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Evaluation and Treatment of Male Infertility

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There have been many years of debate over the causes and therapy of male infertility. Many treatments have been strongly advocated for male infertility during the past four decades, such as clomiphene citrate, testosterone, human menopausal gonadotropin, human chorionic gonadotropin, corticosteroids (for sperm antibodies), cold wet athletic supporters, vitamins, and even more recently the popularly marketed "Proxceed," without any documented evidence of effectiveness.[1] Even the varicocelectomy operation has come into serious question.[2–6] It is becoming clear that most spermatogenic defects in humans are actually genetic in origin and clearly impervious to improvement with any therapy.[7–10] Furthermore, the development of intracytoplasmic sperm injection as an effective therapy for all cases of male infertility that have failed to respond to conventional treatment has caused a major reassessment and critical analysis of the diagnostic and therapeutic approaches to male infertility.[11]

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Evaluation of the Male

The first and most important test for men remains the semen analysis (sperm count). However, a poor semen analysis, or a low sperm count, does not rule out natural conception, and a normal sperm count does not necessarily mean that the husband's sperm can fertilize his wife's eggs. Men with extremely low sperm counts often have no difficulty impregnating their wives, and in a small percentage of in vitro fertilization (IVF) cycles in which the semen analysis is completely normal, there is no fertilization.[12,13,1]

In fact, how many sperm and what quality of sperm are necessary for a man to be fertile, is not at all a simple question. Twenty-five years ago, it was thought that a sperm count of <40 million spermatozoa per milliliter meant that the husband was infertile and the urologist gave such couples a poor prognosis for pregnancy in this situation. When the wife did get pregnant, this happy result was usually attributed to whatever otherwise ineffective treatment was actually being administered to the so-called infertile husband. Acknowledging that low sperm counts can be compatible

TABLE 1. Frequency Distribution of Sperm Counts in 1,000 Fertile Men and 1,000 Infertile Men

Sperm Count (10^6/ml)	Fertile Men (%)	Infertile Men (%)
<20	5	16
20–39	12	13
40–59	12	11
>60	71	60

From MacLeod and Gold, 1951.

with fertilization, the World Health Organization (WHO) in 1992 issued a reduced list of "normal values" for semen analysis that included a sperm concentration of >20 million per milliliter, total sperm count of >40 million per ejaculate, >50% of sperm exhibiting forward progressive motility, and >30% with normal morphology.[14] However, even this new, lower table of normal values has been appropriately attacked as very misleading and still implied a fallacious threshold concept for male fertility, above which the man is fertile and below which he is infertile.[15,1,14] When a couple has been unable to achieve a pregnancy during a certain period of time (eg, 1 or 2 yr), all we really know is that the couple is infertile. The important question is, to what extent is the husband's deficient or "abnormal" sperm count contributing to (or not affecting) the couple's infertility?

CORRELATION OR LACK OF CORRELATION OF SPERM COUNT TO SPONTANEOUS PREGNANCY RATE: HOW TO INTERPRET THE SEMEN ANALYSIS

The correlation of sperm count with fertility was originally presented in the classic article by MacLeod and Gold in 1951. These authors studied sperm counts in 1,000 "fertile" and 1,000 "infertile" men (Table 1).[16] Their results indicated that in a fertile population, the vast majority of men had sperm counts >40 x 10^6/ml. Only 17% had sperm counts <40 x 10^6/ml. Only 5% of fertile men had sperm counts <20 x 10^6/ml. This distribution would suggest that a normal count is >40 x 10^6 spermatozoa/ml, and this had been the assumption for many decades.

Rehan et al in 1975 reported results similar to MacLeod and Gold.[17] In 1,300 fertile men, the percentages were remarkably similar to those of MacLeod and Gold, with only 7% of fertile men having sperm counts <20 x 10^6/ml. Eighty-three percent of fertile men had sperm motility of grades 3 and 4, but what perhaps has not been adequately emphasized is that 17% of fertile men had very poor sperm motility of grades 1 and 2. Similarly, 86% of fertile men had >40% motile sperm, but 14% had <40% motile sperm and 4% of fertile men had sperm motility of <20%. Neither of these early studies addressed the possibility that low sperm counts, like high sperm counts, might occur at either end of the bell-shaped population curve and might perhaps be unrelated to the man's fertility.

David et al in 1979 reported on sperm counts in almost 3,000 infertile men with a lop-sided control group of only 190 fertile men (Table 2).[18] The frequency distribution of sperm counts in fertile and infertile men obtained by these authors is shown in Table 2 and is similar to that of MacLeod and Gold in 1951. Thus, the inference remained strong that a sperm count of >40 x 10^6/ml indicates a much greater likelihood of fertility.

That sperm count actually may not correlate closely with the man's fertility was first proposed in 1974. Nelson and Bunge reported in fertile 386 men that low sperm

TABLE 2. Frequency Distribution of Sperm Counts in 190 Fertile Men and in 2,889 Infertile Men

Sperm Count (10^6/ml)	Men Fertile (%)	Men Infertile (%)
<20	6.9	28
20–39	9.5	16.4
40–59	14.7	13.6
>60	69	41.3

From David et al, 1979.

counts are compatible with fertility and that a sperm count of <20 x 10^6 or 40 x 10^6/ml does not indicate a "male factor."[19] In 1977, Zukerman et al reported on several thousand fertile men who had a semen analysis performed prior to vasectomy.[20,13] Twenty-three percent of these fertile men had sperm counts <20 x 10^6/ml and only 40% had sperm counts >60 x 10^6/m.

I have reviewed sperm count and motility indices in men following vasovasostomy whose wives became pregnant in comparison with those whose wives did not become pregnant (Table 3). The distribution of sperm counts, percentage motility, and total motile sperm per ejaculate was quite similar in both groups.[21,22] Twelve percent of the patients who had successful vasovasostomy and whose partners became pregnant had total motile sperm counts per ejaculate of <10 x 10^6. In fact, the extensive comparison by Jouannet et al of spontaneous pregnancy rates in infertile couples with varying sperm parameters showed results similar to my long-term follow-up of patients who have had vasovasostomy performed: above 5 x 10^6 sperm, the difference in pregnancy rate related to differences in sperm count is not dramatic.[23]

Nonetheless, although a low sperm count and a low sperm motility do not necessarily indicate infertility in any particular couple, control studies have shown that lower motile sperm counts are still associated with lower spontaneous conception rates over the course of time in couples who are infertile. Schoysman and Gerris in 1983 studied the spontaneous pregnancy rate over the course of time in 1,327 oligozoospermic couples (Table 4).[24] When the motile sperm count was $\leq 1 \times 10^6$/ml (even as low as 100,000/ml) with no treatment of either the husband or wife, in 5 years, there was a 4% spontaneous pregnancy rate and in 12 years, the wives of 9% of these couples spontaneously conceived. When the motile

TABLE 3. Frequency Distribution of Motile Sperm Count and Pregnancy Rates After Vasovasostomy in Men Whose Wives Did or Did Not Become Pregnant (Silber, 1989)*

Total Motile Sperm Count (10^6/ejaculate)	Total Patients (frequency distribution)	No. Pregnant (frequency distribution)	Pregnancy Rate
0–10	32 (12%)	25 (11%)	78%
10–20	31 (12%)	27 (12%)	87%
20–40	32 (12%)	30 (13%)	94%
40–80	79 (31%)	68 (30%)	86%
>80	84 (33%)	78 (34%)	93%
Totals	258 (100%)	228 (100%)	88%

* 10-year follow-up.

TABLE 4. Pregnancy Rates in 1,327 Men With Oligozoospermia

Motile Sperm Count (10^6/ml)	% Pregnancy	
	5 Years	12 Years
0.1–1	3.9	8.7
1–5	11.9	26.6
5–10	22.1	34.3
10–15	45.0	58.5
15–20	68.6	82.0

From Schoysman and Gerris, 1983.

sperm count was between 5 and 10 x 10^6, 22% conceived within 5 years and 34% within 12 years. When the motile sperm count was between 15 and 20 x 10^6, 69% conceived within 5 years and 82% within 12 years.

Baker (1986) constructed a life table pregnancy curve for infertile couples with varying degrees of oligozoospermia comparing them to various fertile control populations (Fig. 1).[25–28] Pregnancy rates were compared for couples with a sperm count of <5 x 10^6/ml, 5 to 20 x 10^6/ml, >20 x 10^6/ml with <60% motile, and >20 x 10^6 with >60% motile. These four groups were compared graphically with the life-table conception rate of Kovacs' donor insemination group,[26] Vessey's women discontinuing the intrauterine device,[27] and the spontaneous pregnancy rate reported in 1953 by MacLeod and Gold for fertile couples.[28]

Again, quite remarkably, with <5 x 10^6 spermatozoa/ml regardless of motility, the pregnancy rate at 2 years was 26% (Fig. 1). When the sperm count was 5 to 20 x 10^6/ml, the pregnancy rate at 2 years was 42%. When the sperm count was >20 x 10^6/ml with <60% motile, the pregnancy rate at 2 years was similar to the results obtained when the count was 5 to 20 x 10^6. When the sperm count was >20 x 10^6 and the motility >60%, the pregnancy rate at 2 years was 63%. When any of these pregnancy rates is compared with that of donor insemination or to otherwise fertile couples discontinuing an intrauterine device, it is clear that no matter how high the sperm count, the subsequent pregnancy rate of couples attending a fertility clinic is lower than that of a normal control population. Furthermore, even though women may become spontaneously pregnant with extremely low sperm counts, nonetheless, among infertile couples, a higher

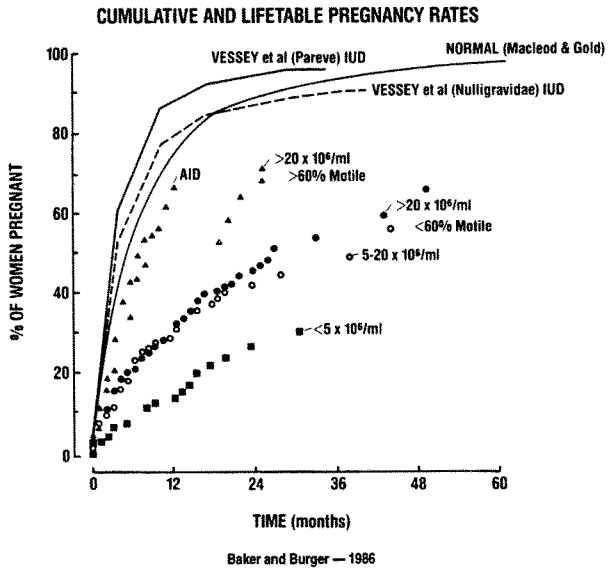

FIG. 1. Cumulative and life table pregnancy rates. (From: Baker HWG, Burger HG. Male infertility in reproductive medicine. In: Steinberger E, Frajese G, Steinberger A, eds. Reproductive Medicine. New York: Raven, 1986:187–197).

*TABLE 5. Mathematical Model to Predict a Couple's Fertility for Given Sperm Concentration and Motility and Allowing for Duration of Involuntary Infertility: Percentage Chance of Conception for the Next Year**

	Motile Density	Duration of Infertility (months)			
		12	24	48	96
Azoospermia		0	0	0	0
Sperm present (millions of motile sperm/ml)	0	0	0	0	0
	0.5	16	12	9	6
	1	25	19	14	9
	2	34	26	19	13
	5	36	28	21	14
	10+	37	28	21	14

* Wife with normal investigation results.

