

## CONTROVERSIAL PROBLEMS INVOLVING MALE INFERTILITY

Sherman J. Silber, M.D.  
Infertility Center of St. Louis  
St. Luke's Hospital  
224 South Woods Mill Road, Suite 730  
St. Louis, MO 63017  
Telephone: 314-576-1400  
FAX: 314-576-1442  
Email: [silber@infertile.com](mailto:silber@infertile.com)

### LEARNING OBJECTIVES

1. The ART physicians and laboratory personnel should be able to discern evidence-based practices in male infertility from popular myth.
2. The ART physician should be in a position to evaluate more critically the role of the male factor in the couple's infertility.
3. The ART laboratory personnel and physician should better be able to manage azoospermia, both obstructive and non-obstructive, including sperm retrieval and processing for ICSI.
4. The ART physician and laboratory personnel should be better able to tackle the question of when to use ICSI rather than conventional IVF, and to evaluate the risks of unanticipated failed fertilization.
5. ART physicians should be able to deal with the patient's and public's concern that we may be transmitting his infertility to the next generation with our treatment.
6. ART physicians will better understand the role of vasectomy reversal versus sperm retrieval and ICSI.

## PRE-COURSE QUESTIONS

1. The objective of ART for patients with azoospermia is:
  - a. To obtain immature sperm for ICSI in the absence of mature sperm.
  - b. To distinguish between obstructive and non-obstructive azoospermia.
  - c. To locate tiny foci of spermatogenesis in a testis otherwise devoid of sperm.
  - d. To obtain more mature epididymal sperm from distal regions of the epididymis in order to improve delivered pregnancy rate.
2. Specific treatment of oligoteratoasthenospermia (OAT) with either Clomid, hormonal stimulation, or varicocelectomy:
  - a. Should be instituted before ART to encourage the possibility of spontaneous conception.
  - b. Should be instituted before ART in order to improve delivered pregnancy results with ART.
  - c. Should be limited to men with an ultrasound proven varicocele.
  - d. Should be combined with proper nutritional supplementation.
  - e. None of the above.

### Answers:

1. C
2. E

## INTRODUCTION

There have been many years of debate over the causes and therapy of male infertility. Many treatments have been strongly advocated over 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 very popularly marketed "Proxceed," without any documented evidence of effectiveness (1). Even the varicocele operation, long proclaimed as the one treatment for male infertility that had any validity, has now come also into serious question (2-6). It is becoming clear that most spermatogenic defects in the human are likely to be 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 which 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).

## EVALUATION OF THE MALE: IS THERE ANY USE TO IT?

### Sperm Count

The first and most important test for the male 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 wife, and in a small percentage of 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 ml meant that the husband was infertile and the urologist gave such

couples a very poor prognosis for pregnancy. 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 with fertilization, the World Health Organization, in 1992, issued a reduced list of "normal values" for semen analysis which included a sperm concentration of >20 million per cc, 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 over a certain period of time (e.g., one or two years), 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, conversely, 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 paper by MacLeod and Gold in 1951. These authors studied sperm counts in 1000 'fertile' and 1000 'infertile' men (Table 1) (16). Their results indicated that in a 'fertile' population, the vast majority of men had sperm counts  $>40 \times 10^6/\text{ml}$ . Only 17% had sperm counts  $<40 \times 10^6/\text{ml}$ . Only 5% of fertile men had sperm counts  $<20 \times 10^6/\text{ml}$ . This distribution would suggest that a normal count is  $>40 \times 10^6$  spermatozoa/ml, and this had been the assumption over many decades.

Rehan *et al.*, in 1975, reported results similar to MacLeod and Gold (17). In 1300 'fertile' men, the percentages were remarkably similar to those of MacLeod and Gold, with only 7% of 'fertile' men having sperm counts  $<20 \times 10^6/\text{ml}$ . Eighty-three percent of 'fertile' men had sperm motility of grades 3 and 4, but what has perhaps 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 3000 'infertile' men with a lopsided 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  $>40 \times 10^6/\text{ml}$  indicates a much greater likelihood of fertility.

That sperm count might actually not correlate closely with the man's fertility was first proposed in 1974. Nelson and Bunge reported in 386 'fertile' men that low sperm counts are compatible with fertility and that a sperm count of  $<20 \times 10^6$  or  $40 \times 10^6/\text{ml}$  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  $<20 \times 10^6/\text{ml}$  and only 40% had sperm counts  $>60 \times 10^6/\text{m}$ .

