentation. With occasional exceptions, the promise of endocrine manipulation of male infertility never has been fulfilled. The second point is just as simple: ART delivers results. Not even its critics dispute the success rates of ART. Careful consideration must be taken before applying this technology indiscriminately for male infertility, however. ART requires treatment of the normal partner instead of the affected man, and there are other safety and cost considerations. In an ideal world, more effort would be devoted to identifying the underlying disorders in men who have idiopathic infertility and to reducing the need for so-called “empiric therapy.” Despite current clinical practice, it is important not to dismiss endocrine therapy of male infertility as a topic of merely academic interest. Although it now occupies a relatively minor position, hormonal therapy may assume a more prominent role in the future with continued identification of genetic mutations associated with infertility. Although these disorders are rare, mutations, small deletions, or polymorphic expansions of genes involved in the endocrine regulation of sexual development may impair male fertility [1]. Point mutations and small deletions of the androgen receptor gene resulting in androgen insensitivity syndromes are good examples of genetic endocrine disorders affecting fertility [2]. At the very least, endocrine therapy may serve as an adjunct aiding in the sperm retrieval process and thus allowing successful ART.

The most important distinction for endocrine therapy for male infertility is between specific and nonspecific treatment. Men who have specific diagnoses such as hypogonadotropic hypogonadism (HH) are treated appropriately and successfully with targeted therapy. About 1.7% of infertile men have clinically significant endocrine diagnoses [3]. These men are an important subset of fertility patients because specific medical therapies are available for their conditions, and there is very little dispute about the role of diagnosis-specific treatment. Given this small proportion of men who have specific diagnoses, one study found no prognostic value of routine examination of the male partner of infertile couples [4]. This approach is problematic because of the lost opportunity to apply diagnosis-specific therapy and because serious medical illnesses beyond infertility could remain undiagnosed. In one study, significant medical pathology was detected in 6% of men presenting for an infertility evaluation [5]. Endocrine disorders such as...
diabetes mellitus, genetic abnormalities such as cystic fibrosis mutations, and testicular tumors were among the medical problems detected.

The empiric use of hormones for idiopathic male infertility is an entirely different matter, and it is this application that has earned hormonal therapy its dubious reputation. When an infertile couple presents with a male component, as indicated by abnormal semen analyses, and there are no known male diagnoses such as obstruction and varicoceles, clinicians have used endocrine agents, usually with little or no success. Idiopathic male infertility, the presumed target of endocrine therapy, is complex and can have various causes. In this article, the label “idiopathic infertility” is applied to men who have abnormal semen parameters without an identifiable cause based on history, physical examination, and currently available laboratory studies and radiographic examinations. Nieschlag [6] evaluated a cohort of 10,469 consecutive infertile men. Thirty-one percent were diagnosed as having idiopathic infertility, the most common diagnosis among infertile men. This series underscores the limitations in the understanding of the pathophysiology of male infertility, despite remarkable technological advances in the field.

Clinical studies have referred to “idiopathic infertility” and “subfertility.” These terms have been determined based on semen analysis parameters [7]. This point highlights another potential pitfall in the discussion of hormonal therapy for idiopathic male infertility. The lack of standardization of terminology, study design, and methodology makes it difficult to accumulate sufficient data for evidence-based assessment of efficacy. Without a standardized, well-defined diagnosis, it is difficult to select appropriate study assays and outcome measures. Difficulty in defining this heterogeneous group of disorders probably contributes to the often equivocal and disappointing results in many of the clinical trials. Both specific and nonspecific endocrine therapies of male infertility are discussed in this article.

The hypothalamic-pituitary-gonadal axis

Reproductive hormones are under the control of the hypothalamus. Gonadotropin-releasing hormone (GnRH) is released in a pulsatile fashion, on average every 90 to 120 minutes, from the preoptic and arcuate nuclei of the hypothalamus. GnRH enters the portal hypophyseal venous system to stimulate the anterior pituitary gland, which releases luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates the production of testosterone by Leydig cells, and FSH supports spermatogenesis in Sertoli cells. Androgens and estrogens inhibit LH release. Sertoli cells produce inhibin that decreases FSH release [8]. Disruption at any point along the HPG axis may result in fertility problems. Patients who have these disorders can undergo targeted (specific) endocrine therapy.

Specific endocrine therapy

Targeted endocrine therapy may be applied to patients who have altered levels of GnRH, gonadotropins, androgens, and associated hormones such as prolactin, growth hormone, and thyroid-stimulating hormone. Systemic illnesses, medications, and environmental factors such as chemotherapy can impair fertility by disrupting the HPG axis; specific endocrine therapies potentially can modulate the effects of these disorders on fertility [9]. Specific disorders can be congenital or acquired and can be categorized by their defect along the HPG axis.

Gonadotropin-releasing hormone release and receptor defects

Whether the lesion is centered in the hypothalamus or in the pituitary, one main subset of male infertility associated with endocrine factors is defective action of GnRH. This category can be represented broadly by HH, perhaps the best example of a family of disorders amenable to specific endocrine therapy for male infertility. HH may be acquired, congenital, or idiopathic. One cause of HH is GnRH receptor 1 mutations in the pituitary, but the best-characterized inherited form is Kallmann’s syndrome, which is HH associated with midline defects. This genetic disorder may be transmitted in an X-linked (KAL gene) autosomal dominant or autosomal recessive pattern [10]. Kallmann’s syndrome is a failure of GnRH secretion secondary to failure of the olfactory axons to establish connections with the developing olfactory bulb, hence the association with anosmia. GnRH neurons are dependent on proper olfactory nerve migration for their own travel to the forebrain [11]. These patients usually present with delayed pubertal development. Androgen therapy initiates and sustains virilization in these patients, but
Additional gonadotropin therapy is required for spermatogenesis.

Several treatment regimens are available for patients who have HH and who desire fertility. Human chorionic gonadotropin (hCG) can be administered intramuscularly or subcutaneously two to three times per week, at doses ranging from 1000 to 2500 IU, in combination with human menopausal gonadotropin (hMG), 75 to 150 IU three times a week. If the patient has received only androgens in the past, hCG monotherapy is given until normal serum testosterone levels are achieved. Months of hCG administration may be required before the addition of hMG. Spermatogenesis is observed in more than 90% of patients on this regimen [12]. Alternative regimens include the use of intermittent injections or pulsatile infusion of GnRH (in men who have intact pituitary function) and highly purified FSH or recombinant FSH (rFSH). Those who do not respond to one regimen may benefit from another. Currently available gonadotropin preparations include hMG, urinary FSH (uFSH), highly purified urinary FSH (uFSH-HP), and rFSH [13]. rFSH is derived from genetically modified cells, whereas the other gonadotropins are prepared from urine from postmenopausal women. Unlike the others, hMG contains both FSH and LH. HMG and uFSH are less than 5% pure, whereas uFSH-HP and rFSH achieve greater than 95% and 99% purity, respectively [13]. The purity of urine-derived hormones is compromised primarily by undefined urinary protein contaminants. Given the range in cost of these preparations, several studies have evaluated relative efficacies of the available gonadotropins, predominantly in women undergoing ovarian hyperstimulation. One main comparison group is hMG versus FSH, because hMG contains both FSH and LH. In women, the precise role of LH in follicular growth and maturation is uncertain [13]. A subsequent meta-analysis detected no difference between FSH and hMG, but the validity of this study has been questioned because of problems with methodology [15–17]. In a Cochrane review comparing hMG and rFSH for ovarian stimulation in ART cycles, Van Wely and colleagues [18] found insufficient evidence of a difference between the two gonadotropins on ongoing pregnancy or live birth. The relative efficacy of commercially available gonadotropins has not been studied as extensively in men, but, as in women, a clear advantage of one gonadotropin over another has yet to be determined.

Gonadotropin release and gonadotropin receptor defects

Loss-of-function mutations can occur in genes encoding for gonadotropin and gonadotropin receptors. Hypogonadism can result from defects in the beta subunit of LH [19] and the LH receptor [20–22]. LH receptor gene mutations can result in Leydig cell hypoplasia and undermasculinization in men [20–22]. Defects in the beta subunit of FSH [23] and the FSH receptor [24] also have been reported in the literature. The patient who had the defect in the beta subunit of FSH in the 1998 report by Phillip and colleagues [23] presented with delayed puberty and had low serum testosterone and FSH but high LH concentrations. The diagnosis was made by DNA analysis. Treatment of these patients varies depending on the severity of hypogonadism, which can range widely. Therefore treatment regimens for these uncommon disorders are not standardized and should be tailored to individual clinical presentation. Unfortunately, receptor defects often are untreatable.

Androgen release and androgen receptor defects

Because androgens inhibit gonadotropins via the feedback loop of the HPG axis, exogenous androgen excess can result in hypogonadism and fertility problems. Exogenous androgen excess from anabolic steroid use impairs spermatogenesis by suppressing FSH levels and by depressing intratesticular testosterone [25]. The seminiferous tubules are exposed to testosterone concentrations 20 to 100 times higher than serum levels, because androgens normally are produced locally by the Leydig cells [26]. Testosterone supports spermatogenesis and maintains the tubular microenvironment [27]. Androgen excess also can result from endogenous sources. The most common cause is congenital adrenal hyperplasia, which results from defects in cortisol synthesis. Low levels of cortisol cause increased release of adrenocorticotropic hormone, which in turn stimulates adrenal androgen production. Men who have congenital adrenal hyperplasia may or may not have fertility problems [28,29]. Adrenal or testicular tumors also can produce excess...
endogenous androgens. Defects in androgen production, in the conversion of testosterone to dihydrotestosterone (5-alpha reductase deficiency), and in the androgen receptor also can cause infertility.

Numerous mutations of the androgen receptor gene, collectively described as “androgen insensitivity syndromes,” can disrupt normal virilization and potentially impact fertility, [30]. A relatively rare condition, androgen insensitivity syndromes affect about one in 20,000 to 64,000 live male births [31]. More than 300 mutations of the androgen receptor gene have been documented so far [32]. The phenotype of affected individuals depends on the severity of the mutation. Complete androgen insensitivity syndrome (testicular feminizing syndrome) results in the complete feminization of genetically male individuals [32]. Less severe mutations that cause partial androgen insensitivity can present with various degrees of ambiguous genitalia, including partial labial-scrotal fusion, hypospadias, bifid scrotum, and gynecomastia [33–35]. Mild androgen receptor gene mutations with minimal androgen insensitivity can manifest with impaired spermatogenesis without associated deficiencies of secondary male sexual development [36,37]. Although the phenotypic presentation varies with the severity of the gene defect, most individuals who have androgen insensitivity have similar hormone profiles [31]. Serum testosterone and LH concentrations are at or above the normal male range during the first 3 months of life and normalize during the prepubertal period [38,39]. In adult patients who have in situ testes, LH concentrations usually are elevated because of the reduced sensitivity of the hypothalamus and pituitary to feedback inhibition by sex steroids; testosterone and FSH concentrations are normal or elevated [40,41]. In partial androgen insensitivity an hCG test is used to confirm normal testosterone and dihydrotestosterone production, which distinguishes this diagnosis from defects in testosterone biosynthesis and 5 alpha-reductase deficiency [31].

Other defects

Disturbances in levels of other hormones such as estrogens, prolactin, thyroid hormone, and glucocorticoids are other endocrinologic causes of infertility that potentially are amenable to directed therapy. Diabetes mellitus is a systemic endocrinopathy that can impair fertility at many levels, including spermatogenesis and ejaculatory and erectile dysfunction [42].

Idiopathic infertility

More than 30% of infertile men have no identifiable cause for their abnormal semen analyses in the setting of a completely normal work-up (with the possible exception of mild FSH elevation) [6]. In these men who have “idiopathic” infertility, semen analyses may reveal oligo-, astheno-, or teratospermia or a combination of these conditions. Greater understanding of the factors that control spermatogenesis probably will reveal the underlying pathology of many of these disorders. Genetic mechanisms, especially autosomal disorders, may account for many cases heretofore labeled “idiopathic” [43,44]. Interest has been renewed in genetic studies to determine underlying causes of idiopathic male infertility, but the overwhelming trend has been to sidestep improvements in diagnostic evaluation in favor of a more expensive, albeit efficacious option, ART. Ideally, cause-specific therapy of male-factor infertility would minimize the use of ART, avoiding the costs, complications, and treatment of the unaffected female partner.