From Hargreave and Elton, 1983.

motile sperm count does increase the chance for spontaneous conception.

MALE INFERTILITY AND THE FEMALE FACTOR

The major variable in the oligospermic couple's chances for pregnancy is not the sperm count, but rather the wife. Before the era of intracytoplasmic sperm injection (ICSI), when donor insemination was performed for couples with both azoospermia and severe oligospermia, the pregnancy rate was always higher in the wives of men with azoospermia than in the wives of men with oligospermia.[29] Thus, a man with severe oligospermia might have initiated a pregnancy even with his small number of spermatozoa if the woman herself did not also have reduced fertility.[21] Thus, when couples with oligospermia in the pre-ICSI era would opt for donor sperm, the pregnancy rate was lower than with azoospermic men because the wife was more likely to be infertile herself. As Schoysman said, "The subfertile female reveals the subfertile male."[24]

Hargreave and Elton studied the spontaneous pregnancy rate in couples with varying degrees of oligospermia in relation to the duration of prior infertility.[30] Men with an extremely low sperm count successfully impregnated their wives without any treatment if the duration of prior infertility was only 1 year (Table 5). If the duration of prior infertility was much longer, then the outlook with oligospermia was much worse. In fact, the most critical factors that determined pregnancy prognosis, in couples with oligospermia, were the age of the wife and the duration of prior infertility, more so even than the sperm count.

In an extensive study of the various factors affecting pregnancy rate with sperm retrieval and ICSI in men with azoospermia, neither the quality of the sperm nor the site of retrieval had any affect on the pregnancy rate. The only factors that affected implantation and pregnancy rate were the age of the wife and her ovarian reserve.[31] Nieshlag, in his controlled studies of couples undergoing varicocelectomy or being deferred for counseling, the treatment of the varicocele and the sperm count had no effect on the pregnancy rate. The only factor that was judged to be significant was the age of the wife when the duration of prior infertility was equivalent.[2,3] In fact, according to Collins' studies, the woman's age is

the single most important determinant of the couple's fertility.[32]

In the era of IVF, it became clear that couples with reduced or abnormal standard semen parameters have lower fertilization rates (eg, 68% vs 23%), which leads to lower pregnancy rates.[33] However, it was still impossible to predict from the semen analysis which couples with reduced semen parameters would have normal fertilization and which would have reduced or no fertilization. A lower transfer rate, fewer embryos, and lower pregnancy rates were obtained in couples with abnormal semen parameters, but there was no way to predict which couples with reduced semen parameters would fertilize and which couples would not.[34]

More Specialized Tests of Sperm Function

MORPHOLOGY

Frustration with the inability of the ordinary semen analysis to accurately predict fertility of the couple and a clear absence of a threshold value below which one can definitely determine that the man is infertile, have led to the introduction of many other more specialized tests to evaluate sperm function. One of the simplest of these tests is the "strict criteria" evaluation of sperm morphology.[35] The WHO has for years defined the lower limit of normal for morphology of sperm in the semen analysis as 30%.[1] This parameter has not been very successful in predicting fertility.[15] However, the simple categories of normal (oval-head), amorphous (irregular-head), tapered-head, and small-headed sperm, have now been replaced by strict criteria.[35,36] The "strict criteria" method of determining morphology specifically measures the length and width of the oval spermatozoa head to a more exacting degree, and a sperm head could only be called "normal" if it fits within this narrow range (2.5–3.5-μm wide and 5–6-μm long). The acrosome had to represent 40% or more of the sperm head, and other perhaps less important measurements of the mid-piece (<1-μm wide and 7.5–9- μm long) and tail of the sperm (45-μm long and uncoiled) had to be "strictly" applied. With these strict criteria, it was suggested that the lower limit of normal was 14% rather than 30%. Those with <4% normal morphology by strict criteria had only a 7.6% fertilization rate with IVF, those with 4–14% normal forms by strict criteria had a 64% fertilization rate with IVF, and those with >14% normal morphology by strict criteria had a 91% fertilization rate.[37] This simple system has not really been perfectly consistent either.[36,38]

The concept of trying to predict fertilization by the strict evaluation of sperm morphology has been enthusiastically embraced in theory but has not worked well in practice. The original criteria that defined normal sperm were based on an esthetically pleasing oval shape.[39,40] What really matters, however, in assessment of morphology for predicting fertilization capability of sperm is: 1) whether the acrosome can function properly in zona binding and zona penetration; and 2) whether the abnormal morphology is related to any basic DNA defect in the sperm head. The strict criteria approach to assessing morphology was an effort to apply the metric standards first established by WHO regarding sperm head length and width, and then to exclude more sperm from that normal category because of subtle abnormalities in sperm head shape and staining properties.[41–44] However, the subjective nature of the visual morphology assessment still contributes to a considerable variation both within the same laboratory with different technicians and between the technicians of different laboratories. Some would argue that whether you use the WHO criteria (which are also objective and metric) or the strict criteria, the same basic methodology is used, and it is just a matter of whether a smaller number of sperm can

be considered normal because of various subtle differences in shape and staining.[14,37]

The theoretic basis for the predictability of fertility by evaluation of sperm morphology by strict criteria is that it is indirectly indicative of acrosomal function, which is necessary for sperm-binding to the zona pellucida and penetration through the zona pellucida. Sperm with abnormally-shaped heads do not bind to the zona and cannot penetrate the egg.[45–48] This is a sound theoretic foundation for reliance on morphology. Nonetheless, even with evaluation of morphology by strict criteria, the range of what is found in fertile and infertile men still only represents a spectra with no clear threshold.[36,38] It is logical to expect that unless there is truly 100% abnormal morphology (which is extremely rare), strict morphology suffers from the same dilemma of all the other sperm parameters in the semen analysis.[23] Results of strict morphology evaluation is certainly related to fertilization rate in vitro, but patients with poor morphology do fertilize, and at least 25% of patients who do not fertilize have perfectly normal morphology by the strictest criteria.[45–48] Thus, there still seems to be no easy way to eliminate the possibility of a man being infertile despite a normal semen analysis, or that he may be fertile despite an abnormal semen analysis.

ZONA BINDING, SPERM PENETRATION, AND IN VITRO FERTILIZATION

Because failure of fertilization is unexplained in at least 25% of patients, Liu and Baker made an extensive study of the sperm of patients with unexplained "failed fertilization" in IVF who had otherwise completely normal semen parameters, including normal morphology by strict criteria.[45–48] They noted that: 1) sperm with abnormal morphology did not bind to or penetrate the zona pellucida; and 2) sperm with normal morphology did bind to the zona pellucida but, in cases of failed fertilization, did not penetrate it. A failure of the zona-induced sperm acrosome reaction thus explained the failure of fertilization in men with otherwise normal semen parameters. General "acrosome reaction" assays that are not induced by zona-binding are unphysiologic and, therefore, it is no surprise that they are of no predictive value.[49] They have nothing to do with how a sperm fertilizes an egg, which begins with the zona-induced acrosome reaction. The studies by Liu and Baker thus seemed to eliminate a great deal of confusion about sperm testing problems and provided an explanation for unexplained failed fertilization and also clarified why and how sperm morphology affects fertility.[50] Human spermatozoa must first bind to the zona pellucida to fertilize the egg, and they do this with an intact normal sperm head that has not yet undergone the acrosome reaction. Once the sperm head is bound to the zona pellucida, the zona becomes an efficient inducer of the acrosome reaction, which then allows the sperm to penetrate through it. Sperm with normal morphology that are capable of binding to the zona pellucida, but then cannot penetrate, have a specific failure of the zona-induced acrosome reaction. Therefore, however sound the rationale, for strict morphology, much like the rest of the semen parameters, it provides no assurance of whether the sperm can or cannot fertilize.

OTHER TESTS OF SPERM FUNCTION

Many other tests of sperm function have been developed in an effort to solve this enigma of "male-factor," but none have become very popular. The hamster egg–sperm penetration assay, the cervical mucous sperm penetration assay (as well as the simpler postcoital test), computerized sperm motility analysis, and hemizona-binding assay were all developed because of the apparent inadequacy of the routine semen analysis.[51] Most of these tests have fallen into disfavor either because they yielded no greater information than the standard semen analysis (or sperm morphology evaluation) or because they in-

volved a great deal of equipment and expense that could not be justified by what, at best, was a controversial and debatable effectiveness.[52–56,46] It is probably the diverse population of spermatozoa in the semen of each man that makes such testing problematic because most infertile men who are do not have azoospermia represent a spectra of fertility. The development of IVF and ICSI and the lack of reliability of semen analysis in providing prognostic information to predict fertilization led to the proliferation of all of these more complicated and expensive functional tests for spermatozoa. However, most clinicians today favor the use only of routine semen analysis with morphology and motility assessment, recognizing full well its limitations.[57,58]

Nonetheless, for determining the likelihood of fertilization with IVF, we favor the approach recommended by the Adelaide group in 1993, and simply look at the percentage progressive motility and normal morphology in the postpercoll insemination droplet, paying no heed to the prewashed semen.[59] After the percoll wash and separation, the sperm concentration should improve and the motility should be more than 98%, with most being rapidly progressive. This is quite simple and as predictive as any elaborate testing for the likelihood of fertilization with IVF.

RECONSIDERATION OF ANDROLOGIC TESTING AND CONVENTIONAL TREATMENT OF MALE INFERTILITY

With the arrival of ICSI, Devroey has argued that none of these complex andrologic tests are of great importance any longer anyway.[1] For the most part, treatment of male infertility, prior to IVF and ICSI, has been authority-based and not evidence-based. It is highly doubtful whether the fertility of any male with oligospermia, or oligoasthenoteratospermia, can be improved by any treatment whatsoever, including antiestrogens such as Clomid and Tamoxifen, androgens, gonadotropins, or even varicocelectomy.[60–62,1–7] It has been argued that with the exception of an occasional testicular cancer that may be detected, even physical examination has no impact on therapeutic results for oligoasthenoteratospermia.[62]

Baker et al found that couples who underwent varicocelectomy, as well as couples who did not undergo varicocelectomy, had a conception rate within 1 year of approximately 30%, and by 2 years of approximately 45% (Fig. 2). Nieschlag in his varicocele control study of 125 infertile couples found that 25% of couples with varicocele who did not undergo varicocelectomy became pregnant within 1 year and a similar percent that had undergone varicocelectomy became pregnant within 1 year (Fig. 3). Therefore, it is impossible to assess the effectiveness of any of the popularly advocated treatments for male infertility during the last 40 years without rigorously controlled studies.[63,2,3,24]

There is probably no subject that is more controversial in the area of male infertility than varicocele. Most nonurologist infertility specialists around the world are extremely skeptical of the role of varicocele or varicocelectomy in the treatment of male infertility, despite the fact that most urologists are enthusiasts. The directors of most assisted reproductive technology programs view the enthusiasm with which urologists approach varicocelectomy as a potential impediment to the couple that is getting older and often does not have much time left for having good pregnancy rates with assisted reproductive technology. They feel that these couples are being inappropriately delayed in obtaining assisted reproductive technology with the hope that varicocelectomy will solve their problem. Often during that time, years are wasted while the woman becomes older.

There are quite a few controlled studies that show no effect of varicocelectomy on male infertility. These papers are generally given much greater credence by infertility specialists who are not urologists.[2–6,60,64–67] The only "controlled" studies that favor varicocelectomy were extremely flawed by obvious patient

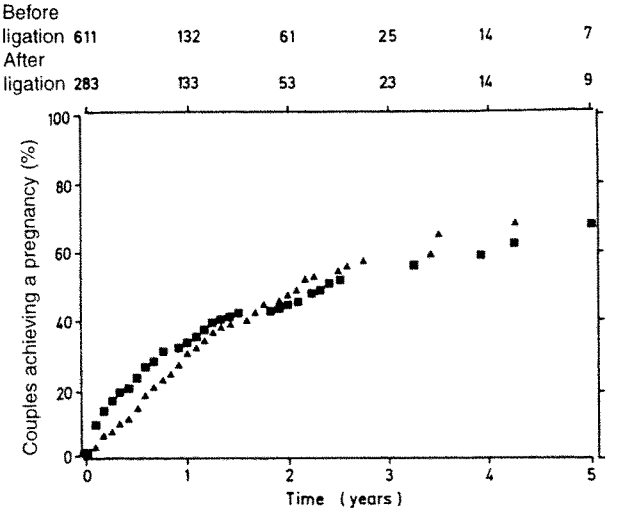

FIG. 2. Life table curves of pregnancy rates for before ligation (■) and after ligation (▲) groups. Number of patients initially and those followed-up to the end of each year is shown at top of figure. Symbols indicate those months in which the life table changed, that is, when pregnancies occurred. Although some patients were followed-up for more than 5 years (those in before ligation group for maximum of 92 mos, after ligation group for 108 mos), the longest duration of follow-up to pregnancy was 60 months. There was no significant difference between the two curves by log-rank test (Baker HWG, Burger HG, deKretser DM, et al. Testicular vein ligation and fertility in men with varicoceles. Reprinted with permission from Br Med J. 1985,291:1678–1680).

selection. Nonetheless, these flawed studies are the ones which urologists often quote to support their enthusiasm for varicocelectomy. One study involved 455 patients undergoing varicocelectomy with only 19 controls.[68] Another study involved 1,500 men with infertility who underwent varicocelectomy and only 47 controls who did not.[69] Finally, the third controlled "study" involved 238 couples who were separated from the original WHO study of more than 7,000 couples. Of these 238 couples selected from the original 7,000, only 45 were actually studied and the remaining 193 were dismissed from the study for a variety of reasons. The other 7,000 or more WHO study participants discontinued participation in the study because of protocol deviations.[70,2,3] Thus, the evidence in favor of varicocelectomy for male-factor infertility is very thin. Even the claim that semen parameters are improved by varicocelectomy is much weakened by the failure of most papers to consider the variability of semen analysis in infertile men and its regression toward the mean.[71–73,63]

A meta-analysis of all the published controlled trials of various treatments of male infertility fails to support any conventional treatment for male infertility with the exception of the rare cases of Kallman syndrome and hypopituitarism.[61] The few properly controlled studies of various treatments for male infertility (including Clomid, gonadotropin, and varicocelectomy) failed to provide any solid evidence-based support.[71–74,1–4,63] It is easy to be deluded into thinking that whatever treatment we

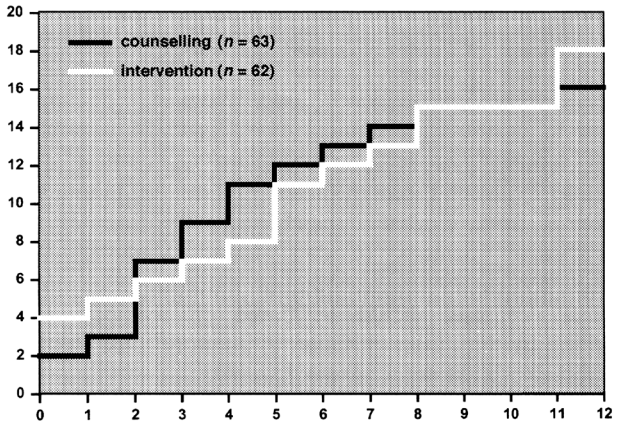

FIG. 3. Life table analysis of pregnancy rate in patients with varicocele who underwent counseling only, and who underwent varicocelectomy, and showing no difference in pregnancy rate. (From: Nieschlag E, Hertle L, Fischedick A, et al. Update on treatment of varicocele: Counseling as effective as occlusion of the vena spermatica. Hum Reprod. 1998,13:2147–2150. Also from: Nieschlag E, Hertle L, Fischedick A, et al. Treatment of varicocele: Counseling as effective as occlusion of the vena spermatica. Hum Reprod. 1995,10:347–353).

apply to the male, including vitamin C, erythromycin, or Proxceed, is actually having an impact because of the relatively high pregnancy rate in a control group undergoing no treatment at all. Therefore, it is easy to incorrectly think that our treatment was effective just because a pregnancy occurs.

It is also easy to be deluded into thinking the sperm count has increased, because careful longitudinal studies of semen analysis in untreated patients often appear to increase because of the phenomenon known as "regression toward the mean."[73] Whenever one measures a test result that is extremely variable, such as semen analysis with the same patient performed at different times, the purely mathematic phenomenon of regression toward the mean will make it appear that a patient who initially consulted because of a low sperm count will appear over the course of time to have an improvement without any treatment at all. This phenomenon was recognized as early as the original study of McLeod and Gold[16] and was mathematically elucidated with carefully controlled longitudinal trials by Baker in 1985, which serve as a model for evaluating ineffective treatments for male infertility that are mistakenly advocated with misguided enthusiasm.[30,52–55,71–73,63]

Intracytoplasmic Sperm Injection

In the face of previously miserable results in both the diagnosis and treatment for various causes of male infertility, the development of ICSI has been a *deus ex machina* (Fig. 4). Any type of male-factor infertility can be treated simply and effectively by ICSI.[75,11,7] The most severe cases of oligoasthenoteratozoospermia have resulted in the same pregnancy rates with ICSI as with mild cases, and these results were no different from those of couples with normal sperm undergoing conventional IVF.[76,77] Neither severe morphologic defects nor the tiniest number of spermatozoa (even "pseudo azoospermia") had any negative effect on the pregnancy rate

FIG. 4. Intracytoplasmic sperm injection has completely revolutionized treatment of male infertility. It was introduced at approximately the same time that there was a greater awareness among reproductive clinicians that none of the popular conventional treatments for male infertility were consistent with evidence-based control studies. (From: Silber SJ. Intracytoplasmic sperm injection (ICSI) today: A personal review. Hum Reprod. 1998,13:208–218).

with ICSI (Table 6). It appeared that all of the characteristics of the sperm, whether low numbers, poor motility, or abnormal morphology and deficient zona induced acrosome reaction, had no impact on the fertilizing capacity of the sperm, or the delivery of healthy offspring. Furthermore, the source of spermatozoa and the cause of the sperm defect appear to have no significant effect on the success of the procedure, whether the spermatozoa was from the epididymis, fresh or frozen, testicular, ejaculated, or from the testicles of men with severe defects in spermatogenesis.[76,31]

TABLE 6. Results of Intracytoplasmic Sperm Injection Using Ejaculated Spermatozoa Categorized According to Sperm Quality

	No. of Cycles	2 PN (%)	Transfer (%)	Clinical Pregnancies (%)
Sperm count (total)				
'0'	57	58	86	25
>0 to 1 × 10^6	97	64	96	26
>1 to 5 × 10^6	128	70	96	22
>5 × 10^6	684	71	93	30
Motility (%)				
0*	12	10	42	0
0	54	69	87	13
>0 to 5	19	68	100	32
>5 to 50	479	70	88	31
>50	337	74	95	26
Morphology				
0	48	68	88	31
>1 to 3	125	70	96	33
>4 to 13	307	71	94	26
>14	203	75	95	29

* Nagy et al, 1995.

2 PN = oocytes that had two pronuclei.

Results are categorized according to sperm quality.

The only exception was absolute immotility of ejaculated sperm, which is extremely rare. In a man who has the appearance of absolutely no motility in any of the sperm, in most instances, a careful search will find occasional weakly-twitching sperm, and again the success rate with such sperm is no different than in men with normal semen parameters undergoing IVF. In fact, it is not the immotility of the sperm that has any negative effect on the results, but rather the nonviability. Completely nonmotile sperm, which are viable, are still capable of normal fertilization and pregnancy rates.

It was immediately apparent that ICSI was the answer for which we were all searching for so many decades. The treatment of oligoasthenoteratospermia in the present era is simply ICSI with ejaculated sperm, and, for the most part, the diagnostic dilemmas of oligospermia have been made largely irrelevant by ICSI. It quickly became apparent that ICSI would be equally successful in combination with sperm retrieval techniques even for the treatment of azoospermia.[78–83,7,31,76]

Azoospermia

Approximately 20% of couples in the United States are infertile,[84,85] and approximately 25% of all infertile couples have a low sperm count.[86] Approximately 2% of infertile couples have azoospermia.[86] Thus, azoospermia represents approximately 8% of the cases of male infertility. One can therefore estimate that approximately one out of every 200 men in the population (excluding those who have had a vasectomy) have azoospermia. Approximately 5% of men who have previously undergone vasectomy (perhaps 10 million in the U.S. alone) become remarried and then wish to have children again.[87] Thus, there is a huge population of infertile men who have azoospermia.

We classify azoospermia as "obstructive" and "nonobstructive." Obstructive azoospermia includes patients who have had a vasectomy, patients with congenital absence of the vas deferens, those who have had accidental surgical interruption of the vas or epididymis during a hernia or hydrocele operation, or patients who have primary epididymal blockage from previous infections. They all have normal spermatogenesis in the testes. Even before the arrival of ICSI, all of these patients, with the exception of congenital absence of the vas, were amenable to microsurgical repair.[88–100] For obstructive azoospermia, ICSI simply adds a new dimension in that those who have failed with reconstructive attempts or those with congenital absence of the vas (which is not reconstructable) can now also have children.[101,31,76] In fact, because of ICSI, virtually any man with obstructive azoospermia can now father his own child, with the only limitation being the fertility of the wife.[31]

EVALUATION OF THE AZOOSPERMIC MAN

The diagnosis of obstructive versus nonobstructive azoospermia should be quite simple. However, it is sometimes approached in a confusing way that can lead to misjudgments, such as attempting to perform a vasoepididymostomy on a patient who has no obstruction. If the diagnosis is obstructive azoospermia, the management is quite different than if it is nonobstructive. Adherence to a few simple principles will avoid these difficulties and allow a proper preoperative decision to be made: if a patient has a testicle biopsy that shows normal spermatogenesis and if he has azoospermia, his infertility must be caused by obstruction. Everything else is superfluous. If in addition to these two criteria he also has a palpable vas deferens on physical examination, he is a candidate for surgical exploration and probable vasoepididymostomy. All other data are irrelevant.