Empiric endocrine therapy

One approach for treating male-factor infertility has been empiric medical therapy. In fact, medical treatment for idiopathic male infertility was used for many years before the introduction of ART. Unfortunately, the efficacy of empiric therapy has been underwhelming in most clinical trials. Evaluating empiric therapy for idiopathic infertility is difficult for many reasons. Who is being treated, and what is being treated? What are the inclusion and exclusion criteria? Which treatment(s) and which dosing regimen are used? What are the outcome measures? Why perform a clinical trial when antecedent studies have borne disappointing results and with the availability of ART? Perhaps because of these obstacles, many trials conducted to date have been nonrandomized and uncontrolled. In a previous review of medical management of infertility, Siddiq and Sigman [45] point out the significant background pregnancy rate (26%) for untreated couples with abnormal semen parameters [46]. The frequency of treatment-independent pregnancies brings into question the results of
previous clinical trials that were conducted without appropriate control data.

Gonadotropin-releasing hormone agonists

One strategy of empiric therapy is to stimulate native gonadotropin secretion with GnRH agonists, which must be administered in a pulsatile manner. Very few controlled studies have been conducted. Most published trials have small sample sizes without a control arm and have varying outcome measures including gonadotropin, androgen, and estrogen levels, semen parameters, and pregnancy rate. Both GnRH analogues and synthetic luteinizing hormone-releasing hormone (LHRH) have been evaluated. The dosing regimens of hormonal agents for several trials discussed are listed in Table 1. A few randomized, controlled trials have been completed. In 1988, Badenoch and colleagues randomly assigned 19 men who had idiopathic oligozoospermia to receive one of two different dosages of buserelin (D-Ser(TBU)6-GnRH ethylamide) for 12 weeks. No effect was noted for gonadotropin levels or sperm concentrations with therapy. Crottaz and colleagues subsequently reached a similar conclusion. Twenty-eight men who had idiopathic normogonadotropic oligozoospermia (INOA) were assigned randomly to received GnRH or placebo. GnRH treatment did not improve sperm quality. Matsumiya and colleagues noted modest but statistically significant improvement in sperm parameters with buserelin therapy for men who had INOA, but more important outcome measures such as pregnancy rate were not evaluated. Although a few studies report improved semen parameters and even successful pregnancies on therapy, most trials do not have sufficient power to demonstrate a significant benefit of these medications for idiopathic therapy. There is consensus that GnRH and LHRH agonists have no role in empiric therapy.

Gonadotropins

Although the mechanism of action of FSH on spermatogenesis is unclear, FSH deficiency adversely affects sperm quantity and quality. Gonadotropins, effective in targeted therapy for HH, have been evaluated for INOA. Various urinary, purified, and recombinant forms of gonadotropins have been used, including hCG (LH analogue), hMG (FSH analogue), FSH, LH, and various combinations. There are not enough data to tease out the relative efficacies of these different agents. Multiple studies with varying methodology have presented contrasting results, some indicating benefit of gonadotropin therapy for improving semen parameters and pregnancy rates, both spontaneous and in association with ART. To clarify these heterogeneous reports, a Cochrane meta-analysis in 2006 reviewed four randomized, controlled trials with 278 participants assessing systemic gonadotropin administration for idiopathic male subfertility. This analysis focused on randomized, controlled trials using clinically relevant outcomes such as pregnancy rates instead of semen parameters. Although there were too few studies to achieve adequate power to draw definitive conclusions even with a collective sample, the analysis found a significant increase in pregnancy rates within 3 months of gonadotropin therapy, 13.4% versus 4.4% (odds ratio [OR], 3.03; 95% confidence interval [CI], 1.30–7.09). Three trials assessed spontaneous pregnancy rates (9.3% in the treatment group versus 1.7% in the control group) (OR, 4.18; 95% CI, 1.38–12.37) and pregnancy rates after intracytoplasmic sperm injection (ICSI) cycle (33.3% in the treatment group versus 20% in the control group) (OR, 1.93; 95% CI, 0.52–7.2). This ICSI trial did not achieve a statistically significant improvement in the pregnancy rate. In a related study, Ashkenazi and colleagues demonstrated improved fertilization, pregnancy, and implantation rates when purified FSH was given to 39 men who had severe (< 5 million/mL) oligospermia for 50 days before ICSI, as compared with 39 control subjects. The benefit in fertilization rate (68% in the treatment group versus 59% in the control group) and pregnancy rate (36% versus 18%, respectively) did not reach statistical significance, but the improvement in implantation was barely significant (15.5% versus 6.5%, respectively; P = .05). Of note, the doses of FSH used in these trials are far higher (usually > 150 IU/d) than typically given for specific treatment of HH. This finding may suggest the need for higher treatment doses than previously provided.

Androgens

Exogenous androgen treatment for male infertility is not indicated. As discussed previously, application of exogenous testosterone suppresses the HPG axis and results in decreased levels of intratesticular testosterone. Several potential
Table 1
Dosing regimens of hormonal agents in clinical trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Year</th>
<th>Preparation Used</th>
<th>Dose</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH/LHRH</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Schwarzstein et al [51]</td>
<td>1975</td>
<td>LHRH</td>
<td>Mean dose 500 µg/d IM</td>
<td>100–135 days</td>
</tr>
<tr>
<td>Aparicio et al [52]</td>
<td>1976</td>
<td>LHRH</td>
<td>100–500 µg/d IM</td>
<td>Long term (60 days or more) or short term (30-day intervals)</td>
</tr>
<tr>
<td>Schwarzstein et al [53]</td>
<td>1982</td>
<td>LHRH</td>
<td>5 µg IM every 2 days or 10 µg IM daily or 10 µg IM every 2 days</td>
<td>90 days</td>
</tr>
<tr>
<td>Fauser et al [54]</td>
<td>1985</td>
<td>LHRH</td>
<td>5 µg or 20 µg SC every 90 minutes</td>
<td>3 months</td>
</tr>
<tr>
<td>Honigl et al [55]</td>
<td>1986</td>
<td>LHRH</td>
<td>5 µg SC every 2 hours</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Badenoch et al [47]</td>
<td>1988</td>
<td>Buserelin</td>
<td>1 µg or 10 µg twice weekly</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Aulitzky et al [56]</td>
<td>1989</td>
<td>LHRH</td>
<td>4 µg SC every 120 minutes</td>
<td>6 months</td>
</tr>
<tr>
<td>Bals-Pratsch et al [48]</td>
<td>1989</td>
<td>GnRH</td>
<td>5 µg SC infused for 1 minute every 120 minutes or 5 µg SC infused for 1 minute every 90 minutes</td>
<td>24 weeks</td>
</tr>
<tr>
<td>Crottaz et al [49]</td>
<td>1992</td>
<td>GnRH</td>
<td>0.2 mg every 2 hours IN from 8 AM to 8 PM</td>
<td>3 months double-blind, then 1 month no treatment, then 3 months open</td>
</tr>
<tr>
<td>Matsumiya et al [50]</td>
<td>1998</td>
<td>Buserelin acetate or Clomiphene citrate</td>
<td>15 µg once daily IN 50 mg once daily</td>
<td>3 months 3 months</td>
</tr>
<tr>
<td>Gonadotropins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knuth et al [59]</td>
<td>1987</td>
<td>hMG &amp; hCG</td>
<td>150 IM 3 times per week &amp; 2500 IM twice weekly, respectively</td>
<td>13 weeks</td>
</tr>
<tr>
<td>Kamischke et al [60]</td>
<td>1998</td>
<td>rFSH</td>
<td>150 IU SC daily</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Ashkenazi et al [63]</td>
<td>1999</td>
<td>purified FSH</td>
<td>75 IU FSH ( &lt; 1 IU LH)</td>
<td>50–71 days</td>
</tr>
<tr>
<td>Baccetti et al [62]</td>
<td>2004</td>
<td>uFSH-HP</td>
<td>150 IU SC daily</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Foresta et al [61]</td>
<td>2005</td>
<td>rFSH</td>
<td>100 IU IM on alternate days</td>
<td>3 months</td>
</tr>
<tr>
<td>Androgens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aafjes et al [71]</td>
<td>1983</td>
<td>Mesterolone</td>
<td>75 mg daily</td>
<td>6 months, then cross-over</td>
</tr>
<tr>
<td>Wang et al [64]</td>
<td>1983</td>
<td>Clomiphene citrate</td>
<td>25 or 50 mg daily or 100 mg daily or 100 or 250 mg on alternate weeks</td>
<td>6 months 6 months 4 months</td>
</tr>
<tr>
<td>Hargreave et al [72]</td>
<td>1984</td>
<td>Mesterolone</td>
<td>100 mg daily</td>
<td>9 months</td>
</tr>
<tr>
<td>Pusch [65]</td>
<td>1989</td>
<td>Testosterone undecanoate</td>
<td>120 mg daily</td>
<td>100 days</td>
</tr>
<tr>
<td>World Health Organization [73]</td>
<td>1989</td>
<td>Mesterolone</td>
<td>75 or 150 mg daily</td>
<td>6 months</td>
</tr>
<tr>
<td>Comhaire [66]</td>
<td>1990</td>
<td>Testosterone undecanoate</td>
<td>240 mg daily</td>
<td>3-month double-blind period followed by 3 month open phase</td>
</tr>
<tr>
<td>Gerras et al [74]</td>
<td>1991</td>
<td>Mesterolone</td>
<td>150 mg daily</td>
<td>12 months</td>
</tr>
<tr>
<td>Comhaire et al [68]</td>
<td>1995</td>
<td>Testosterone undecanoate</td>
<td>120 mg daily</td>
<td>3 months</td>
</tr>
</tbody>
</table>

(continued on next page)
<table>
<thead>
<tr>
<th>Trial</th>
<th>Year</th>
<th>Preparation Used</th>
<th>Dose</th>
<th>Duration</th>
</tr>
</thead>
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<tr>
<td><strong>Antiestrogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ronnberg [81]</td>
<td>1980</td>
<td>Clomiphene citrate</td>
<td>50 mg daily</td>
<td>3 months (cross-over trial)</td>
</tr>
<tr>
<td>Abel et al [78]</td>
<td>1982</td>
<td>Clomiphene citrate</td>
<td>50 mg daily</td>
<td>6 months</td>
</tr>
<tr>
<td>Micic and Dotlic [82]</td>
<td>1985</td>
<td>Clomiphene citrate</td>
<td>50 mg daily</td>
<td>6–9 months</td>
</tr>
<tr>
<td>Torok [84]</td>
<td>1985</td>
<td>Tamoxifen citrate</td>
<td>20 mg daily</td>
<td>3 months</td>
</tr>
<tr>
<td>AinMelk et al [85]</td>
<td>1987</td>
<td>Tamoxifen citrate</td>
<td>20 mg daily</td>
<td>6 months</td>
</tr>
<tr>
<td>Sokol et al [79]</td>
<td>1988</td>
<td>Clomiphene citrate</td>
<td>25 mg daily</td>
<td>12 months</td>
</tr>
<tr>
<td>World Health Organization</td>
<td>1992</td>
<td>Clomiphene citrate</td>
<td>25 mg daily</td>
<td>6 months</td>
</tr>
<tr>
<td>Adamopoulos et al [69]</td>
<td>2003</td>
<td>Tamoxifen citrate and testosterone</td>
<td>20 mg daily and 120 mg</td>
<td>6 months</td>
</tr>
<tr>
<td>Hussein et al [83]</td>
<td>2005</td>
<td>Clomiphene citrate</td>
<td>Dose titrated to achieve serum testosterone levels between 600 &amp; 800 ng/dL</td>
<td>3–9 months</td>
</tr>
<tr>
<td><strong>Aromatase inhibitors</strong></td>
<td></td>
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</tr>
<tr>
<td>Clark and Sherins [95]</td>
<td>1989</td>
<td>Testolactone</td>
<td>2 g daily</td>
<td>8 months, then cross-over for another 8 months</td>
</tr>
<tr>
<td>Pavlovich et al [89]</td>
<td>2001</td>
<td>Testolactone</td>
<td>50–100 mg orally twice daily</td>
<td>5 months mean</td>
</tr>
<tr>
<td>Raman and Schlegel [94]</td>
<td>2002</td>
<td>Testolactone or Anastrozole</td>
<td>50–100 mg twice daily</td>
<td>6 months mean</td>
</tr>
<tr>
<td><strong>Oxytocin</strong></td>
<td></td>
<td></td>
<td>1 mg daily</td>
<td>4.7 months mean</td>
</tr>
<tr>
<td>Byrne et al [100]</td>
<td>2003</td>
<td>Oxytocin</td>
<td>0.75 IU</td>
<td>Two separate occasions</td>
</tr>
<tr>
<td><strong>Special uses</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cryptorchidism</td>
<td></td>
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</tr>
<tr>
<td>Hadziselimovic and Herzog</td>
<td>1997</td>
<td>Buserelin acetate</td>
<td>10 µg IN every other day</td>
<td>6 months</td>
</tr>
<tr>
<td>Huff et al [109]</td>
<td>2001</td>
<td>Naferelin</td>
<td>200 µg IN biweekly</td>
<td>6 months</td>
</tr>
<tr>
<td><strong>Cancer therapy</strong></td>
<td></td>
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</tr>
<tr>
<td>Johnson et al [122]</td>
<td>1985</td>
<td>LHRH</td>
<td>50 µg daily SC</td>
<td>Started median 5 days before chemotherapy and continued for at least 1 week following completion of chemotherapy</td>
</tr>
<tr>
<td>Fossa et al [125]</td>
<td>1988</td>
<td>Medroxyprogesterone acetate</td>
<td>500 mg daily</td>
<td>Started on day 1 of the first chemotherapy cycle</td>
</tr>
<tr>
<td>Kreuser et al [126]</td>
<td>1990</td>
<td>LHRH</td>
<td>0.4–0.5 mg three times/d</td>
<td>Started 14 days before chemotherapy and continued for 15 days after completion of chemotherapy</td>
</tr>
<tr>
<td>Masala et al [121]</td>
<td>1997</td>
<td>Testosterone (blend of enanthate and propionate esters)</td>
<td>100 mg IM every 15 days</td>
<td>Started 30 days before cyclophosphamide therapy and continued for duration of therapy</td>
</tr>
</tbody>
</table>

*Abbreviations: FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropin; IN, intranasally; IM, intramuscularly; LH, luteinizing hormone; LHRH, luteinizing hormone–releasing hormone; rFSH, recombinant follicle-stimulating hormone; SC, subcutaneously; uFSH-HP, highly purified urinary follicle-stimulating hormone.*
mechanisms of action had been proposed. Historically, androgens were among the first empiric treatments for idiopathic male infertility, based on the premise that raising serum testosterone levels would improve epididymal maturation and boost spermatogenesis. Another rationale for the use of androgens is the so-called “rebound phenomenon.” Exogenous testosterone inhibits the HPG axis and results in azoospermia; a transient increase in gonadotropins upon stopping testosterone administration has been observed. A third hypothetical potential benefit of testosterone administration has been in the treatment of men who have androgen insensitivity. The resistance of these patients could be overcome with higher circulating testosterone levels. No data suggest this treatment approach is effective. More than 11 randomized, controlled trials have evaluated whether androgen therapy improves pregnancy rates. Testosterone enanthate [64], testosterone undecanoate [65–69], or mesterolone (orally active dihydrotestosterone derivative) [64,70–74] were used in these trials. Liu and Handelsman [75] performed a meta-analysis pooling data from 10 of these studies involving more than 1000 men and found no improvement in pregnancy rate with androgen therapy (OR, 1.09; 95% CI, 0.73–1.62). Two prior meta-analyses also did not demonstrate efficacy of androgens for idiopathic infertility [76,77]. Available evidence strongly argues against any role of androgen monotherapy for idiopathic male infertility.

Antiestrogens

Clomiphene citrate and tamoxifen citrate are two nonsteroidal selective estrogen receptor modulators commonly used for idiopathic infertility. They block the estrogen receptor, including those in the hypothalamus and pituitary, preventing estrogenic inhibition of gonadotropin secretion. Tamoxifen is a weak estrogen agonist, although it is stronger than clomiphene. Multiple clinical trials, including many uncontrolled studies, have been performed for both clomiphene and tamoxifen and have demonstrated mixed efficacy. Of the randomized, controlled trials completed for clomiphene, several detected no benefit of treatment for idiopathic infertility [78–80]. Others indicated some improvement in semen parameters with clomiphene therapy but no statistically significant improvement in pregnancy rates [64,81,82]. A meta-analysis of these clomiphene studies detected no benefit of therapy on pregnancy rates (OR, 1.48; 95% CI, 0.84–2.61) [75]. In a more recent study, Hussein and colleagues [83] evaluated the potential benefit of clomiphene for men who had nonobstructive azoospermia. Forty-two men (43% of whom had maturation arrest and 57% of whom had hypospermatogenesis) were treated with clomiphene, with dosing titrated to maintain serum testosterone levels between 600 ng/dL and 800 ng/dL. Sixty-four percent of patients achieved sperm in their analyses with a mean sperm density of 3.8 million/mL (range, 1–16 million/mL). Sperm for ICSI was retrieved successfully in all patients. In those requiring testicular biopsy, evaluation demonstrated a statistically significant improvement in histologic criteria after treatment. The lack of a control arm severely limits the potential application of clomiphene therapy in azoospermic men undergoing ART.

Several randomized, controlled trials have assessed tamoxifen’s performance for idiopathic infertility [84–86]. Liu and Handelsman’s [75] meta-analysis of these studies demonstrated no significant treatment effect (OR, 1.64; 95% CI, 0.81–3.34); combining the clomiphene and tamoxifen results did not change the outcome (OR, 1.54; 95% CI, 0.99–2.40). A Cochrane meta-analysis of 10 randomized, controlled studies involving 738 men who had idiopathic infertility found no difference in pregnancy rate with antiestrogen therapy [87]. Pregnancy rates in the five trials with secure randomization were 15.4% in the treatment arm versus 12.5% in the control arm (OR, 1.26; 95% CI, 0.99–1.56). In 2003, Adamopoulos and colleagues [69] randomly assigned 212 men who had idiopathic oligozoospermia to treatment with 120 mg/d of testosterone undecanoate and 20 mg/d of tamoxifen citrate for 6 months or to placebo treatment. Eighty-two normozoospermic men were observed during the trial along with the treatment groups. Semen analyses and pregnancy incidence were evaluated 3, 6, and 9 months after the initiation of therapy. Sperm parameters improved in the group treated with testosterone and tamoxifen, and, more importantly, there was a significantly higher rate of spontaneous pregnancy in this group than in the placebo group (RR, 3.20; 95% CI, 2.62–3.77). This study explored combination therapy using two agents that act at different levels along the HPG axis, with the hypothetical benefit of synergy. It is possible that tamoxifen and antiestrogens in combination
with other agents could be useful for treating idiopathic infertility.

Aromatase inhibitors

Aromatase is a cytochrome P-450 enzyme that converts testosterone to estradiol and androstenedione to estrone. In addition to the female reproductive tract and adipose tissue, aromatase can be found in the testis, liver, and brain, among other tissues. In the testis, aromatases are localized to Leydig and Sertoli cells and are found in germ cell tumors [88]. The effects of aromatase can be blocked by inhibitors. Reflecting the interplay of aromatase with its inhibitors, investigators have used the ratio of testosterone to estrogen (T/E2) instead of absolute levels of each to assess patients’ hormonal status. Pavlovich and colleagues [89] demonstrated a significantly lower T/E2 ratio in men who had severe infertility (6.9 versus 14.5 in age-matched reference subjects; \( P < .01 \)). Aromatase inhibitors have been applied to idiopathic infertility with the intent of reducing estrogenic effects on the male reproductive system, especially by reducing feedback inhibition of the HPG axis [90]. Estrogens also have direct adverse effects on the germinal epithelium [91]. High estrogen levels, perhaps in combination with low androgen levels, have been shown to impair spermatogenesis [92]. One animal study, however, found that local expression of aromatase is essential for spermatogenesis, pointing to a role of estrogen in male germ cell development [93].

Aromatase inhibitors are available in steroidal and nonsteroidal oral formulations. Testolactone, a steroidal inhibitor, has been studied in several trials for idiopathic male infertility. The study by Pavlovich and colleagues [89] noted a statistically significant increase in the T/E2 ratio as well as improvement in sperm parameters when men who had severe male infertility were treated with testolactone. In this study, the treatment group was selected with specific hormonal criteria using an age-matched, fertile control reference group.

Raman and Schlegel [94] evaluated the effects of anastrozole, a more selective aromatase inhibitor, on hormonal and semen parameters of infertile men who had abnormal T/E2 ratios. This was not a study of empiric treatment, because it did not focus on patients who had INOA; some of the patients had other male-factor diagnoses such as Klinefelter’s syndrome and varicoceles. The study included 140 infertile men who had abnormal T/E2 ratios. Seventy-four patients were given 50 to 100 mg of testolactone twice daily for a mean duration of 6 months, and 104 men were given 1 mg of anastrozole daily for a mean duration of 4.7 months. No untreated group was included. Subgroup analyses were performed on overweight patients, patients who had Klinefelter’s syndrome, and those who had past or current varicoceles. Both treatment groups had a statistically significant increase in T/E2 ratios, and improved semen parameters were noted in those who underwent semen analyses before and after treatment. In men treated with testolactone, the T/E2 ratio increased from 5.3 ± 0.2 to 12.4 ± 1.1 (mean ± standard error of the mean) \( (P < .001) \). Sperm concentration increased from 5.5 to 11.2 million sperm per mL \( (P < .01) \), motility increased from 14.7% to 21.0% \( (P < .05) \), morphology increased from 6.5% to 12.8% \( (P = .05) \), and the motility index increased from 606.3 to 1685.2 million motile sperm per ejaculate \( (P < .05) \). Similarly, in men treated with anastrozole, the T/E2 increased from 7.2 ± 0.3 to 18.1 ± 1.0 \( (P < .001) \). Sperm concentration increased from 5.5 to 15.6 million sperm per mL \( (P < .001) \), and the motility index increased from 832.8 to 2930.8 million motile sperm per ejaculate \( (P < .005) \). The men who had Klinefelter’s syndrome did not improve during anastrozole therapy, perhaps because anastrazole does not inhibit adrenal steroid. Pregnancy rates were not evaluated, because many men had nonobstructive azoospermia; this clinically important outcome may be addressed in future randomized, controlled trials.

One randomized, controlled trial already has investigated the use of testolactone in men who have INOA [95]. Twenty-five men who had INOA were assigned randomly to testolactone, 2 g/d, or placebo for 8 months followed by crossover to the other treatment arm for another 8 months. Oddly, estradiol and testosterone levels did not change with treatment during the trial. Sex hormone–binding globulin, however, decreased by 30%, and free testosterone increased by 36% with treatment. There was not a statistically significant increase in free estradiol levels. The increase in serum levels of LH, FSH, and 17-alphahydroxyprogesterone was 15%, 20%, and 90%, respectively. No improvement in semen parameters was noted, and no pregnancies occurred in either group. It is difficult to reach a conclusion about the use of aromatase inhibitors for the treatment of idiopathic infertility based on these studies. Further evaluation is needed.
Oxytocin

Oxytocin is best known for its role in parturition and lactation, but it has endocrine and paracrine roles in the male reproductive tract [96]. Oxytocin is a neurohypophysial hormone mainly released into the systemic circulation by the posterior pituitary. Oxytocin also is synthesized within the reproductive tract, where oxytocin receptors have been localized. Ogawa and colleagues [97] found increased plasma oxytocin levels after ejaculation. Filippi and colleagues [98] found oxytocin receptors within the human epididymis where it stimulated in vitro contractility. They also found that oxytocin promoted sperm progression through the reproductive tract and increased sperm retrieval in oligozoospermic men. Oxytocin also increases the conversion of testosterone to dihydrotestosterone [99]. With this background information, Byrne and colleagues [100] hypothesized that oxytocin’s role in epididymal contractility may improve motile sperm output in severely oligozoospermic men. They randomly assigned 49 men who had sperm concentrations below 0.2 million/mL to receive intravenous saline or oxytocin (0.75 IU) injections on two separate occasions. The injections were administered in random order 5 minutes before collection of masturbatory samples. Single-dose oxytocin had no effect on semen parameters. Oxytocin is a relative newcomer to the hormonal arsenal in the treatment of male infertility. Undoubtedly, the potential role of oxytocin as a therapeutic agent will be explored further.