A normal follicle-stimulating hormone (FSH) level does not necessarily indicate normal spermatogenesis or obstruction. In fact,

more commonly it indicates maturation arrest and nonobstructive azoospermia. The serum FSH level correlates most closely with the total number of spermatogonia, and less well with the number of mature spermatids, or the sperm count.[102,103,80] The most common diagnosis for patients with azoospermia and a normal serum FSH level is maturation arrest, not obstruction. Follicle-stimulating hormone level is in the normal range because the total number of spermatogonia in these cases is normal. It is true that an increased FSH level usually means inadequate spermatogenesis associated with Sertoli cell only, but even this axiom is not always true. Thus, endocrine evaluations are only modestly helpful in the diagnosis of obstruction.

A vasogram should be performed only as part of the operative procedure for correcting obstruction. It should not be used to make a diagnosis or to determine the need for surgery. Performing a vasogram as an isolated diagnostic procedure creates many problems. First, a scrotal exploration is not needed to ascertain that the vas is present; that should be easily discernible by physical examination. Second, unless performed as part of a careful microsurgical procedure, any injection or transection of the vas in performing a vasogram could result in obstruction where originally there was none. Third, the vasogram data are not necessary for preoperative planning. Most importantly, the test indicates nothing about the epididymis and can lead to a false-positive diagnosis of obstruction as well as a false-negative diagnosis of no obstruction. If a diagnosis of obstruction is certain, based on testicle biopsy and sperm count, the most logical time to perform a vasogram is at the time of vasoepididymostomy, once the vas is transected, to make sure that the vas empties distally into the ejaculatory duct and prostatic urethra. It is not necessary to know this information ahead of time.

Physical examination of the epididymis and testes, as well as a history or lack of history of infection, can be misleading as well. Testicles that produce a normal amount of sperm may be small, and those that produce no sperm (that have maturation arrest) may often be large. Historic data can be similarly confusing. At least 50% of our patients who were found to have epididymal obstruction from inflammatory causes had no previous history of clinical epididymitis. We must assume that whatever infection caused their epididymal obstruction must have been subclinical.

In conclusion, most of the ancillary medical information that we routinely consider in male fertility evaluation is irrelevant to the question of whether the patient has obstruction. The physical examination is only relevant in that if a vas deferens is not palpable (ie, congenital absence of the vas), then no surgical anastomosis can be planned. With that exception, the history and physical examination, serum FSH, luteinizing hormone, testosterone levels, and vasography are irrelevant to the diagnosis.

DIAGNOSTIC TESTICLE BIOPSY

The open technique for diagnostic testicle biopsy, which we recommend, is very simple, and should be a quick outpatient procedure using local anesthesia. The spermatic cord is injected with approximately 6 ml of 0.5% Marcaine (bupivacaine) via a 25-gauge needle just distal to the external inguinal ring. Then an additional 2 ml of 0.5% Marcaine is injected over the anterior scrotal skin in the area where a 1-centimeter incision is made down to the tunica albuginea. With this method, a small "window" is created through which the testis can be visualized. A 0.5-centimeter-long piece of testicular tissue is excised and placed in Zenker's (or Bouin's) fixative with an atraumatic "no touch" technique. This is a thoroughly painless clinical procedure (except for the initial injection of local anesthetic). The patient is able to get up and walk away immediately afterward with no more pain than if he had had a vasectomy performed.

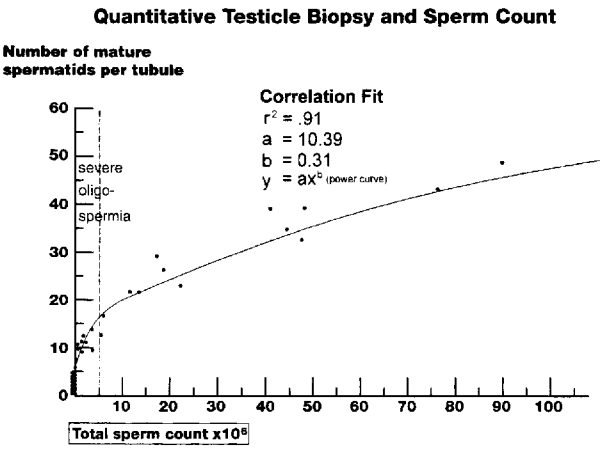

FIG. 5. An exponential curve relating sperm count in the ejaculate to the average number of mature spermatids seen in each seminiferous tubule. A threshold of three to six mature spermatids per tubule had to be exceeded for sperm to appear in the ejaculate. (From: Silber SJ, Nagy Z, Devroey P, et al. Distribution of spermatogenesis in the testicles of azoospermic men: The presence or absence of spermatids in the testes of men with germinal failure. Hum Reprod. 1997,12:2422–2428).

Needle biopsy is another alternative, but it is no less painful than the open biopsy as described previously, and the open biopsy always yields a sufficient number of seminiferous tubules (>20 cross sections) to perform an adequate quantitative analysis. Needle biopsy cannot yield this amount unless performed multiple times, which is ironically much more invasive and dangerous than the open biopsy technique.

The biopsy must be of adequate quality to determine: 1) Does the patient has normal spermatogenesis, and, therefore, obstruction that may be amenable to microsurgical repair; or 2) If he has nonobstructive azoospermia, will TESE provide a good or poor prognosis? Many testis biopsies are fixed incorrectly in formalin, or so traumatized as to create artifacts and absurd readings like "sloughing and disorganization."[103–106] Testicle biopsy has been used by most clinicians in a nonquantitative manner only. This has severely limited its usefulness and has led to many errors in interpretation.[107–110]

A simplified quantitative evaluation of the testicle biopsy is based on the normal histology and kinetics of spermatogenesis in the human.[111] The rate, or speed, of spermatogenesis in humans, or in any species, is constant for any variety of sperm counts, high or low. Reduced sperm production is always caused by lower numbers of sperm, not by a diminished rate of sperm production. Therefore, the daily quantity of sperm being produced for the ejaculate by the testicle is reflected quite accurately by the testicle biopsy. Thus, testicle biopsies of patients with both oligospermia and normal sperm counts have been found to be predictive of mean sperm count in the ejaculate. Patients with severe oligospermia after a strictured vasovasostomy have normal spermatogenesis. A testicle biopsy should clarify whether blockage or just poor spermatogenesis is causing the poor semen quality, and indeed for azoospermic cases, whether there is any mature sperm production at all (Fig. 5).

The testicle biopsy is performed bilaterally, and at least 20 seminiferous tubules are included in the count on each side. The mature spermatids (the oval cells with dark, densely stained chromatin) and large pachytene spermatocytes are the easiest to count. Previous studies have shown that these cells have the greatest correlation with sperm count and are the easiest ones to recognize. All of the steps of spermatogenesis from spermatogonia through leptotene, zygotene, pachytene spermatocytes, and early spermatids are observed, of course, but what is most important clinically is the number of mature spermatids in a minimum of 20 tubules, divided by the number of tubules (Fig. 6a).

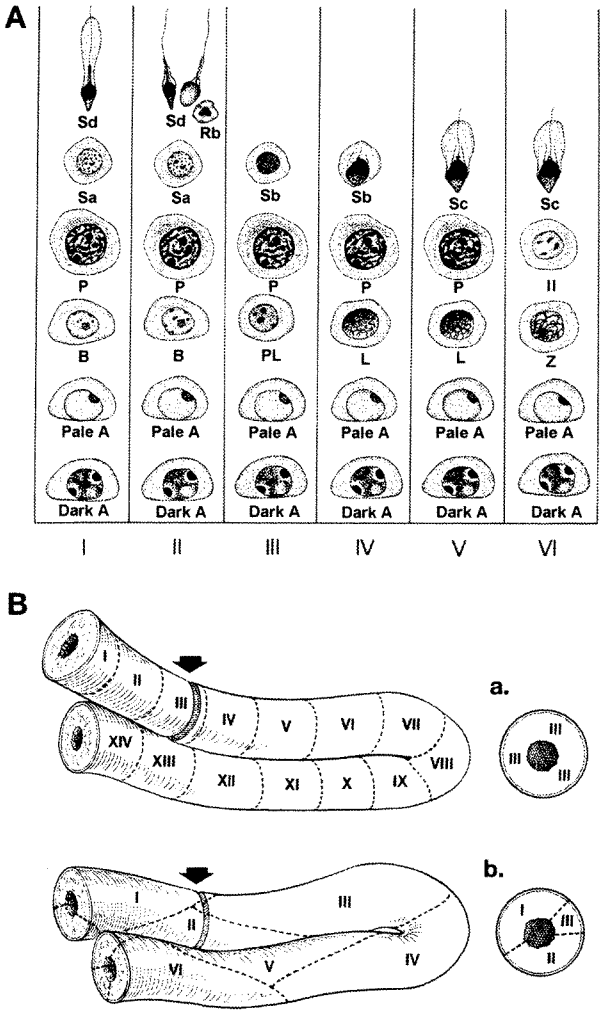

FIG. 6. A: The six stages of spermatogenesis in the human testicle. (From Silber SJ. Reproductive Infertility Microsurgery in the Male and Female. Baltimore, MD: Williams & Wilkins and Waverly Press Inc, 1984). B: Drawings of the progression of stages of spermatogenesis in the rat seminiferous tubule (a) and in the human seminiferous tubule (b). In most animals, there is a wave of spermatogenesis going in an orderly manner down the seminiferous tubule. In the human, however, there is a mosaic arrangement of the six stages of spermatogenesis. (From: Silber SJ. Reproductive Infertility Microsurgery in the Male and Female. Baltimore, MD: Williams & Wilkins and Waverly Press Inc, 1984).

Using an exponential curve (Fig. 5), the number of mature spermatids per tubule can be used to predict the anticipated sperm count. In the absence of obstruction, the correlation is remarkably close. For example, if the patient has 40 mature spermatids per tubule, the sperm count should be just less than 60 million/ml; if there are 45 mature spermatids, the sperm count should be just more than 85 million. The patient with a sperm count of <3 million would be expected to have only 6 to 10 mature spermatids per tubule.

Frequently, patients undergo vasoepididymostomy inappropriately because the pathology report incorrectly indicates normal spermatogenesis. Such readings often are not quantitative, but rather qualitative impressions that tubules are filled with spermatocytes and some mature sperm. If the biopsy shows thick tubules with large numbers of spermatocytes but only two or three mature spermatids per tubule, obstruction is not the cause of the patient's "azoospermia." Such patients require TESE with ICSI for nonobstructive azoospermia caused by maturation arrest.

Some clinicians have attempted to use the serum FSH level to monitor the amount of spermatogenesis: a normal FSH level in

a patient with azoospermia would supposedly indicate obstruction. Unfortunately, this correlation is poor.[102] Patients with maturation arrest causing azoospermia have a normal FSH level. The FSH level correlates most closely with the total number of spermatogonia and with the testicular volume, but not with the number of mature sperm.

Ironically, it is the scattered mosaic arrangement of the various stages of spermatogenesis in the human seminiferous tubule (as opposed to the orderly wave moving across the tubule in most other species) that makes quantifying the human testicular biopsy so simple. In rats, a cut through any particular seminiferous tubule shows only one particular stage (Fig. 6B). In humans, a cut through any area of the testicle reveals a scattered array of all the various stages of spermatogenesis. Thus, in humans, unlike most other animals, it requires only 20 seminiferous tubules for a good statistical sample of the total range of spermatogenesis in the entire testicle.