Special applications of endocrine therapy

Cryptorchidism

The association between cryptorchidism and infertility is well known [101]. Histologic analyses of undescended testes (and contralateral descended testes) demonstrate diminished germ cell counts, which correlate with abnormal semen analyses in adulthood [102]. It is thought that germ cells are dependent on two stages of maturation, with the first step occurring at 2 to 3 months of age [103]. At this stage, gonocytes transform into adult dark spermatogonia. Adult dark spermatogonia then transform into primary spermatocytes at 4 to 5 years of age. Both steps occur during gonadotropin surges. These surges are attenuated in patients who have cryptorchidism [104,105], impairing the maturation of germ cells especially in the ipsilateral testis [106,107]. In a preliminary study, the GnRH analogue buseralin resulted in improved germ cell counts and semen parameters compared with controls [108]. Hadziselimovic and Herzog evaluated 10 men (current mean age ± SD of 22.1 ± 2.8 years) who had previously had orchiopexy at a mean age of 9.4 ± 2.8 years for unilateral (seven men) or bilateral (three men) cryptorchidism after they failed to respond to hormonal treatment with hCG. These patients received low-dose buseralin (10 μg every other day) as a nasal spray for 6 months after orchiopexy. Twenty-three men (mean age, 20.9 ± 2.5 years) who had not received hormonal treatment following orchiopexy for unilateral (13 men) or bilateral (10 men) cryptorchidism served as a comparison group. The authors noted an increased number of normal forms of spermatozoa per ejaculate and improved motility in the treatment group. Huff and colleagues [109] treated 12 boys who had cryptorchidism (six unilateral and six bilateral) and severely reduced germ cell counts with the GnRH analogue naferelin after orchidopexy with biopsy. The patients ranged in age from 7 months to 14 years during the time of the study. Eight of the 12 patients (five of the six who had unilateral disease and three of the six who had bilateral disease) had improved total germ cell counts in one or both testes. Prognosis was better in unilateral disease. Additional controlled studies are needed.

Cancer therapy

Each year 17,000 men between the ages of 15 and 45 years are diagnosed as having Hodgkin’s disease, lymphoma, bone and soft tissue sarcomas, testicular cancer, or leukemia, and 3000 of them undergo treatment with alkylating agents, platinum-based chemotherapy, or radiation therapy [110,111]. Following successful cancer treatment, the consequences of gonadotoxic therapy, including infertility, are important issues for patients in this age group. The degree and duration of impairment of spermatogenesis depend on the therapeutic agents and doses used. Although it is thought that Leydig and Sertoli cells generally survive cytotoxic therapies, several studies found evidence of Leydig cell insufficiency following chemotherapy [112,113]. Without question, germ cells, especially differentiating spermatogonia, are particularly vulnerable to the effects of cytotoxins. Histologic evaluation of testicular specimens after cytotoxic
therapy often demonstrates Sertoli cell–only pathology [111]. Germ cells must survive the treatment course and retain their capacity for differentiation. Spermatogenesis may be impaired for 1 to 6 years or more, if not permanently, depending on the agent and dose used.

In recent years much attention has been focused on applying hormonal therapy to help preserve or restore fertility following cytotoxic therapy. Studies in rats exposed to radiation [114] or procarbazine [115] have demonstrated impairment of spermatogenesis as well as elevation of serum gonadotropins and intratesticular testosterone [114–116]. These exposures depleted all the germ cells except spermatogonia; the spermatogonia continued to proliferate after application of the cytotoxins but underwent apoptosis when ready to differentiate [116]. Hormonal evaluation of the rats after cytotoxic exposure revealed elevated LH and FSH levels, normal serum testosterone levels, but markedly increased intratesticular testosterone [115,116] levels. From this observation it was hypothesized that FSH and testosterone were inhibiting germ cell differentiation. Rats were treated with a GnRH analogue immediately after radiation [117] or procarbazine [115] exposure, resulting in increased numbers of differentiated germ cells. Differentiation of the germ cells stopped at the round spermatid stage because of low testosterone. When therapy ceased, however, tubules demonstrated almost complete recovery of spermatogenesis [118]. Pretreatment of rats with hormones before cytotoxic exposure similarly improved spermatogenesis [119,120]. Only one of seven human trials has demonstrated a protective effect of hormone suppression therapy given before and during cytotoxic therapy [111,121–127]. Masala and colleagues [121] randomly assigned 15 patients who had nephrotic syndrome undergoing cyclophosphamide therapy for 6 to 8 months to receive daily oral cyclophosphamide, monthly intravenous cyclophosphamide boluses, or monthly intravenous boluses of cyclophosphamide and testosterone (100 mg intramuscular injections every 15 days). There were five patients in each group. All 15 patients became azoospermic or severely oligospermic during therapy. All five patients receiving testosterone had normal sperm counts 6 months after cessation of cyclophosphamide treatment, versus only 1 of 10 in the nontestosterone groups.

Shetty and Meistrich [111] offer a few possible explanations for the failure of many of the other human trials. The human trials tested several different hormone regimens, including GnRH agonist, testosterone, medroxyprogesterone acetate, and combination therapy. These authors point out the counteractive effect of testosterone supplementation and the low efficacy of medroxyprogesterone acetate in the trials on rats. Also, the successful human trial involved cyclophosphamide therapy, whereas the other trials included very few, if any, patients treated with this agent. Cyclophosphamide may have only moderate stem cell kill. The different cytotoxic therapies and hormonal treatment regimens make it difficult to compare these studies. Overall, the promising results of animal studies have not been duplicated in human trials. Alternative methods for preserving fertility after cancer treatment are being pursued, including pretreatment harvest and cryopreservation of testicular tissue or dispersed germ cells. The transplantation of cryopreserved spermatogonia is being evaluated [128], and initial rodent studies point to the beneficial effects of GnRH analogues in the survival of transplanted cells [129,130].

Summary

Although male infertility with a primary endocrine etiology is encountered infrequently in clinical practice, endocrinopathies represent an important category of illnesses amenable to available medical treatments. Further research endeavors with genetic analyses probably will identify more endocrine disorders that previously had been classified as idiopathic. In contrast to endocrine diagnoses, idiopathic infertility represents the most frequently encountered male diagnosis and generally is not responsive to available medical treatments. Although most clinical trials have not yielded promising results, a few studies demonstrate a potential for future development and application of endocrine therapy. Further trials, especially randomized, controlled trials with adequate sample size, are needed. Specific subgroups of men, including those undergoing ART, may be good candidates for trials of empiric hormone therapy. Special attention must be paid to study design for future trials, because idiopathic infertility represents a heterogeneous population of patients. Methodologies and outcome measures must be selected with care, perhaps in collaboration among multiple centers. A better understanding of the role of hormones in the pathophysiology of male infertility may allow the judicious application of both endocrine therapy and ART.
References


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Reactive oxygen species (ROS) are free radicals that are derived from the metabolism of oxygen. The production of ROS, such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and the hydroxyl radical (OH$^-$), normally occurs in cells. ROS play an important role in multiple cellular physiologic processes, such as phagocytosis, and in many signaling processes. In the male reproductive tract, small amounts of ROS are involved in many sperm functions. Hydrogen peroxide stimulates sperm capacitation, the acrosome reaction, and hyperactivation. Free radicals are also involved in the fusion of spermatozoa with the oocyte [1,2].

Oxidative stress is a condition that occurs when high concentrations of ROS exist relative to antioxidant capacity. In 1943, John MacLeod first made the observation that oxidative stress could be a significant cause of male infertility [3,4]. Neutrophils, macrophages, and immature spermatozoa are the major sources of ROS in the male reproductive tract. Leukocyte production of ROS in the setting of infection and inflammation may lead to the propagation of excessive ROS levels in surrounding tissues [5]. In higher concentrations, ROS may cause varying degrees of sperm dysfunction, depending on the extent of oxidative stress. Damage from ROS occurs primarily through two routes: First, ROS may be responsible for the DNA fragmentation commonly seen in the spermatozoa of infertile men by causing single- and double-stranded DNA breaks [6].

Aitken and colleagues [7] described how a low concentration of hydrogen peroxide did not affect sperm motility but did suppress sperm-egg fusion. Second, higher levels of ROS also may cause damage through a chain of chemical reactions that result in lipid peroxidation of the sperm plasma membrane [8]. Lipid peroxidation results in loss of the membrane fluidity, which is essential for sperm motility and sperm-oocyte fusion. Griveau and Le Lannou [1] have shown that the acrosome reaction in human spermatozoa was susceptible to ROS. Several studies have shown that levels of ROS inversely correlate with sperm motility.

**Antioxidants**

Normally, antioxidant scavengers constantly inactivate ROS. Seminal plasma contains significant amounts of antioxidants. The human body uses three general systems of antioxidants for protection against free radicals: endogenous antioxidants, dietary antioxidants, and metal-binding proteins [9]. Endogenous antioxidants can be categorized as low molecular weight molecules, such as bilirubin, thiols, uric acid, and coenzyme Q-10 (CoQ10), and larger molecular enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. All three of the larger molecular enzymes are essential for adequate antioxidant defense. Dietary antioxidants include vitamin C, vitamin E, carotenoids, and flavonoids. The mechanism of action of the dietary antioxidants includes scavenging free radicals and interfering with the chain of chemical reactions that lead to lipid peroxidation. Antioxidants that act through the last mechanism are known as chain-breaking antioxidants. Metal-binding proteins, such as
albumin, ceruloplasmin, metallothionein, transferrin, ferritin, and myoglobulin, inactivate the transition metal ions (eg, iron) that catalyze the production of free radicals [10,11]. Twigg and colleagues demonstrated the protective effect of adding albumin to sperm in vitro by binding to lipid peroxides or binding to ferrous ion promoters [11,12].

### Evaluation of oxidative stress

Oxidative stress occurs when there is an imbalance between ROS production and antioxidant capacity. High levels of seminal ROS have been found in 30% to 80% of infertile men [7]. Elevated levels of ROS and decreased levels of total antioxidant capacity have been noted in patients with varicoceles [13]. A positive correlation between ROS and varicocele grade also has been noted [14]. With infection of the male reproductive tract, one can expect elevated levels of ROS caused by the presence of leukocytes. Patients with prostatovesiculoepididymitis have been shown to have higher ROS levels than patients without infection [15–17].

Because oxidative stress has been shown to play a key role in male infertility, it is important to be able to quantify the level of oxidative stress to determine if it is a significant contributor to sperm dysfunction in a given patient. There are numerous methods to determine the ROS levels in semen. Chemiluminescence, one of the most widely used methods to assess the level of ROS, discriminates between the production of superoxide and hydrogen peroxide by spermatozoa by the reagent used (luminol and lucigenin are the most common reagents) [7,18]. Flow cytometry uses the principles of light scattering, light excitation, and emission of fluorochrome molecules to generate specific multiparameter data measures. By measuring the fluorescent intensity of a particular dye after oxidation by ROS, one can quantify the amount of ROS present in the sample [19]. The total antioxidant capacity of the semen can be determined by either the enhanced chemiluminescence assay or the calorimetric assay. In both of these assays, ROS production is chemically induced, which results in the development of a chemiluminescence signal or a color change. The ability of the seminal plasma to inhibit the production of the signal or color change is compared with known amounts of a control antioxidant. The greater the ability of the seminal plasma to inhibit the production of the signal or color change, the greater the antioxidant capacity of the semen sample. By comparing the degree of inhibition of the reaction by seminal plasma to the ability of known amounts of the control antioxidant to inhibit the reaction, the amount of antioxidant capacity can be quantified [20,21].

Because the ultimate amount of oxidative stress and subsequent damage may be a function of the total amount of ROS and the total antioxidant capacity, an ROS-total antioxidant capacity score was developed. This measurement mathematically combines the ROS and total antioxidant capacity measurements of a patient’s semen and control semen. The controls consisted of healthy men with low ROS levels who had achieved pregnancy with their partner in the past 2 years [5]. A low ROS-total antioxidant capacity score (< 30) indicates the presence of high oxidative stress and sperm dysfunction. Despite the different methods available to measure ROS, currently no standard exists for estimating oxidative stress. Allamaneni and colleagues [22] defined the basal levels of ROS in normal donors in whole, unprocessed semen specimens and in mature and immature spermatozoa. It is hypothesized that the determination of what the basal level of ROS is in human semen may be used to identify pathologic ROS levels in infertile men and ultimately guide treatment.

### Treatment of infertility caused by oxidative stress

Several approaches have been suggested to manage infertility caused by oxidative stress. Unfortunately, most clinical trials of antioxidants do not select patients based on measured levels of oxidative stress in semen, and many do not correlate treatment to changes in oxidative stress. Attempts may be made to decrease endogenous ROS production by changes in behaviors and lifestyle habits that have been associated with increased ROS production. Antioxidant supplements have been proposed and are commonly sold to increase the antioxidant capacity of semen, thereby scavenging excess ROS and resulting in less oxidative stress. Finally, specific laboratory techniques may be used to decrease ROS production, which is often stimulated when sperm are handled in the laboratory.

#### Behavior and lifestyle modification

Various behaviors and lifestyles have been associated with increased ROS production.
Cigarette smoking has been shown to increase ROS in semen and decrease semen quality (density, total count, and motility) [23]. Exposure to environmental pollution also has been linked to increased ROS production. A study of 85 middle-aged tollgate workers exposed to traffic pollutants showed significant decreases in total sperm viability, motility, and membrane function compared with age-matched controls living in the same area [24]. Finally, several systemic diseases, such as diabetes mellitus, cancer, cardiovascular problems, and infection, are known to increase the production of ROS [25]. These data suggest that behavior and lifestyle modification and treatment of a patient’s underlying pathology should be the first steps in reducing ROS. Unfortunately, few data link changes in these exposures to decreased oxidative stress and subsequent increases in human fertility. Although it is likely good medical practice to recommend modifications of unhealthy lifestyles or exposures, definitive evidence awaits further studies.

Dietary antioxidants

Dietary antioxidants are present in fruits and vegetables and daily dietary supplements. The National Academy of Sciences recommends 90 mg/d of vitamin C for an adult man and 15 mg/d of vitamin E [26,27]. Carotenoids and selenium have recommended daily allowances of 900 and 55 μg/d, respectively. Despite the daily recommendations for dietary antioxidants, randomized trials have largely failed to show an effect of antioxidant vitamins on the risk of other disease processes, such as cardiovascular disease. The Women’s Antioxidant Cardiovascular Study was a randomized trial that tested the effects of vitamin C (500 mg/d), vitamin E (600 IU every other day), and beta-carotene (50 mg every other day) on the combined outcome of myocardial infarction, stroke, coronary revascularization, and cardiovascular disease–related death among 8171 female healthcare professionals. There were no overall effects of vitamin C, vitamin E, or beta-carotene on cardiovascular events among women at high risk for cardiovascular disease [28]. This study suggested that at least for cardiovascular disease, antioxidants in pill form are not effective in modifying risk. The possibility of adverse events with therapy also should be considered.

A recent meta-analysis of trials of antioxidants to prevent gastrointestinal cancers found an increase in mortality with the use of most supplements [29]. Menezo and colleagues [30] reported that in an uncontrolled study, treatment with a combination of oral antioxidants led to decreased sperm DNA fragmentation and an increase in sperm DNA decondensation, which is likely not advantageous. The finding of a link between oxidative stress and sperm DNA fragmentation has led to studies looking for changes in semen DNA fragmentation as an outcome rather than just changes in semen parameters. Although the most desirable outcome measure is pregnancy rate, this is rarely reported. Although obtaining antioxidants through a diet rich in these compounds may be considered, a recent study reported no relationship between dietary antioxidant intake and the degree of sperm DNA fragmentation [31]. A positive relationship between dietary antioxidant intake and better semen parameters has been reported, however [32]. Unfortunately, comparison of individual studies is often difficult because many studies use combinations of different antioxidants and endpoints.

Vitamin C (ascorbic acid), a major antioxidant present in extracellular fluid, is present at a high concentration in seminal compared with blood plasma (364 versus 40 μM) and is present in detectable amounts in the sperm themselves. Vitamin C is known to be an effective scavenger of hydroxyl, superoxide, and hydrogen peroxide radicals. It also has been shown to recycle tocopherol (vitamin E) by repairing its tocopheroxyl radical, thereby allowing vitamin E to function as a free radical chain-breaking antioxidant [33]. Vitamin C has been found in reduced quantity in the seminal plasma of infertile men [34]. In a randomized controlled trial of 75 fertile, heavy smokers divided into placebo, 200-mg, and 1000-mg vitamin C supplementation group, both supplementation arms of the study showed a significant improvement in sperm concentration, morphology, and viability (P < .01 and P < .001 for the 200- and 1000-mg groups, respectively) [35]. Although an uncontrolled study reported an improvement in semen parameters of infertile men after treatment with vitamins C and E, a randomized controlled trial demonstrated no effect (Table 1) [6,40].

In a randomized, placebo-controlled trial, Greco and colleagues [36] found that treatment of men with unexplained infertility associated with elevated sperm DNA fragmentation (≥15%) with oral vitamin C and E, led to decreased DNA fragmentation without a change in semen parameters. It is important to note that these
<table>
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<th>Study</th>
<th>Antioxidant</th>
<th>Study design</th>
<th>N</th>
<th>Study population</th>
<th>Dose and duration</th>
<th>Outcome</th>
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<td>Greco et al [36]</td>
<td>vitamin C, vitamin E</td>
<td>R/C/B</td>
<td>64</td>
<td>infertile men with elevated percentage of DNA-fragmented spermatozoa</td>
<td>1 g vitamin C, 1 g vitamin E daily, 2 mo</td>
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<td>men with an elevated percentage of DNA-fragmented spermatozoa with one failed ICSI attempt</td>
<td>1 g vitamin C, 1 g vitamin E daily, 2 mo</td>
<td>no difference in cleavage or fertilization rates or embryo morphology; significant improvement in pregnancy and implantation rates</td>
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<tr>
<td>Keskes-Ammar et al [38]</td>
<td>vitamin E and selenium</td>
<td>R/C/NB</td>
<td>53</td>
<td>infertile male volunteers</td>
<td>400 mg vitamin E, 225 µg selenium daily, 3 mo</td>
<td>improved motility, decreased MDA concentration decreased ROS, decreased 8-hydroxy-deoxyguanosine; no improvement in concentration, morphology, or motility</td>
</tr>
<tr>
<td>Comhaire et al [39]</td>
<td>vitamin E, vitamin A, acetyl-cysteine</td>
<td>NR/C/NB</td>
<td>27</td>
<td>infertile men</td>
<td>600 mg acetylcysteine or 30 mg β-carotene, 180 mg vitamin E daily, 6 mo</td>
<td>decreased ROS, decreased MDA concentration, decreased DNA damage; no improvement in morphology or motility</td>
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<td>Rolf et al [40]</td>
<td>vitamin C, vitamin E</td>
<td>R/C/B</td>
<td>33</td>
<td>asthenozoospermia</td>
<td>1000 mg vitamin C and 80 mg vitamin E daily, 56 d</td>
<td>no improvement in concentration, motility, morphology, viability</td>
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<td>infertile men</td>
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<td>R/C/B</td>
<td>30</td>
<td>infertile men with high ROS</td>
<td>600 mg/d, 3 mo</td>
<td>improved zona-binding assay; no improvement in motility, concentration, morphology or ROS</td>
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**Abbreviations:** ART, assisted reproduction technique; B, blinded; C, controlled; MDA, malondialdehyde; NB: nonblinded; NC, no controls; NR, nonrandomized; R, randomized.
studies did not choose patients based on abnormal ROS levels. An uncontrolled study of 38 men with an elevated percentage of fragmented spermatozoa (≥15%) and one prior failed intracytoplasmic sperm injection (ICSI) attempt who underwent oral supplementation with vitamins E and C demonstrated a significant improvement in pregnancy (48.2% versus 6.9%) and implantation (19.6% versus 2.2%) when compared with their prior ICSI attempt [37]. No current randomized controlled trials show an improvement in the semen parameters or pregnancy rates of healthy infertile men who take oral supplementation of vitamin C.

Vitamin E (α-tocopherol), which is present within the cell membrane, is one of the major membrane protectants against ROS. It neutralizes hydrogen peroxide and protects the plasma membrane from lipid peroxidation. Suleiman and colleagues [42] showed that oral administration of 300 mg/d of vitamin E in 52 asthenospermic patients significantly decreased malondialdehyde concentration (a measure of lipid peroxidation) in spermatozoa and improved sperm motility versus placebo. Furthermore, 11 of the 52 spouses in the treatment group became pregnant in the 6-month treatment period, but there was none in the placebo group. In a double-blind, randomized, placebo cross-over controlled trial, Kessopoulou and colleagues [43] showed that oral administration of vitamin E (600 mg/d) in a population of 30 men with high levels of ROS in their semen (measured using the chemiluminescent method) improved sperm function as assessed by the zona pellucida binding assay. It should be noted that a randomized, placebo-controlled, double-blind study by Rolf and colleagues [40] did not show an improvement in conventional semen parameters or 24-hour sperm survival rate in 32 patients with asthenozoospermia or moderate oligoasthenozoospermia treated with high-dose oral vitamins C and E for an 8-week period. Studies on oral vitamin C and vitamin E supplementation for treatment of male infertility are summarized in Table 1. Although more well-designed studies (randomized controlled trials) examine the effects of oral vitamin E supplementation than any other antioxidant, the studies showed conflicting results when the endpoint was improvement in semen parameters (Table 1) [6,36–38,40–42,44].

Selenium, a trace element, is necessary for the synthesis of glutathione peroxidase. Vitamin E has been closely linked to selenium metabolism, and it has been shown that vitamin E and selenium work synergistically as antioxidants. Keskes-Ammar and colleagues [38] found that 225 μg/d of oral selenium in combination with 400 mg/d of oral vitamin E over a 3-month period significantly decreased malondialdehyde concentrations (a lipid peroxidation marker) in seminal plasma and improved sperm motility. These findings were not confirmed in another study, however [45]. Few studies looking at the effects of selenium supplementation on male infertility, and the results of these studies are conflicting.

Carotenoids, such as beta-carotene and lycopene, are an important component of antioxidant defense [46]. Beta-carotenoids protect the plasma membrane against lipid peroxidation [47]. Lycopene, which is found in tomatoes, has a suggested daily intake of 5 to 10 mg/d. Lycopene has been shown to be twice as potent as beta-carotene and ten times more potent than vitamin E in scavenging singlet oxygen and inhibiting lipid peroxidation in serum plasma [48]. A recent study by Goyal and colleagues [49] showed a significant increase in seminal plasma levels of lycopene after oral supplementation with a processed form of tomatoes (22.8 mg of lycopene) daily for a 2-week period. No net increase in the radical scavenging capacity of the oral lycopene-enriched seminal plasma was noted, however. Astaxanthin, a carotenoid extracted from the algae Hemaococcus pluvialis, is an antioxidant that is a much higher singlet molecular oxygen quencher than vitamin E. A small, double-blinded, randomized controlled trial of men with infertility who received 16 mg/d of Astaxanthin for 3 months showed significantly higher sperm linear velocity and total and per cycle pregnancy rates when compared with placebo [44]. Of note, this study included patients who achieved pregnancies by intercourse whereas others followed IUI. Other treatments, including varicocele repair, anti-estrogens, and antibiotics were used in some patients. The number of studies investigating oral supplementation of carotenoids or lycopene in the setting of male infertility is sparse. More studies are needed to determine if carotenoid or lycopene supplementation significantly affects semen parameters in men who have infertility.

Glutathione is one of the most common antioxidants found in the body. It plays an important role in protecting lipids, proteins, and nucleic acids against oxidative damage. It combines with vitamin E and selenium to form glutathione peroxidase (the main enzyme involved
in removing hydrogen peroxides in the epididymis). It is found at physiologically significant concentrations in seminal plasma and, although it cannot cross cell membranes, its concentration can be increased in biologic fluids by parenteral administration [27]. In a placebo-controlled, double-blinded crossover trial, 600 mg glutathione was administered for 2 months by intramuscular injection in 20 infertile men. Glutathione therapy significantly increased sperm motility, particularly forward progression [50].