Microsurgical Vasectomy Reversal: Vasovasostomy Versus Vasoepididymostomy

The majority of patients who have had their vasectomy in the late 80s and 90s are found to have secondary pressure-induced epididymal blockage (whether caused by blow-outs or by inspissation) in addition to blockage of the vas. This results in absence of sperm in the vas fluid, which correlates with a zero success rate no matter how good the vasovasostomy technique.[93–96,99,112] This problem of secondary epididymal obstruction has become much more common (at a much earlier date after vasectomy) since the adoption of "better" techniques for vasectomy that result in no occult leakage of sperm at the vasectomy site.[87,97,113] When microsurgery with vasovasostomy was first popularized in the mid-70s, sperm was present in the vas fluid in the majority of patients, and vasovasostomy (if performed microsurgically and accurately) resulted in remarkably high success rates.[93–97] However, in the late 80s and 90s, the "improved" vasectomy techniques being used by most urologists allowed no occult leakage of vas fluid and a lower incidence of sperm granuloma at the vasectomy site. Ironically, this has created a much earlier occurrence of secondary epididymal obstruction from pressure build-up proximal to the vasectomy site. Thus, in the modern era, without addressing the problem of secondary epididymal blockage when performing a vasectomy reversal, the results with microsurgical anastomosis of the vas are likely to be very poor.

However, with specific tubule microanastomosis of vas to the epididymis, first described in 1978, this problem of secondary epididymal blockage can be circumvented.[92,99] In fact, the only way vasectomy reversal can be recommended in preference to sperm retrieval and ICSI today is if a high success rate can be achieved, and it can only be achieved by resorting to vasoepididymostomy with a specific tubule microtechnique whenever there is no sperm found in the vas fluid[22,89,92,98,99] (Figs. 7A and B). Unless the urologist is well-trained in microsurgery of the epididymis, he is better-off not attempting any procedure, not even vasovasostomy, because of the high likelihood that there will be epididymal damage indicated by the finding of no sperm in the vas fluid.

Thus, there are four major considerations for vasectomy reversal: 1) techniques for obtaining a reliable reanastomosis of the vas deferens (with modern microsurgical techniques, accurate reanastomosis should be achievable in almost every instance); 2) the detrimental secondary effects of vasectomy (pressure-induced epididymal damage); 3) microsurgical bypass of this secondary epididymal obstruction; and 4) freezing of epididymal sperm for later use with ICSI as a back-up if the reversal operation should fail.[82]

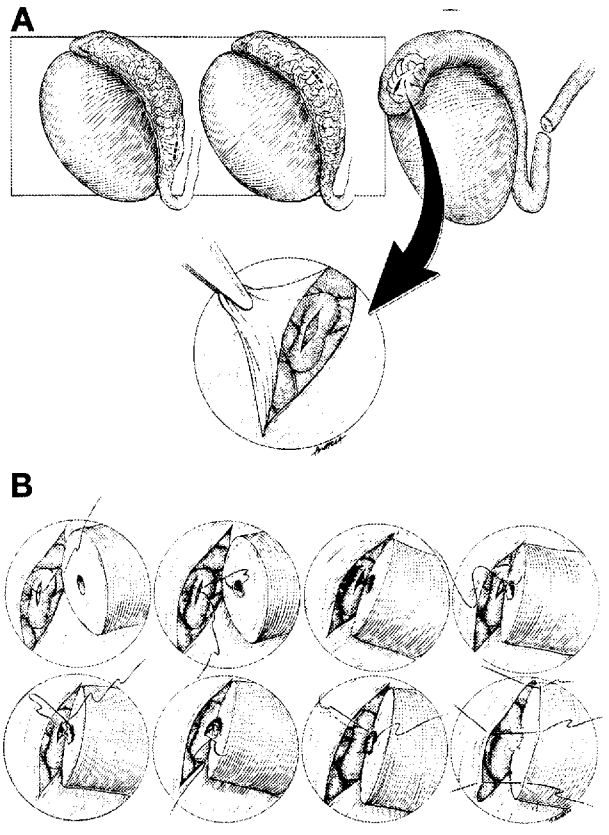

FIG. 7. A: The microsurgical-specific tubule vasoepididymostomy technique requires first transection of the vas, and then locating of the distal-most site that is proximal to the area of blockage in the epididymis, freeing the tubule, and making a tiny longitudinal slit for aspirating sperm and subsequent anastomosis. B: A depiction of the eight stages of the microsurgical-specific tubule anastomosis of the vas to the epididymis, bypassing epididymal blockage.

A microscope is necessary for the accuracy of the operation. Loupes can, at best, provide x2.5 to x4 magnification.[93,95,96,114–122] Visualization of the inner lumen of the vas deferens for easy and accurate placement of stitches requires x16 magnification. Other advantages of a microscope are that the depth of focus is clear, the light is constantly supplied directly to the patient, and the instrument rests on a stand and is immobile. The operators can move their heads or necks from time to time without disturbing the steadiness of the view of the subject. The technique of vasovasostomy is crucial for a nonstrictured anastomosis and is depicted in the figure from our original publication[90,91,93] (Fig. 8).

RESULTS OF VASOVASOSTOMY

The sperm count and quality should tend to improve gradually with time. If the anastomosis is strictured, however, the count may increase briefly but then eventually reduce to oligospermia or azoospermia. If the patient is still azoospermic 3 months or more after vasovasostomy, then either the vas anastomosis or the epididymis is obstructed.

More than 98% of patients with sperm in the vas fluid at the time of vasovasostomy have sperm in the ejaculate postoperatively (Table 3);[22] 82% of their wives conceived spontaneously, and this is dependent only on the fertility of the wife. Most of the confusion in the literature about vasovasostomy stems from the lack of documentation of preoperative sperm quality in the vas fluid, sparse observations of the epididymal ductal system, poor testis biopsy studies among men who have undergone vasectomy, and the failure to bypass secondary epididymal blockage. The group on whom we operated participated in such a careful

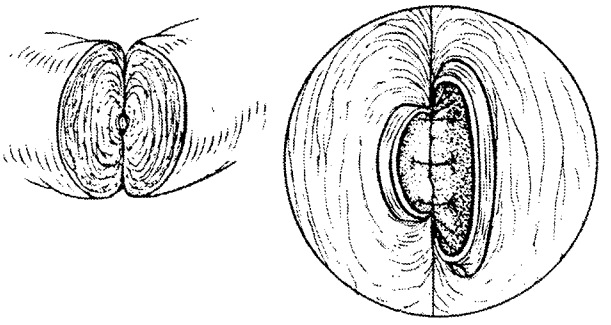

FIG. 8. An original depiction of the two-layer microscopic vasovasostomy, using interrupted 10–0 nylon mucosal sutures to anastomose the undilated abdominal side lumen of the vas to the dilated testicular side lumen of the vas with no leakage and no stricturing. (From: Silber SJ. Microscopic technique for reversal of vasectomy. Surg Gynecol Obstet. 1976,143:630–631).

study. Seminal fluid was sampled form the testicular side of the obstructed vas from each patient at the time of reanastomosis. The age of the patient, time since vasectomy, type of vasectomy, and area in which it was performed were correlated to subsequent sperm count and pregnancy of the partner. Appearance and quantity of vas fluid as well as sperm morphology (electron and light microscopy), quantity, and motility were recorded and correlated with postoperative results.

The distribution of sperm counts among patients with a patent vas postoperatively does not bear a statistically significant difference from that among control populations of fertile men (Table 3). The sperm count postoperatively was closely correlated with the results of quantitative testis biopsy. The woman's age was the critical determinant of pregnancy rate. These data led us to conclude that spermatogenesis is not substantially harmed by obstruction and that failure to achieve fertility after an accurate vasovasostomy is caused by dilatation and then perforation of the epididymal duct (or inspissation) with subsequent secondary epididymal obstruction (Fig. 9).

Patients without viable intact sperm in the vas fluid rarely had successful vasovasostomy, as measured by sperm in the ejaculate. All men with sperm granulomas had abundant, morphologically normal sperm in the vas fluid. Even when the vasectomy

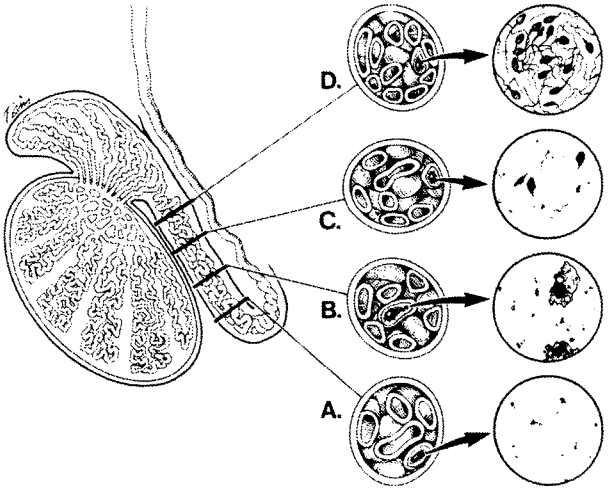

FIG. 9. A diagram of the serial transection of the epididymis in a patient who had undergone vasectomy but has no sperm in the vas fluid. At some point proximally in the epididymis, secondary epididymal obstruction is bypassed and then normal motile sperm are seen. (From: Silber SJ. Reproductive Infertility Microsurgery in the Male and Female. Baltimore, MD: Williams & Wilkins and Waverly Press, Inc, 1984).

had been performed more than 10 years previously, none of these men had poor-quality sperm. The presence of a sperm granuloma at the vasectomy site represents persistent and continual leakage of sperm, which alleviates the deleteriously high intravasal and epididymal pressure that otherwise always occurs after vasectomy. The high pregnancy rate with vasovasostomy was only achieved in couples whose husband had abundant sperm in the vas fluid at the time of the reversal procedure.

MICROSURGICAL VASOEPIDIDYMOSTOMY

The microsurgical-specific tubule technique of vasoepididymostomy was described in 1978.[91,92,95,96,98,99,105,120–124] The tunica vaginalis is opened and the testis and epididymis are everted from the hydrocele sac. The dilated epididymal tubule is usually approximately 0.1 to 0.2 mm in diameter. The epididymal duct is extraordinarily delicate with a wall thickness of approximately 30 μm. Earlier nonmicroscopic approaches made a deep longitudinal incision into the outer epididymal tunic, cutting through what looks like as many as 20 or 30 tiny tubules. Then, a fistula was created by suturing the vas to the epididymal tunic. The results were terrible.

The proper approach for reestablishment of continuity of the ductal system is to perform a specific anastomosis between the inner lumen of the vas deferens and the epididymal tubule (Figs. 7A and B). Our original approach was end-to-end, but now an end-to-side approach is preferred. The requirements for success and results are the same. The results with this approach to vasoepididymostomy are similar to those of vasovasostomy when there is sperm in the vas fluid.[89,22] The objective is to explore the epididymis more and more proximally (usually mid to proximal corpus) until we get beyond the secondary obstruction and find good-quality motile sperm and perform the specific tubule anastomosis at that level.