CoQ10, an energy-promoting agent, is concentrated in the mitochondria in the sperm midpiece. CoQ10 recycles vitamin E and prevents its pro-oxidant activity [51]. The reduced form of CoQ10 ubiquinol, also acts as an antioxidant preventing lipid peroxidation. CoQ has been shown to inhibit hydrogen peroxide formation in the seminal fluid and seminal plasma of infertile men [52]. In an in vitro study on semen samples of men with asthenozoospermia, incubation with 50 µM of CoQ significantly increased sperm motility. In the same uncontrolled study, in vivo supplementation of 60 mg/d of oral CoQ for a mean of 103 days in 17 infertile men improved their fertilization rate via ICSI without changing their semen parameters [53]. An open, uncontrolled study of 22 men with idiopathic asthenozoospermia who were given 400 mg/d of oral CoQ for 6 months also showed a significant improvement in forward motility compared with pretreatment results [54]. The administration of oral CoQ may be beneficial in the treatment of asthenozoospermia because of its role in mitochondrial respiratory chain and as an antioxidant; however, randomized controlled trials are needed to further substantiate the positive effect of oral CoQ supplementation on sperm motility.

Zinc and copper are trace elements that constitute a part of the antioxidant enzyme superoxide dismutase. Adequate intake of these elements is important to maintain the function of these enzymes. The estimated average daily intake in the United States is 12.3 mg zinc and 900 µg of copper. Although both of these metals are important constituents of an antioxidant enzyme, high levels of these metals may catalyze reactions that lead to an increase in ROS. In an in vitro study on salmon sperm DNA, Lloyd and colleagues [55] showed that at concentrations of 20 to 50 µM and more, these metal ions caused maximum DNA strand breaks. Currently, no studies area available in humans, and the in vivo dosage required for these concentrations in seminal plasma is still unknown.

Carnitine is a dietary antioxidant that decreases ROS by removing extracellular toxic acetyl-CoA that is responsible for mitochondrial ROS [56]. Seventy-five percent of carnitine that is present in humans is derived from diet [57]. The highest concentration of carnitine occurs in the epididymis, with epididymal concentrations approximately 2000-fold higher than in plasma [58]. A multicenter, uncontrolled clinical trial by Costa and colleagues [59] showed that oral administration of l-carnitine (3 g/d over a 4-month period) in 134 patients with asthenozoospermia led to a significant improvement in sperm motility, linear index, rapid linear progression, and mean velocity. A placebo-controlled, double-blinded, randomized trial of the use of combined L-carnitine (2 g/d) and L-acetyl-carnitine (1 g/d) treatment in a 2-month period in 56 men with asthenozoospermia found no statistically significant improvement in sperm concentration or motility [60]. A placebo-controlled, double-blind crossover trial of 86 infertile patients showed that L-carnitine supplementation (2 g/d over a 2-month period) led to a significant increase in motility (11% increase) versus placebo (8.8% increase), but only after the exclusion of 5 patients deemed outliers. With the inclusion of these 5 patients, no significant increase in motility was observed [61].

A smaller randomized, double-blinded, placebo-controlled trial by Sigman and colleagues [62] reported that oral carnitine supplementation (2 g L-carnitine and 1 g L-acetyl-carnitine daily) in men with idiopathic asthenospermia demonstrated no clinically or statistically significant effect on sperm motility or total motile count. It should be noted that oral carnitine therapy has not been shown to increase seminal plasma or sperm carnitine or acetyl carnitine levels [61]. Although earlier uncontrolled studies have shown an increase in sperm motility with carnitine supplementation, randomized controlled studies have not shown any consistent significant increase in sperm motility or count (Table 2) [17,59–65].

**Iatrogenic oxidative damage**

High ROS levels are associated with a reduced pregnancy rate after in vitro fertilization or ICSI and arrested embryo growth. A meta-analysis by Agrawal and colleagues [66] showed that ROS levels correlated significantly with the in vitro fertilization rate; thus the measurement of ROS...
Table 2
Studies using carnitine in the treatment of male infertility

<table>
<thead>
<tr>
<th>Study</th>
<th>Antioxidant</th>
<th>Study design</th>
<th>N</th>
<th>Study population</th>
<th>Dose and duration</th>
<th>Semen parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigman et al [62]</td>
<td>L-carnitine, L-acetyl-carnitine</td>
<td>R/C/B</td>
<td>21</td>
<td>asthenozoospermia</td>
<td>2 g L-carnitine, 1 g L-acetyl-carnitine daily; 6 mo</td>
<td>no improvement in motility or sperm count</td>
</tr>
<tr>
<td>Lenzi et al [60]</td>
<td>L-carnitine, L-acetyl-carnitine</td>
<td>R/C/B</td>
<td>56</td>
<td>infertile men</td>
<td>2 g L-carnitine, 1 g L-acetyl-carnitine daily; 6 mo</td>
<td>no improvement in motility or sperm count</td>
</tr>
<tr>
<td>Lenzi et al [61]</td>
<td>L-carnitine</td>
<td>R/C/B</td>
<td>86</td>
<td>oligoastheno-teratozoospermia</td>
<td>2 g L-carnitine daily; 6 mo</td>
<td>significant increase in motility if 5 patients were excluded</td>
</tr>
<tr>
<td>Vicari et al [17]</td>
<td>L-carnitine, L-acetyl-carnitine</td>
<td>R/C/NB</td>
<td>98</td>
<td>infertile men with</td>
<td>2 g L-carnitine, 1 g L-acetyl-carnitine daily; 4 mo</td>
<td>improvement in motility, viability, ROS, and seminal WBC; no improvement in</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>prostato-vesiculoepidiymitis</td>
<td></td>
<td>concentration or morphology</td>
</tr>
<tr>
<td>Vicari and Calogero [63]</td>
<td>L-carnitine, L-acetyl-carnitine</td>
<td>NR/C/NB</td>
<td>54</td>
<td>infertile men with</td>
<td>2 g L-carnitine, 1 g L-acetyl-carnitine daily; 3 mo</td>
<td>improvement in motility, viability, ROS, and seminal WBC; no improvement in</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>prostato-vesiculoepidiymitis</td>
<td></td>
<td>concentration or morphology</td>
</tr>
<tr>
<td>Vitali et al [64]</td>
<td>L-carnitine</td>
<td>NR/NC/NB</td>
<td>47</td>
<td>asthenozoospermia</td>
<td>3 g L-carnitine daily; 3 mo</td>
<td>37/47 showed improved motility, 3 patients with no change in motility, 7 patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>with worsened motility (no statistics provided)</td>
</tr>
<tr>
<td>Costa et al [59]</td>
<td>L-carnitine</td>
<td>NR/NC/NB</td>
<td>100</td>
<td>asthenozoospermia</td>
<td>3 g L-carnitine daily; 4 mo</td>
<td>significant increase in motility and sperm count</td>
</tr>
<tr>
<td>Moncada et al [65]</td>
<td>L-acetyl-carnitine</td>
<td>NR/NC/NB</td>
<td>20</td>
<td>oligoasthenozoospermia</td>
<td>4 g L-acetyl-carnitine daily; 2 mo</td>
<td>significant increase in motility</td>
</tr>
</tbody>
</table>

*Abbreviations: ART, assisted reproduction technique; B, blinded; C, controlled; MDA, malondialdehyde; NB, nonblinded; NC, no controls; NR, nonrandomized; R, randomized.*
<table>
<thead>
<tr>
<th>Study</th>
<th>Antioxidant</th>
<th>Controls</th>
<th>N</th>
<th>Study population</th>
<th>Dose</th>
<th>In vitro effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rossi et al [72]</td>
<td>superoxide dismutase, catalase</td>
<td>C</td>
<td>25</td>
<td>infertile men</td>
<td>100 U/mL superoxide dismutase, 100 U/mL catalase</td>
<td>decreased lipid peroxidation by ROS after freezing-thawing</td>
</tr>
<tr>
<td>Donnelly et al [73]</td>
<td>glutathione, hypotaurine</td>
<td>C</td>
<td>45</td>
<td>infertile men</td>
<td>10 mM glutathione, 10 mM hypotaurine</td>
<td>no effect on motility or baseline DNA integrity; significant protection against ROS</td>
</tr>
<tr>
<td>Hughes et al [77]</td>
<td>vitamin C, vitamin E, vitamin C + vitamin E, acetyl bysteine or urate</td>
<td>C</td>
<td>150</td>
<td>healthy men, samples then x-ray irradiated with dose of 30 Gy</td>
<td>300 or 600 μM vitamin C, 30 or 60 μM vitamin E, 200 or 400 μM urate, 30+300 or 60+600 μM vitamin E+C, 5 or 10 μM acetyl cysteine</td>
<td>significant improvement in DNA integrity at both concentrations for vitamin C, vitamin E, or urate; vitamin E + vitamin C or acetyl cysteine resulted in increased DNA damage compared with controls</td>
</tr>
<tr>
<td>Oeda et al [75]</td>
<td>N-acetyl-L-cysteine</td>
<td>NC</td>
<td>73</td>
<td>infertile men</td>
<td>1 mg/mL of N-acetyl-L-cysteine</td>
<td>significant improvement in motility and decreased ROS; no improvement with respect to acrosome reaction</td>
</tr>
<tr>
<td>McKinney et al [74]</td>
<td>pentoxifylline</td>
<td>C</td>
<td>10</td>
<td>asthenozoospermia</td>
<td>3.6 mM and 7.2 mM pentoxifylline</td>
<td>both concentrations caused comparable decreases in MDA concentrations</td>
</tr>
<tr>
<td>Aitken et al [76]</td>
<td>vitamin E</td>
<td>C</td>
<td>64</td>
<td>healthy men, normal semen parameters, A23187 ionophore added to stimulate oxygen radical production</td>
<td>10 mM vitamin E</td>
<td>significant improvement in sperm-oocyte fusion</td>
</tr>
<tr>
<td>Aitken and Clarkson [69]</td>
<td>vitamin E or BHT</td>
<td>C</td>
<td>122</td>
<td>healthy men, normal semen parameters, A23187 ionophore added to stimulate oxygen radical production</td>
<td>10 mM vitamin E, 10 mM BHT</td>
<td>no improvement in motility for BHT or vitamin E; significant improvement in sperm-oocyte fusion for vitamin E</td>
</tr>
</tbody>
</table>

*Abbreviations: ART, assisted reproduction techniques; C, controlled; N, number of samples; NC, no controls.*
levels in semen specimens before in vitro fertilization may be useful in predicting in vitro fertilization outcome. The use of specific sperm preparation techniques has greatly reduced the oxidative stress associated with sperm handling and cryopreservation. Sperm separation techniques, such as density gradient centrifugation, migration-sedimentation, and glass-wool filtration, significantly reduce the level of ROS by removing leukocytes, which are the major source of ROS [67]. Ejaculates with high ROS production separated by means of conventional swim-up technique may lead to damage to the spermatozoa [68]. Separation techniques, such as percoll density gradient, may minimize iatrogenic ROS damage, but centrifugation without prior removal of poorly motile sperm may lead to increased ROS damage [69]. The use of testicular sperm obtained by testicular sperm extraction instead of epididymal sperm in assisted reproductive technology also may decrease ROS-induced damage because DNA fragmentation has been reported to be significantly higher in epididymal compared with testicular sperm [70].

In vitro supplements used during sperm preparation and assisted reproductive technique also help to protect spermatozoa against ROS. Adding antioxidants to culture media neutralizes ROS produced by leukocytes and immature spermatozoa and improves sperm-oocyte fusion [71]. In vitro supplementation with superoxide dismutase and catalase prevented lipid peroxidation of the sperm plasma membrane by ROS and contributed to the recovery of high-quality spermatozoa after freezing-thawing procedures [72]. Adding glutathione and hypotaurine has been shown to protect spermatozoa against oxidative damage induced by hydrogen peroxide [73]. Pentoxifylline, a nonspecific inhibitor of phosphodiesterase, is approved by the US Food and Drug Administration and is used in the treatment of patients who have cardiovascular disease. It has been shown to have a beneficial effect on sperm motility and acrosome reaction and reduces the O$_2^-$ release by human spermatozoa [74]. N-acetyl-L-cysteine, a precursor of glutathione, reduces the ROS production in human ejaculate and ROS-induced DNA damage [75]. The use of vitamin E in vitro has been reported to improve sperm motility and viability [69,76]. A study by Hughes and colleagues [77] determined that in vitro supplementation of urate (a powerful antioxidant that binds transition metals), vitamin C, and vitamin E separately has protective effects on sperm DNA integrity measured using the comet assay in the setting of x-ray irradiation. Although many of these compounds have demonstrated in vitro effects on sperm, studies demonstrating improvement in pregnancy rates using these in vitro studies are lacking (Table 3) [69,72–77].