CONGENITAL ABSENCE OF VAS DEFERENS AND MICROSURGICAL EPIDIDYMAL SPERM ASPIRATION

Congenital absence of the vas deferens occurs in approximately 1% of infertile couples.[104] Until recently, it was a frustrating and dismal problem. Since the first successful use of epididymal sperm aspiration and IVF for congenital absence of the vas deferens was reported, ICSI has now made it possible for all these men to have children.[31,125] In fact, with ICSI, the pregnancy rate with epididymal sperm retrieval (microsurgical epididymal sperm aspiration) is only related to female factors.[31,78,79]

The men undergo a simple "window" scrotal exploration using local anesthesia immediately after their partners undergo oocyte aspiration. Using x10 to x40 magnification with an operating microscope, a 0.5-cm incision is made with microscissors into the epididymal tunic to expose the tubules in the most proximal portion of the congenitally blind-ending epididymis. Sperm are aspirated with a micropipette (0.7 mm/22 mm; Cook Urological, Spencer, IN) on a tuberculin syringe directly from the opening in the epididymal tubule. The specimens are immediately diluted in HEPES-buffered Earle's medium, and a tiny portion is examined for motility and quality of progression. If sperm motility is absent or poor, another aspiration is made 0.5-cm more proximally. Sperm are obtained from successively more proximal regions until progressive motility is found (Fig. 10). Motile sperm are usually not obtained until the most proximal portion of the caput epididymis or vasa efferentia is reached. Once the area of motile sperm is found, an aliquot of epididymal fluid is used for ICSI, and the remainder is frozen.

The present state of the art appears to be that there are virtually no cases of obstructive azoospermia that cannot be successfully treated with sperm retrieval methods and ICSI, so long as the female does not have insurmountable problems herself. For obstructive azoospermia, we prefer to use

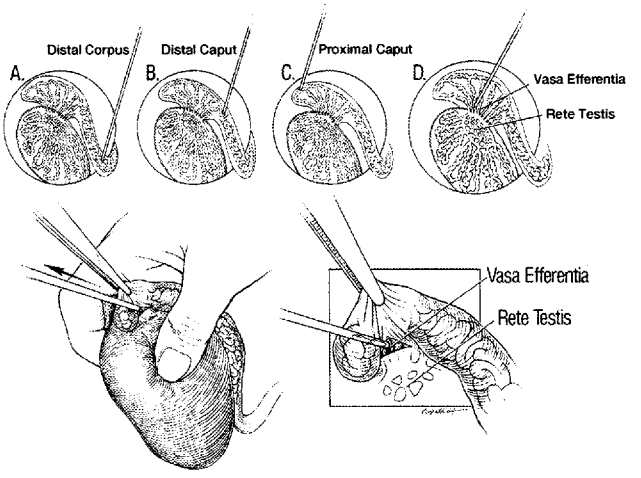

FIG. 10. A depiction of microsurgical epididymal sperm aspiration beginning at the distal corpus (A) and moving proximally to the distal caput, the proximal caput, and the vasa efferentia (B, C, and D). With obstructive azoospermia, there is an inversion of the usual physiologic location of greatest and least sperm motility. With obstruction, the most motile sperm are always the most proximal. Distal sperm, because of senescence, are the least motile. (From: Silber SJ. Congenital absence of the vas deferens. N Engl J Med. 1990,323:1788–1792).

epididymal sperm, although testicular sperm works just as well. The advantage of epididymal sperm as a first choice is that it freezes so easily and represents such a simple, clean, easy, indefinite supply of sperm for the laboratory to use for that particular patient without any need for future invasive procedures.

There have been many trivial debates about how to best collect epididymal or testicular sperm from patients with azoospermia for ICSI. The reader can decide what works best in his own particular setting, but our preference is described in the next few paragraphs.

For obstructive azoospermia, there is usually some epididymis present no matter how severe the congenital defect. In these instances, we prefer microsurgical epididymal sperm aspiration (MESA). We perform all sperm retrieval using local anesthesia without sedation. Although the approach is microsurgical and careful, it is an outpatient procedure performed with minimal postoperative discomfort. The spermatic cord is first grasped between thumb and forefinger by the urologist, a manner quite similar to performing vasectomy. The cord is then infiltrated with several milliliters of 0.5% Marcaine. This produces anesthesia of the testicle, but not of the scrotum. Then, several milliliters of 0.5% Marcaine are used to infiltrate the anterior scrotal skin with a 25-gauge needle along a proposed 1-cm to 2-cm incision line. Once the tunica vaginalis line is entered, the epididymis and testicle are exposed and brought into the field of an operating microscope. The patient, indeed, can watch the whole procedure on a video monitor and should be wide-awake and comfortable.

The advantage of epididymal sperm retrieval performed in this way is the huge number of the most motile sperm that can readily be obtained from the most proximal duct and frozen for an unlimited number of future ICSI cycles. There is often only one specific area of the proximal epididymis where motile sperm can be retrieved, and this can be found more easily through microsurgery than via a blind needle stick (which, in truth, is more painful than this microsurgical epididymal sperm aspiration procedure). For nonobstructive azoospermia, the epididymal sperm can never be retrieved because the walls are collapsed and there is no obstruction to allow epididymal sperm collection to take place. Nonetheless, for nonobstructive azoospermia, an open testicular biopsy performed using the microscope can still be accomplished in the same way using the same type of local anesthetic with the patient wide-awake and with minimum postoperative discomfort.

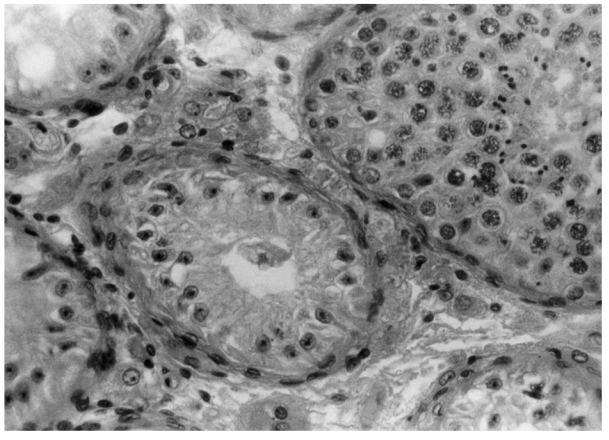

FIG. 11. This is a histologic section of testicle biopsy in a patient with Sertoli cell only, increased FSH, and occasional tubules with normal spermatogenesis. Upper right-hand tubule exhibits normal spermatogenesis, but all of the other tubules are Sertoli cell only. (From: Silber SJ, Johnson L, Verheyen G, et al. Round spermatid injection. Fertil Steril. 2000,73:897–900).

Testicular Sperm Extraction

Soon after introducing sperm retrieval for obstructive azoospermia, we made the observation that even in men with the most severe spermatogenic defects, causing complete azoospermia, there were often a minute number of sperm sparsely present in an extensive testicular biopsy, and these occasional testicular sperm could be used for ICSI.[76,83,7,103,79,80] We called this procedure testicular sperm extraction (TESE). This approach was based on a quantitative study of spermatogenesis dating back to the late 1970s.[105,106,126–128] Examination of the testicular histology of men with azoospermia, with oligospermia, and with normal sperm counts show that the number of sperm in the ejaculate is directly correlated to the number of mature spermatids found quantitatively in the testis. The average mature spermatid count per tubule in a large number of tubules is predictive of the sperm count in the ejaculate. Intriguingly, however, many patients with complete azoospermia have been found to have a few mature spermatids in their testis histology (Fig. 11). These studies of quantitative spermatogenesis, in the late 70s and early 80s, provided the theoretic basis for our efforts to extract sperm, however few, from men with azoospermia caused by Sertoli cell only or maturation arrest and allowed for the use of these few sperm for ICSI. An extremely diminished quantity of sperm production in the testis will result in absolute absence of sperm in the ejaculate even though there are some sperm being produced in the testicle. There is simply a low threshold of sperm production needed for any sperm to actually spill into the ejaculate. Thus, severe oligospermia, which is readily treated with ICSI, is just a quantitative variant of azoospermia, and there is some minute presence of spermatogenesis in 60% of azoospermic men (Fig. 12). The amount of spermatogenesis, however, is below the threshold necessary for these few sperm to spill into the ejaculate.[103]

The initial approach to TESE for nonobstructive azoospermia was crude, often involving numerous extensive biopsies from multiple areas of the testis until sperm were located. Legitimate concerns were apparent, including: 1) how do you counsel the couple to be prepared for IVF and ICSI (with all that it entails for the woman) when there is only a 55–60% chance that you will find any sperm?; 2) can you prognosticate which patients will have sperm successfully retrieved and which will not, so you may better advise who should and should not go through this procedure?; 3) with such severely compromised testes, how do we assure the couple that they can undergo multiple repeat procedures with successful sperm retrievals in future cycles?; and 4) is it possible to simply

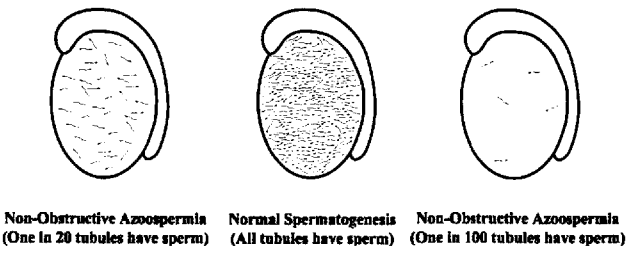

FIG. 12. Various "degrees" of azoospermia. Normal spermatogenesis (center drawing) is associated with obstructive azoospermia. With nonobstructive azoospermia, TESE may be easy as in the drawing depicted on the left, or difficult as depicted in the drawing on the right.

freeze the unused sperm derived from a TESE procedure without diminishing the results, and thereby avoid the necessity of having to time the woman's stimulation cycle to the man's sperm retrieval?

Whereas it is clear that often good results can be obtained with frozen thawed testicular sperm for cases of obstructive azoospermia, sperm retrieved from the testicle in nonobstructive azoospermia cannot be reliably frozen and thawed with result equivalent to that of fresh. Therefore, two major goals of ours were to determine: 1) whether a previous diagnostic biopsy or any other test could predict the success or failure of testicle sperm extraction; and 2) whether a technique for TESE could be used that would be relatively painless and would not compromise future attempts at fresh sperm retrieval. A small previous diagnostic testis biopsy is predictive of the likelihood of finding sperm in a TESE procedure in 85% of patients,[103] but in 15% of patients, a small previous diagnostic testis biopsy was not predictive. Our solution to this dilemma is a microsurgical approach to TESE (microsurgical TESE).

Microsurgical Testicular Sperm Extraction and Intracytoplasmic Sperm Injection for Nonobstructive Azoospermia

In nonobstructive azoospermia, occasional mature spermatids are noted in the testis biopsy of men who might have been thought to have had no spermatogenesis (Fig. 11). At least three mature spermatids per tubule must be present in the testis biopsy for any spermatozoa to reach the ejaculate. More than 50% of patients with azoospermia with germinal failure thus have some minute foci of spermatogenesis, which are not of sufficient quantity to produce spermatozoa in the ejaculate. When spermatogenesis exceeds three mature spermatids per tubule, the patient has sperm "spill-over" into the ejaculate, and then has oligospermia rather than azoospermia (Fig. 5).