Although numerous reports have suggested the benefit of antioxidant treatment of infertile men, many studies also show no effect. Although commercial preparations generally contain a mixture of antioxidants, studies of these combinations are absent. Most studies in the literature are not randomized, placebo controlled, or double blinded in design, which makes it difficult to differentiate regression toward the mean from true positive treatment effects. The small patient sample sizes and varying male populations (from fertile men to men with varying levels of infertility) also add to the difficulty in comparing studies. The combination of different antioxidants in differing dosages for varying durations further complicates the picture. Pregnancy, the most relevant outcome parameter, is rarely reported. Multicenter, well-designed studies with adequate sample size are needed to provide a better level of evidence on the benefit of antioxidants as a treatment modality for patients with male infertility. Ideally, patients would be selected based on oxidative stress levels, and improvement in these levels would be correlated to improvement in pregnancy rates. Until those studies are performed, the use of antioxidants for the treatment of male infertility remains empiric.

References


The Effect of Aging on Spermatogenesis and Pregnancy Outcomes

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In women there is a well-established link between advanced age and (1) reduced fertility and (2) increased risk of birth defects. The effect of age on male fertility and pregnancy outcomes has been less well studied. Until fairly recently, it had been assumed that paternal age had only a minor impact on reproductive outcome. This view has been reinforced by the routine occurrence of older men becoming fathers and the occasional news report of documented paternity in men more than 80 years old. Unlike women, there is clearly no universal or abrupt age-related decline in fertility in men.

Recent publications, however, have suggested that aging men do indeed demonstrate a decline in fertility, and several provocative studies have raised the specter of a causal association between paternal age and significant medical conditions in the offspring. This article reviews the available data on this topic, with an eye toward providing a basis for clinical counseling of the older man who wishes to have a child.

Changing patterns in the age of parenting in the United States

In the mid-1960s, the National Survey of Family Growth (NSFG) initiated the collection and analysis of data from families and relationships across socioeconomic and racial lines. The strengths of this survey lie in its breadth and volume of participants and the length of time over which it has been conducted. The most recent analysis of NSFG data from 2002 [1] has shown a trend toward later parenting.

Since the 1970s, the average age at first marriage for women has increased by almost 5 years, accompanied by an increase in mean maternal age at first birth from 21.4 to 25.1 years. Additionally, the percentage of first-time mothers aged 30 years or older has increased dramatically, from 3.9% to 25.1%. There are multiple factors contributing to these changes including an increasing number of women completing their education, entering the workforce, and establishing careers before choosing to have children [1,2].

Mean paternal age is more difficult to establish because the age of the father usually is not listed on birth certificates. It is important to note that 31% of first-time fathers are aged 30 years and older [1,2]. It generally is recognized that fathers’ ages have increased also. By age 40 to 44 years, 22% of men have not had a child, 20% have had one child, 25% have had two children, and 33% have had three or more.

The effects of advancing paternal age on fertility and pregnancy outcomes

Advancing maternal age has been shown to have a great impact on fertility and on the rate of birth defects and genetic abnormalities of the offspring. Fertility rates clearly decline by age 37 years and are greatly reduced by age 40 years. This decline seems to result from loss of oocytes and a decline in oocyte quality [3–7].

In contrast, no dramatic or sudden changes in male reproductive physiology mirror the changes seen in women as they approach the fifth decade of life, and thus the impact of advancing...
paternal age on fertility outcomes has been less certain. In clinical practice it is not unusual to see men in their fifties and even sixties who wish to achieve a pregnancy with younger female partners. These men and their partners often inquire whether paternal age should play a role in their decisions.

Concerns regarding the impact of paternal age on pregnancy and its outcomes has become even more appropriate as advances in reproductive treatments allow clinicians to assist the creation of pregnancies in an ever-widening pool of men with compromised fertility. Intrauterine insemination, in vitro fertilization, and intracytoplasmic sperm injection, for instance, have increased the reproductive options and potential for couples suffering from infertility caused by a significant male and/or female factor. For management of male-factor infertility, significant advances in techniques such as microsurgical testicular sperm acquisition, vasectomy reversal, and various forms of assisted reproductive technologies have enabled couples who previously had no opportunity to have children using these techniques. Increased investigations into the genetics of male-factor infertility, including Y-chromosome microdeletions, are ongoing [8].

The key questions related to male aging encountered during clinical practice are

1. Does older male age decrease the likelihood of achieving a pregnancy?
2. Does older male age increase the likelihood of genetic abnormalities or other adverse health outcomes in the offspring if a pregnancy occurs?

Age and semen analysis

A number of studies have attempted to determine the impact of age on semen analysis parameters. In one retrospective study, semen analyses results of 66 men aged 50 years and older were compared with those of 134 men aged 21 to 25 years in patients referred to an andrology clinic over a 3-year period [9]. Total sperm count and sperm concentration were unaffected by age. Progressive motility (27%), percentage of morphologically normal spermatozoa (44%), and semen volume (29%) were significantly lower in older men than in younger men. The greater percentage of morphologically abnormal sperm in older men resulted from flagellar abnormalities, such as coiled or bent tails, indicating possible epididymal dysfunction. In addition, serum testosterone levels were significantly lower in the group of older men than in younger men (3.0 ng/mL versus 3.6 ng/mL, a decline of 17%).

Levitas and colleagues [10] performed a retrospective study to examine the relationship between age and semen parameters among 6022 men who had sperm concentrations of 20 million/mL or higher. A peak semen volume of 3.51 ± 1.76 mL was observed in men between the ages of 30 and 35 years old, and the lowest volume of 2.21 ± 1.23 mL was observed in men aged 55 years and older. Sperm motility was inversely related to age, with the greatest motility (44%) for men younger than 25 years and the lowest motility (24%) for men 55 years and older. A 54% reduction in total motile sperm was noted between men aged 30 to 35 years and men older than 55 years. Interestingly, sperm concentration increased with advancing age. The lowest mean sperm concentration of 62 × 10^6/mL was observed among men younger than 25 years old, and the highest sperm concentration was noted among men 55 years and older. This latter group had sperm concentrations almost 20% higher than men 45 to 54 years old and almost 35% higher than men younger than 25 years.

Carlson and colleagues [11] performed a 4-year longitudinal study of 158 young men from Copenhagen in which each participant provided a semen sample for analysis at the outset and on an annual basis for the duration of the study. The median age of men entering the study was 19.1 years. Over the 4 years of the study, no statistically significant changes were noted in sperm concentration, total sperm count, or sperm morphology. Variation in methodology over the course of the study made it difficult to assess changes in motility.

Eskenazi and colleagues [12] also reviewed the impact of age on semen parameters. In this cross-sectional study of 97 nonsmoking men aged 22 to 80 years without known fertility problems, semen volume was found to decrease by 0.03 mL for each year of advancing age, and motility decreased by 0.7% per year. No significant relationship between age and sperm concentration was noted when four azoospermic men were excluded from the analysis. Men in their twenties had median sperm concentrations and total sperm counts of 92 × 10^6/mL and 345 × 10^6, respectively, whereas men 50 to 59 years old had a median sperm concentration of 101 × 10^6/mL and total sperm count of 251 × 10^6. Morphology was not examined in the
study. There was thus no evidence of an age “threshold” for any semen parameter nor more than a minor change in sperm values over time. It is worth noting that several of the older men studied had normal semen analysis results.

In a related study that assessed quantitative aspects of sperm motility using computer-assisted technology, aging was associated with diminished linear motion. This observation may indicate some reduction in fertility potential among older men despite normal-appearing motility results, but this concept remains to be demonstrated [13].

Taken together, these studies generally have demonstrated no more than a mild reduction in semen parameters with age. Specifically, sperm concentration seems largely unaffected, with some studies revealing limited and variable changes in motility and morphology. Although, a decrease in ejaculatory volume with age seems to be consistent, this change by itself is unlikely to affect overall fertility.

The effect of age on reproductive physiology

Changes in testicular histology with age

In general, the size of the testicles remains constant throughout a man’s adult life. Histologic studies, however, have shown that age is associated with a decline in the number of Leydig and Sertoli cells, a thickening of the basement membrane of the seminiferous tubules, and an increase in arrested divisions of germ cells. These spermatogenic cells are largely responsible for the majority of testicular mass, and their decline results in smaller testes in some men [14].

It has been suggested that these changes, in turn, may lead to decreased efficacy of spermatogenesis, with fewer mature sperm produced per tubule [14]. These histologic changes exhibit a high degree of variability, however, and some men seem to preserve apparently normal spermatogenesis well into their nineties.

Endocrine changes affecting reproduction

A number of large studies, including the Massachusetts Male Aging Study, have shown a decline in serum testosterone levels in men with advancing age. On average, total testosterone levels decline by 1% to 2% per year. A decrease in bioavailable testosterone and free testosterone occurs with age as well. In contrast, sex hormone-binding globulin levels increase with age in men [15]. This age-related decline in serum testosterone represents the combination of reduced central responsiveness to circulating androgen levels together with decreased steroidogenic capacity of Leydig cells [14].

The relative decline in testosterone production may affect fertility at the testicular level where relatively high androgen levels seem to be important to support spermatogenesis. In addition, changes in testosterone concentrations may influence the actions of seminal proteins, such as heparin-binding proteins, which seem to be involved in sperm motility [16,17].

Testosterone and estradiol are present in the seminal fluid and are involved in the growth and maintenance of the surface epithelium of the male reproductive tract [18–20]. The decline in androgenic stimulation associated with aging thus has the potential to impact male fertility at multiple levels. Because a wide range of serum testosterone concentrations can be seen with normal fertility, however, the impact of the gradual decline of testosterone on male fertility remains to be determined.

Changes in sexual function with aging

Aging may impair sexual function in men, which in turn may impact fertility. In the Massachusetts Male Aging Study, the prevalence of erectile dysfunction increased with advancing age so that more than 50% of men develop moderate or severe erectile dysfunction by the eighth decade. The increasing incidence of erectile dysfunction is multifactorial: the age-related decline in serum testosterone also may cause erectile dysfunction, as well as diminished libido and difficulty achieving ejaculation [21–25].

Pharmacologically mediated male infertility

A large body of clinical experience and published reports in the literature link many commonly prescribed drugs with sexual dysfunction. This dysfunction is more likely to occur in the aging man who may be taking multiple medications. A variety of antihypertensive drugs, antidepressants, and hormonal agents, to name a few, commonly have been associated with sexual dysfunction [26,27].

Medications may affect fertility by reducing libido, inhibiting ejaculation, causing retrograde ejaculation, contributing to erectile dysfunction, or by direct gonadotoxic effects [27,28]. For example, the serotonin reuptake inhibitor class of antidepressants can reduce libido and cause delayed
ejaculation or complete anorgasmia. The alpha-blocker medications used to treat lower urinary tract symptoms can reduce or block seminal emission.

Medications that directly affect sperm production include gonadotropin-releasing hormone agonists used in the treatment of prostate cancer. These agents produce castrate levels of testosterone, which in turn results in severe disruption of spermatogenesis. Paradoxically, exogenous testosterone administration for the treatment of hypogonadism results in similar impairment of spermatogenesis via negative feedback on the hypothalamus and pituitary. The suppressive effect on sperm production with physiologic replacement doses of testosterone generally is reversible. In contrast, the reduction in sperm production may be permanent among athletes and bodybuilders who have a history of anabolic steroid use for performance enhancement, because of the extremely high doses employed and the common practice of combining multiple agents. In addition, several chemotherapeutic agents, such as cyclophosphamide and other alkylating agents, have a direct and often permanent gonadotoxic effect on spermatogenesis.

The association of erectile dysfunction and aging has resulted in the widespread use by older men of the oral phosphodiesterase inhibitors, such as sildenafil, tadalafil, and vardenafil. Although there were initial concerns regarding a possible negative effect of these medications on male reproductive function or sperm values, the evidence available to date has been reassuring.

Analysis of sperm motility after exposure to sildenafil using computer-assisted semen analysis and acrosome-reaction testing by fluorescein staining was performed on 57 men [29]. The sperm were incubated with sildenafil at 37°C for up to 180 minutes. It was found that the number and velocity of progressively motile sperm were significantly increased by sildenafil between 15 and 135 minutes. Sildenafil also caused a significant increase in the proportion of acrosome-reacted sperm. The clinical significance of these results remains to be determined.

In another study looking at the effects of tadalafil [30], spermatogenesis was examined in men taking placebo versus 10 or 20 mg tadalafil administered daily for 6 months to healthy men and to men who had mild erectile dysfunction. Chronic daily administration of tadalafil had no apparent adverse effects on semen volume or on sperm concentration, motility, or morphology.

Lower urinary tract symptoms and reproduction

Lower urinary tract symptoms and sexual dysfunction secondary to benign prostatic hyperplasia (BPH) are common, highly bothersome conditions in men, and the prevalence of both disorders increases with age [31,32].

Current medical treatment of BPH symptoms consists of monotherapy with alpha1-adrenoceptor antagonists, 5-alpha-reductase inhibitors, or a combination of these. The 5-alpha-reductase inhibitors may, rarely, cause erectile dysfunction, ejaculatory disorders, and diminished libido. The use of alpha-blockers is associated with failure of seminal emission or greatly reduced ejaculatory volume in a substantial percentage of men [33,34]. Combined therapy places men at risk for the adverse sexual effects associated with either type of drug.

Finasteride has been used to treat BPH and male-pattern baldness. In men taking finasteride at 5 mg daily for symptoms of BPH, ejaculate volumes decrease by approximately 25%. One study looking at the semen parameters of men taking low doses of finasteride (1 mg) for hair loss found no changes in semen parameters, however [35].

Surgical therapies for BPH, such as transurethral resection of the prostate, are well known to result in retrograde ejaculation and, less often, in postoperative urethral strictures. These adverse events result in diminished or even complete absence of antegrade ejaculation.

Prostate cancer and reproduction

The incidence of clinically detected prostate cancer is increasing, especially in younger male patients. One study showed that small foci of histologic cancer were found at surprisingly common rates of 27% and 34% of male patients in the fourth and fifth decades of life, respectively [36].

As a result of increased detection at earlier ages, more men are being treated for prostate cancer during their reproductive years. Treatments for prostate carcinomas, including surgery, radiotherapy, and hormonal agents, can individually and in combination result in a substantial decline in sexual and reproductive functioning.

Furthermore, recent data also reveal that more aggressive prostate cancers may be associated with low serum testosterone levels and may present initially as sexual dysfunction and/or impaired fertility [37–39]. The impact of these treatments may have a significant impact on sexual function and fertility potential in both
younger and older men desiring to have children. Recently, it has been reported that transrectal ultrasound–guided needle biopsy of the prostate is associated with abnormal semen parameters [40]. This result may be a significant issue for older men who desire to become fathers, because prostate biopsy has become a common procedure for men 50 years and older.

Age and genetic implications

Advanced maternal age has long been shown to increase the risk of Down syndrome and other genetic abnormalities in the offspring. Much less is known about genetic abnormalities and advanced paternal age.

Singh and colleagues [41] examined the relationship between age and sperm DNA damage among men. Sperm samples collected from 66 men between the ages of 20 and 57 years were evaluated for DNA double-strand breaks and apoptosis. The percentage of sperm with damaged DNA was significantly higher in men aged 36 to 57 years than in those aged 20 to 35 years, but, oddly, the percentage of apoptotic cells was significantly lower in the older group. It has been suggested that this increased DNA “fragility” in older men may contribute to an increased risk of genetic abnormalities in the offspring, but this concept remains unproven.

Nonetheless, a number of reports have suggested an association between advanced paternal age and a variety of genetic syndromes in the offspring, including schizophrenia, achondroplasia, Apert syndrome, autism, Down syndrome, and Marfan syndrome [42–46]. A common confounder in these reports, however, is that older fathers tend to be married to older mothers, and thus it can be difficult to isolate the paternal influence.

A review of New York state health records from 1983 to 1997 revealed an increased risk of Down syndrome with maternal age of 35 years and older and suggested a 50% paternal contribution to Down syndrome when the mother was 40 years or older [47]. Although the number of fathers over 40 years of age increased by 73% from 1983 to 1997, however, neither the number of cases nor the incidence of cases of Down syndrome increased during this period. This type of result makes it difficult to isolate paternal age as an independent risk factor for Down syndrome in the offspring.

Perhaps the most provocative study suggesting a link between paternal age and adverse outcomes in the offspring explores the risk of schizophrenia among children of older fathers. Malaspina and colleagues [48] performed a population-based study of the Israel Psychiatric Registry, comprising a birth cohort of almost 88,000 individuals born in Jerusalem from 1964 to 1976. Of the 1337 individuals from this cohort admitted to psychiatric units, 658 were diagnosed as having schizophrenia. The calculated risk of schizophrenia was 1 in 141 births among the offspring of fathers younger than 25 years and was 1 in 47 if the father was 50 years or older, a threefold increase. A significant association with paternal age was maintained even after controlling for maternal age and other confounding factors, including gender, ethnicity, education, and duration of marriage.

One critical limitation of this study, however, was the failure to include any assessment of family history of mental illness in the study. Without including an important risk factor for schizophrenia, the question remains unanswered whether the observed difference between older and younger fathers represents an effect of paternal age itself or of some other risk factor that may have predisposed to older paternal age.

Another Israeli study examined the relationship between advanced paternal age and the risk of autism [49]. The study was conducted based on persons born in Israel during 6 consecutive years during the 1980s. The registry identified 110 cases of autism (incidence, 8.3 cases per 10,000 persons) in the subset of men with complete parental age data. There was a significant association between advancing paternal age and risk of autism. After controlling for year of birth, socioeconomic status, and maternal age, offspring of men 40 years or older were 5.75 times more likely to have autism than offspring of men younger than 30 years. Advancing maternal age showed no association with autism after adjusting for paternal age.

The authors of this study suggest that the increased risk of autism seen with advanced paternal age was the result of accumulated genetic mutations in the germ line over time. As in the previous study, however, no information regarding paternal medical conditions, especially the presence of autistic traits, was obtained. An equally plausible alternative explanation is that underlying paternal traits led to delayed marriage.

A study by Olshan and colleagues [50] evaluated the relationship between paternal age and cardiac defects in the offspring. A total of 4110 cases of congenital heart defects were identified
from the British Columbia Health Surveillance Registry from 1952 to 1973. Prevalence odds ratios for paternal age, adjusted for maternal age and other factors, were estimated for various cardiac defect groups. A “suggestive general pattern of increasing risk” with increasing paternal age was found for ventricular septal defects, atrial septal defects, and patent ductus arteriosus among cases without chromosomal anomalies. The authors estimated that only 5% of cases may be the result of advanced paternal age (> 35 years). Interestingly, there also was an increased risk for ventricular septal defects and atrial septal defects when the father was younger than 20 years.

The authors of this study speculate that the age-associated cardiac malformations may have occurred through de novo autosomal dominant mutations, related to errors in the division of the germ cells that accumulated over time. Other possibilities include epigenetic or “lifestyle factors” such as cigarette smoking and alcohol consumption. In this study, however, the age-related risk was mild and did not reach statistical significance in most of the primary analyses.

A population-based cohort study of couples and their firstborn children was based on the Danish Fertility Database between 1980 and 1996 (n = 71,937) [51]. Diagnoses of congenital abnormalities in children were obtained by linkage to the national hospital registry. To control for maternal age, only children with mothers aged 20 to 29 years were included. This study showed no overall association between paternal age and risk of congenital defects, but the prevalence of extremity malformations, such as syndactyly or polydactyly, and syndromes affecting two or more organ systems were increased by approximately 30% with paternal age greater than 40 years. As with previously cited studies, one must wonder whether this increased risk reflects aging of the germline or some other characteristic of men who become fathers at a more advanced age.

A more general review of birth defects and paternal age was performed by Yang and colleagues [52] of more than 5 million cases from the United States birth registration data from 1999 to 2000. The authors found that 1.5% of the total group had some documented birth defect. Multiple logistic regression revealed a significant but weak relationship to paternal age, with adjusted odds ratios for any birth defect of 1.04, 1.08, 1.08, and 1.15, for infants born to fathers aged 30 to 35 years, 40 to 44 years, 45 to 49 years, and over 50 years, respectively, when compared with infants born to fathers aged 25 to 29 years. Advanced paternal age was associated with increased risks of heart defects, tracheoesophageal fistula, esophageal atresia, musculoskeletal or integumental anomalies, Down syndrome, and other chromosomal anomalies. Conversely, offspring of fathers under 25 years of age also were at increased risks of spina bifida/meningocele, microcephalus, omphalocele/gastrochisis, and other musculoskeletal or integumental anomalies.

**Perspective on studies linking paternal age to birth defects**

As reviewed in the previous section, a number of reports in the literature have found an association between advanced paternal age and an increased risk of birth defects or subsequent adverse health outcomes in the offspring (Table 1). Although it certainly is possible that this risk is real, it should be made perfectly clear that none of these studies, alone or together, have provided compelling evidence to prove the case.

A critical and universal limitation of all of these studies is that they all are observational and therefore cannot control for multiple confounders. Perhaps the most important of these confounders is the possibility that paternal age is simply a surrogate marker for another condition, or set of conditions, that predisposes to increased adverse outcomes in offspring. One possible explanation is that a subset of men in these studies may have had the opportunity to marry or become fathers only at an advanced age because of their own underlying medical, psychiatric, or social conditions. Similarly, these studies cannot exclude the possibility that a greater number of men in peak health condition have children at a younger age. The argument that age may be only a marker for other contributors to adverse health outcomes in offspring is supported by the fact that very young paternal age has also been associated with an increased risk.

Selection bias also may skew results. For instance, cases in which paternal age was unknown were excluded from several studies, including the large study by Yang and colleagues [52].

One unexamined possibility in these studies is that the excluded cases may include a number of offspring with adverse outcomes and for whom the father was unknown or the coupling had been unstable. It could be argued that the risk of this situation is greater among young men than older men. If true, this situation would influence the
Although it is apparent that advanced maternal age carries definite genetic implications, the risks of advanced paternal age on offspring remain a matter of uncertainty. Given the weak association between paternal age and birth defects [52], it is the authors’ practice to reassure couples that the vast majority of children born to older men are healthy, and that the reportedly increased risk of birth defects is small, if present at all.

Counseling the infertile couple with regard to parental age

Increased female age is associated with decreased pregnancy rates and higher miscarriage rates in natural and assisted pregnancies, but the impact of male age on fertility is less certain. Moreover, in contrast to female reproduction, there seems to be no upper age limit on the ability of a man to father children. In general, the recommendations for a 55-year-old man with a given set of semen parameters will be the same as those for a 25-year-old man who has the same semen parameters. Nevertheless, older men may face a number of fertility challenges that are not present for younger men.

Infertility is different for each couple facing it, and therapy and counseling must be individualized. For the older man, it is critical to obtain a complete medical history, with special attention to current medications and prior medical and surgical history. In some men it may be necessary to discontinue the use of medications that may impair ejaculation or spermatogenesis, such as for the treatment of voiding dysfunction or hypogonadism. The use of oral phosphodiesterase inhibitors such as sildenafil or tadalafil may be indicated to allow increased sexual activity.

Understandably, couples may have concerns about the implications of advanced male age on sperm production and risks to offspring. Clearly, couples who have known familial genetic defects also should have the input of geneticists when making decisions regarding fertility and future genetic risks. Any discussion regarding the possibility of an increased risk of conditions such as...
autism or schizophrenia in the offspring of older fathers should include the caveat that no definitive data exist, because of the absence of prospective controlled studies.

Among couples undergoing assisted reproductive techniques, preimplantation genetic screening is becoming more readily available and probably will play an increasing role in assisting couples in decision making, particularly when there is a real or perceived risk of health issues in the offspring [53,54].

Summary

Changes in male reproductive and sexual physiology clearly occur with aging, but the impact of these changes on male fertility seems to be limited. There is an additional concern that advanced paternal age may increase the risk of a variety of congenital or genetic defects or other serious medical conditions in the offspring, but this risk remains a matter of considerable debate. At this time, there is no reasonable basis for denying infertility treatment to men who desire to father children, regardless of their age. Discussions regarding a possible increased risk of birth defects or other medical conditions associated with advanced male age should include the perspectives that no definite association with paternal age has yet been proved, all pregnancies entail some risk of poor outcomes, and, most importantly, the vast majority of births at any parental age result in healthy babies.

References