Extensive multiple biopsies from every area of the testis are often performed in an effort to find sufficient sperm for TESE.[129,130] This can result in a great deal of testicular damage and may even limit "successful" patients to only one attempt.[129,130] Some try to limit damage by using needle rather than open biopsy to obtain sperm for ICSI.[131] However, control studies have shown that for difficult cases of nonobstructive azoospermia in which spermatogenesis is meager, needle biopsy is much less likely to find the rare foci of spermatogenesis for ICSI than is open biopsy.[132,133] Yet, some andrologists prefer not to risk future attempts at TESE with a massive open biopsy procedure.

We studied the distribution of spermatogenesis in men with azoospermia and have outlined a microsurgical approach to TESE that minimizes tissue loss and pain and makes TESE easily repeatable for an indefinite number of cycles. Knowledge of the distribution of spermatogenesis and use of microsurgical technique help to prevent tes-

ticular damage and postoperative pain, making multiple repeat TESE procedures safe and reliable.[103,104]

There is a lot of unnecessary confusion about testicular sperm, mature spermatids, and round spermatids. The tail of the sperm is seldom seen on histology, and only the thicker sperm head shows up in thin sections. It is usually only the oval head that is observed. Mature spermatids at TESE are no different in appearance than sperm. The solution to cases in which there are no sperm to be seen on TESE is not to look for "round spermatids."[134,135] We never see round spermatids in the absence of mature spermatids, which at TESE are what just appear to be sperm (Figs. 13A, B, and C).[136,134,135]

TECHNIQUE OF THE MICROSURGICAL TESTICULAR SPERM EXTRACTION PROCEDURE

All microsurgical procedures are performed using local anesthesia only. This involves both cord block and local infiltration of the incision line in the scrotum. The procedure is truly painless. The tunica vaginalis is opened and the testicle exteriorized. The operating microscope is then used with x16 to x40 magnification. After microdissection and evaluation of tubular dilation, often just a tiny microscopic removal of single dilated tubules can be used to retrieve large numbers of sperm.

However, large strips of tissue (no greater than the total amount of tissue that would have been removed in the conventional "blind" TESE technique) can be excised if necessary, with no damage to blood supply or pressure atrophy. The tunica albuginea is closed with 9–0 nylon interrupted sutures after meticulous hemostasis with micro-bipolar forceps (Figs. 14 and 15). This prevents any increase in intratesticular pressure, resulting in minimal pain and no subsequent atrophy.

Of the total patients subjected to microsurgical TESE for nonobstructive azoospermia, approximately 60% have sperm recovered. In Sertoli cell only, microsurgical dissection allows removal of only a minuscule amount of testicular tissue to find this sperm because normal tubules have full thickness and Sertoli cell only tubules are thin and empty. In maturation arrest, often a larger amount of testicular tissue has to be removed because all tubules are of normal size, and the foci of spermatogenesis are not easily discernible. Nonetheless, even in those microsurgical procedures in which relatively large amounts of tissue have to be removed, minimal damage is incurred because blood supply is not interrupted, microscopic bleeders are meticulously coagulated, tunica albuginea is not encroached on because of the closure with 9–0 nylon interrupted stitches, and consequently, there is no increase in intratesticular pressure (Figs. 14 and 15). This results in no testicular damage and minimal pain.

Our direct mapping provides evidence for a diffuse rather than regional distribution of spermatogenesis in nonobstructive azoospermia.[105,106] Furthermore, the variation in sparseness of spermatogenesis verified by observation of contiguous strips of testicular tissue explains why a single random biopsy may or may not yield sperm and why with obstructive azoospermia removal of small amounts of tissue blindly with a needle has a high success rate, but has a low success rate with nonobstructive azoospermia (Fig. 12). Microsurgical preselection of a small spermatogenic focus observed with the operating microscope truly trivialized the testicular damage.

However, even in patients in which the only solution is removal of a larger amount of testicular tissue, microsurgery still provides a major advance. The formidable testicular deterioration that has been observed with overly aggressive TESE procedures is caused by either direct interference with microvascular supply of the seminiferous tubules or even more commonly, increased intratesticular pressure caused by minor amounts of bleeding within the enclosed tunica albuginea. The tunica albuginea is a nonflexible enclosure. A small degree of intratesticular bleeding causes a noticeable increase in intratesticular pressure. This can

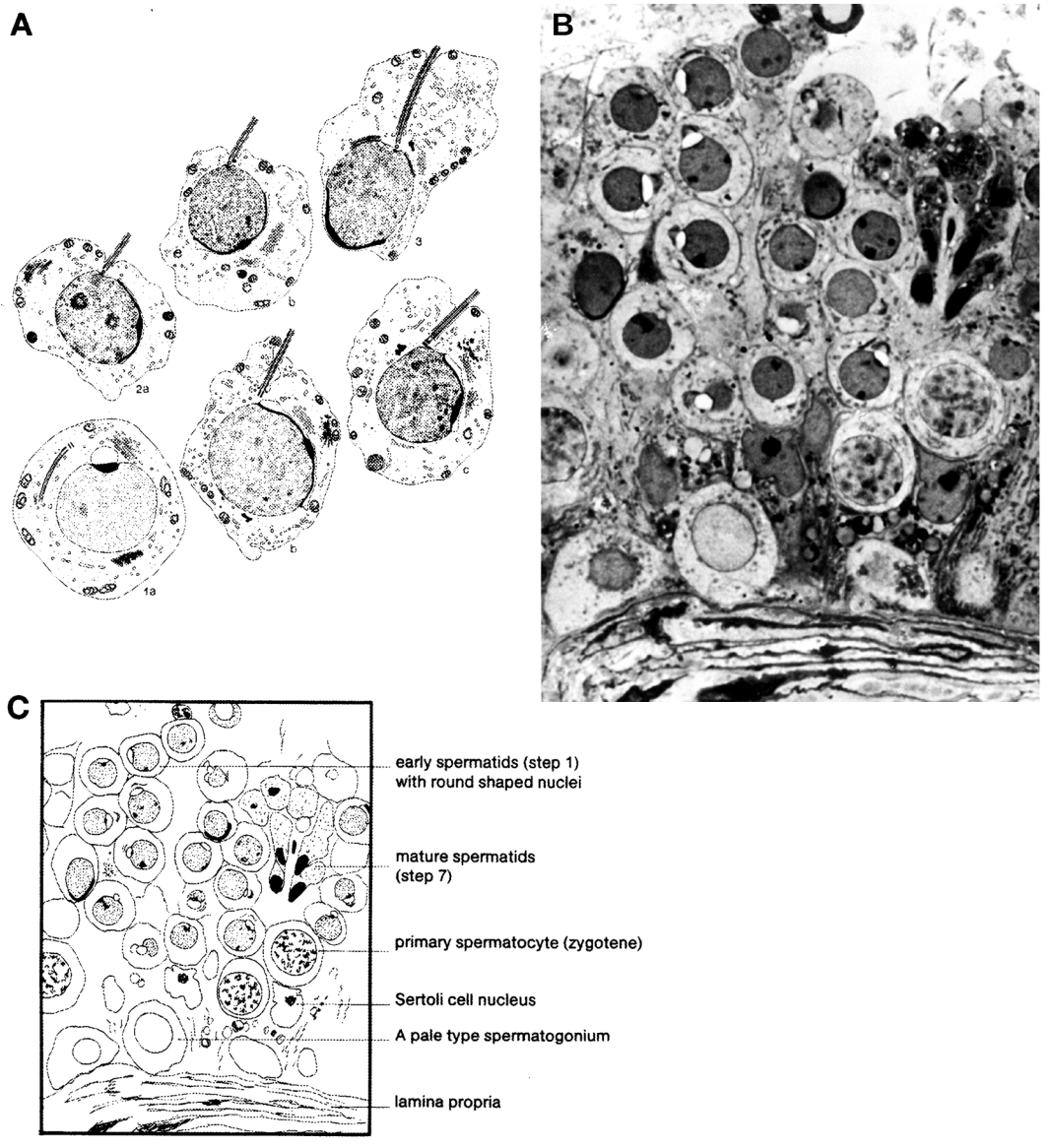

FIG. 13. A: Drawings of the stages of spermiogenesis after the second meiotic division had occurred. Before the formation of the tail, the round spermatid can always be recognized by the prominent acrosomal vesicle (1A). As the acrosomal vesicle recedes, the tail begins to form. B: Electron micrograph of a section of human spermatogenesis demonstrating pale "type A" spermatogonia, Sertoli cell nuclei, pachytene spermatocytes, early round spermatids with acrosomal vesicle, and mature spermatids with an oval, dark staining head. C: Diagrammatic depiction of 13B with labeling of the specific cells involved in spermatogenesis. (From Holstein AF, Roosen-Runge ED, eds. Atlas of Human Spermatogenesis. Berlin: Grosse Verlag, 1981).

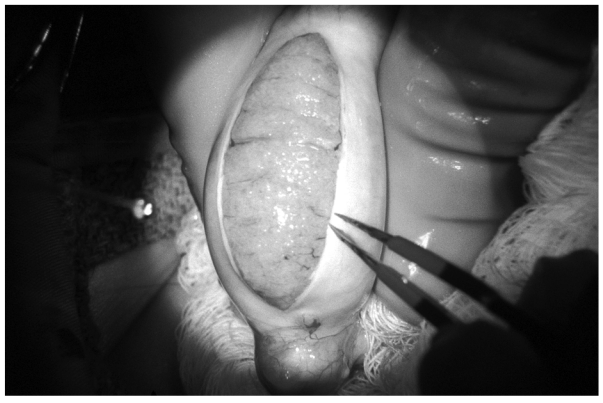

FIG. 14. A single, large, testicular incision for a TESE procedure using the operating microscope results in minimal to no testicular damage, minimal to no postoperative pain, and an ability to analyze each specific seminiferous tubule for the presence of spermatogenesis. (From: Silber SJ. Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. Hum Reprod. 2000;In press).

be readily observed by anybody performing conventional, multiple testicle biopsy samplings for TESE. Furthermore, the closure of open biopsies with the usual nonmicrosurgical suture, particularly in a running fashion with conventional TESE, further compromises the intratesticular volume and thereby adds to the increased pressure.

Genetics of Male Infertility and Intracytoplasmic Sperm Injection

KAROTYPING

A massive summary of karyotyping results in newborn population, reviewed by Van Assche, revealed an incidence of balanced translocations in a normal newborn population of 0.25%. A similar review of 7,876 men with infertility undergoing karyotyping revealed an incidence of balanced translocations of 1.3%, more than four-times that found in normal newborns (Table 7).[137] When the analysis is restricted to men with oligospermia (ie, less than 20 million per ml) some type of autosomal chromosome anomaly, either balanced Robertsonian translocations, balanced reciprocal translocations, balanced inversions, or extra markers is found in 3% of patients. In azoospermic men, the incidence of such translocations was less than in patients with severe oligospermia, but still greater than 1%. Sex-chromosomal anomalies, such as Klinefelter syndrome, were found in more than 5% of azoospermic men and in 1.6% of oligospermic men.

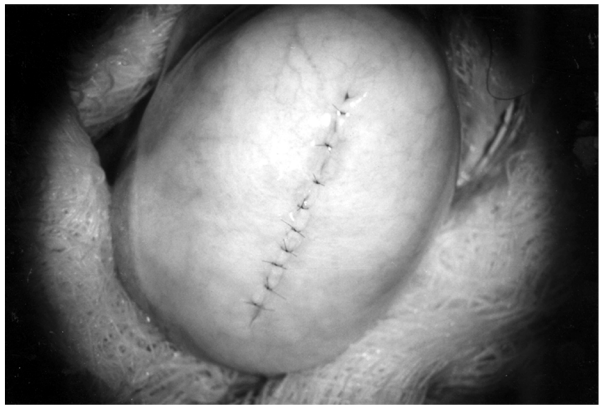

FIG. 15. Microsurgical closure of the tunica albuginea of the testes after a microsurgical TESE procedure results in no increase in intratesticular pressure and subsequently no loss of testicular function. (From Silber SJ. Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. Hum Reprod. 2000;In press).

TABLE 7. Autosome Abnormalities Observed in Infertile Men

	Number	Number (%) Robertsonian Translocation	Reciprocal Translocation	Inversion	Extra Marker	Total
Total	7,876	45 (0.6)	36 (0.5)	8 (0.1)	8 (0.1)	104 (1.3)
Newborn studies	94,465	76 (0.08)	98 (0.10)	23 (0.02)	35 (0.04)	232 (0.25)

Includes men with azoospermia and oligozoospermia.
From Van Assche et al, 1996.

The cytogenetic and pediatric follow-up of the first 1,987 children born as a result of ICSI has been meticulously studied and reported by the originators of ICSI in the Dutch-Speaking Free University.[138,139] In 1,082 karyotypes of ICSI pregnancies, nine (0.83%) had sex-chromosomal abnormalities, including 45 X (Turner), 47 XXY (Klinefelter), 47 XXX and mosaics of 47 XXX, as well as 47 XYY. This is a very low frequency, but nonetheless is four-times greater than the expected frequency of sex-chromosomal abnormalities in a newborn population (0.19%). Four (0.36%) of these 1,082 children had de novo-balanced translocations or inversions (Table 8).[138,139] These children were apparently normal, but this incidence of de novo-balanced translocations is five-times greater than what would be anticipated in a normal newborn population (0.07%). Finally, there were ten cases of translocations inherited from the infertile male (0.92%), and although nine of these ten were balanced translocations in normal newborns, one (0.09%) was an unbalanced translocation that was terminated. This incidence of cytogenetically recognizable chromosomal abnormalities in the offspring of patients undergoing ICSI is extremely low, but much greater than what would be anticipated in the normal newborn population.

TABLE 8. Karyotype Anomalies in 1,082 Prenatal Diagnoses of ICSI Offspring

Abnormal Karyotypes on 1,082 Prenatal Tests	Maternal Age (years)	Number	Percentage	Percentage in Literature
De novo chromosomal aberrations		18	1.66	0.445
Sex-chromosomal		9	0.83	0.19, 0.23
45, X	37			
46, XX/47, XXX	44			
47, XXX (2 children)	32, 37			
47, XXY (4 children)	26, 28, 28, 32			
47, XYY	25			
Autosomal		9	0.83	0.21, 0.61
Trisomy 21 (5 children)	32, 33, 37, 41, 41	5	0.46	0.14
Structural		4	0.36	0.07
46, XXY, t (4;5)	30x			
46, XX, t (2;15)	30			
46, XX, t (2;13)	36			
46, XX, inv (1qh)	39			
Inherited aberrations		10	0.92	0.47
Balanced		9	0.83	0.45
Unbalanced		1	0.09	0.023
Total aberrations (de novo + inherited)	28	2.5	0.92, 0.84	

From Bonduelle et al, 1995 and Bonduelle et al, 1996.

Because approximately 2% of oligospermic men have chromosomal translocations (compared with a control population of 0.25%), it is not surprising that 0.9% of offspring resulting from ICSI would inherit such a translocation from their father.[140] It is somewhat reassuring that only 10% of those inherited translocations were unbalanced, and 90% were balanced. The pregnancy was terminated in one unbalanced transmission of a paternal chromosomal translocation, and this represents an incidence of only 0.1% of offspring resulting from ICSI. However, the other nine translocations that were transmitted in a balanced manner, with "normal" offspring, are likely to potentially have the same infertility defect as their father (0.83%).

Thus, of these first 1,082 offspring resulting from ICSI and undergoing prenatal diagnosis and karyotyping, we can anticipate that almost 2% (based strictly on cytogenetic studies) will be infertile or sterile, which is more than five-fold what would be anticipated in a normal newborn population.

However, the karyotypic study of these offspring is more reassuring than alarming. The incidence of congenital abnormality in children resulting from ICSI (2.3%) is no greater than in every normal population studied.[138,139] Even the few reported ICSI offspring of Klinefelter syndrome patients have had normal chromosomes. Less than 0.1% of fetuses resulting from ICSI have had unbalanced translocations, requiring termination of pregnancy. There is no greater incidence of autosomal aneuploidy than what is predictable from maternal age. Sex chromosome aneuploidy (0.83%) is not a high incidence, although it is clearly greater than normal. Thus, the evidence based on cytogenetic and pediatric follow-up of offspring resulting from ICSI is somewhat reassuring, despite the possible occurrence of infertility and sex-chromosomal disorders in a very small percentage of patients. Molecular study of the Y-chromosome, however, is of greater concern regarding the future fertility of these children.

Genetics of Male Infertility and Intracytoplasmic Sperm Injection

Y-CHROMOSOME DELETIONS

Using molecular mapping techniques, which have much greater resolution than cytogenetics, microdeletions encompassing the AZFc region of the Y-chromosome were originally reported by us in 13% of men with azoospermia and in 7% of men with severe oligospermia.[8] We suspect that these Y-deletions represent only the "tip of the iceberg," and that the current success with ICSI in treating male infertility may result in greater infertility in future generations.[76,9,10,141] Even these subtle "microdeletions" on the Y-chromosome (that are not discernible on cytogenetic examination) represent gross drop-outs of thousands of nucleotides, which is still not a high degree of resolution. Thus, these Y-deletions have implications beyond what we can discern with current methods. Current molecular mapping methods cannot yet pick up smaller mutations. There are probably at least 36 germ cell-specific genes (only one-third of which are primarily on the Y-chromosome) that affect spermatogenesis. Many are on chromosomes other than the Y. Because of the multiple copies that exist for most of the genes that are on the Y, smaller point mutations, which may be much more common than these reported "microdeletions," are naturally much more difficult to find.[140]

Therefore, the AZFa region of the Y-chromosome was recently sequenced and two functional genes that had been previously described were identified, *DFFRY* and *DBY*. This was the first case reported of a point mutation causing a single gene defect associated with spermatogenic failure. This particular region of the Y was amena-

ble to such a mutation search whereas most of the Y (because of multiple DNA repeats) is not; but this gives us a clue to what we may find if we were able to search for these more subtle gene defects in the larger area of the Y-chromosome where most of the testis-specific genes have been located.[140] It now appears that there may be many more germ cell-specific genes on the X-chromosome, and on the autosomes, that may also have a role in spermatogenic failure. That is why this discovery of Y-deletions in men with azoospermia and severe oligospermia may represent just the "tip of the iceberg."

Why the Y

During the course of the last 350 million years of mammalian evolution, the X-chromosome and the Y-chromosome have evolved from what were originally two ordinary autosomes. During that evolution, spermatogenesis genes have transposed, or retropositioned, themselves from autosomes to the Y and amplified into multiple copies.[142] These spermatogenesis genes include *DAZ* and *CDY*. Other spermatogenesis genes have persisted from their original position on the X and achieved greater prominence on the Y, such as *RBM*. Indeed, even the *SRY* gene (the male sex-determining locus) was originally *SOX-3* on the ancestral X and the Y before differentiating into the *SRY*, which actually began the whole process of the Y chromosome's evolution. Genes associated with the nonrecombinant *SRY* region, on the evolving Y-chromosome, that were specifically beneficial for male function, flourished there as a "safe harbor."[143–149]

Our emphasis on the Y-chromosome for locating spermatogenesis genes to help in elucidating the causes of male infertility makes sense because the Y has collected for us genes that otherwise would be hidden throughout the genome. However, it would be naïve to assume, in view of the evolutionary history of the X and the Y, that there are not equally powerful components for regulating spermatogenesis located also on the X-chromosome and on the autosomes. Nonetheless, the instability of the Y-chromosome suggests an inexorable decrease in sperm production in the evolution of any species, unless there is either sperm competition within the mating pattern of the species or a method of continual recruitment of new spermatogenesis genes to the Y-chromosome with subsequent amplification before ultimate degeneration.[142,147]

Because of the effectiveness and widespread adoption of ICSI, with sterile men now becoming fathers, we wished to determine what relationship such Y-deletions may have on the severity of testicular defects in infertile men undergoing TESE and ICSI, what effect these microdeletions may have on ICSI results, and whether this relatively common genetic cause of infertility would be transmitted to offspring as a result of ICSI. All of the ICSI-derived sons of these infertile men were shown to carry the same Y-chromosome microdeletions as their infertile fathers. All of the offspring (boys and girls) of men with Y-deletions had a normal karyotype.[141,9,10]

The idea that the Y-deletion would be transmitted to the son is not as obvious as it may first seem and did require a careful study to elucidate. In fact, further study of more patients is still needed before being certain of this. The reason is that if the presence of a few foci of spermatogenesis in the testis of a severely oligospermic or azoospermic Y-deleted man were caused by testicular mosaicism, it would be likely that the few areas of normal spermatogenesis within such a deficient testis of a Y-deleted man may actually have normal Y-chromosomes. In that event, one could have expected the sons of these patients undergoing ICSI not to have Y-deletions. Thus, it is not at all obvious or clear intuitively, as some have mistakenly assumed, that this Y-deletion had to be transmitted to the son. Our current study, however, seems to indicate that the Y-deletion of the sterile father is, in fact, transmitted to the son, and instead of speculating that infertility would

be transmitted to the offspring, we can now be somewhat more certain of this risk.

There are most likely many spermatogenesis genes involved in male infertility, and we have barely "scratched the surface" with what are at present crude mapping techniques on the Y-chromosome. Whether these gross microdeletions are found in an infertile male patient does not obviate the likelihood of there being a genetic cause for his azoospermia or severe oligospermia. If the defective gene or genes is on his Y-chromosome, then his male offspring will surely inherit his problem. If they are on the X-chromosome, then his sons will not be affected, but his daughters will be carriers, and his grandsons will have a 50% chance of being infertile. Purely autosomal male infertility will be less common (for purely evolutionary reasons), and in those patients, the sons will be at minimum risk.

It is clear that a negative Y-microdeletion assay does not rule out genetic abnormality. Furthermore, it is likely that most causes of nonobstructive azoospermia are related to genetic abnormalities that current routine laboratory testing is not detailed enough or sophisticated enough to determine. Therefore, in my view, genetic counseling should be provided to all males with infertility, whether an abnormality is detected and whether Y-deletion assays are even performed. Although karyotyping certainly should be routinely performed for patients with infertility, Y-deletion testing should not be mandatory from a clinical point of view because it is still simply an important area of research that probably at present only picks up a fraction of the genetic causes of male infertility.

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