I have reviewed sperm count and motility indices in men following vasovasostomy whose wives became pregnant in comparison to 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 'successful' vasovasostomy patients whose wives became pregnant had total motile sperm counts per ejaculate of  $<10 \times 10^6$ . In fact, the extensive comparison by Jouannet *et al.* of spontaneous pregnancy rates in infertile couples with varying sperm parameters showed similarly to my long-term follow-up of vasovasostomy patients, that above  $5 \times 10^6$  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 time in couples who are infertile. Schoysman and Gerris, in 1983, studied the spontaneous pregnancy rate over time in 1327 oligozoospermic couples (Table 4) (24). When the motile sperm count was  $<1 \times 10^6/\text{ml}$  (even as low as 100,000/ml) with no treatment of either the husband or the wife, in 5 years there was a 4% spontaneous pregnancy rate and in 12 years the wives of 9% of these very severely infertile men had spontaneously conceived. When the motile sperm count was  $5-10 \times 10^6$ , 22%

conceived by 5 years, and 34% by 12 years. When the motile sperm count was  $15\text{-}20 \times 10^6$ , 69% conceived by 5 years and 82% by 12 years. Hargreave and Elton in 1983 showed similar results in couples with varying degrees of oligospermia (Table 5).

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  $<5 \times 10^6/\text{ml}$ ,  $5 \text{ to } 20 \times 10^6/\text{ml}$ ,  $>20 \times 10^6/\text{ml}$  with  $<60\%$  motile and  $>20 \times 10^6$  with  $>60\%$  motile. These four groups were compared graphically to the life-table conception rate of Kovacs' donor insemination group (26) Vessey's women discontinuing the IUD (27), and the spontaneous pregnancy rate reported in 1953 by MacLeod and Gold for fertile couples (28).

Again, quite remarkably, with  $<5 \times 10^6$  spermatozoa/ml regardless of motility, the pregnancy rate at 2 years was 26% (Fig. 1). When the sperm count was  $5 \text{ to } 20 \times 10^6/\text{ml}$ , the pregnancy rate at 2 years was 42%. When the sperm count was  $>20 \times 10^6/\text{ml}$ , with  $<60\%$  motile, the pregnancy rate at 2 years was similar to the results obtained when the count was  $5 \text{ to } 20 \times 10^6$ . When the sperm count was  $>20 \times 10^6$  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 IUD, it is clear that no matter how high the sperm count, the subsequent pregnancy rate of couples attending a fertility clinics is lower than a normal control population. Furthermore, even though women may become spontaneously pregnant with extremely low sperm counts, nonetheless, among infertile couples a higher 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 (21,29,24,30).

In an extensive study of the various factors affecting pregnancy rate with sperm retrieval and ICSI in azoospermic men, neither the quality of the sperm, nor the site of retrieval had any affect on the pregnancy rate. The only factors which 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 (Fig. 2 and Fig. 3). 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 age of the wife 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 (e.g., 68% versus 23%), which leads to lower pregnancy rate (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 fertilization, have led to the introduction of 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 World Health Organization 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 very simple categories of normal (oval-head), amorphous (irregular-head), tapered-head, and small-headed sperm, have now been replaced by “strict criteria” (35,36,37,38,39,40,41,42,43,44). 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” that fits within this narrow range (2.5-3.5 microns wide and 5-6 microns long).

The original criteria that defined a normal sperm were based on an aesthetically 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 theoretical 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 theoretical 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 a low percentage of sperm with normal morphology do fertilize, and at least 25% of patients who do not fertilize their wife’s eggs can have greater than 14% 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 cases, 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 which 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 clear up a great deal of confusion about sperm testing problems, provided an explanation for unexplained failed fertilization, and also why and how sperm morphology affects fertility (50). Human spermatozoa must first bind to the zona pellucida in order 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 a very efficient inducer of the acrosome reaction, which then allows the sperm to penetrate through it. Sperm with normal morphology which 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 male partner's 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 post-coital 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 they involved a great deal of equipment and expense (52-56,46). It is probably the diverse population of spermatozoa in the semen of each male that makes such testing problematic, as most infertile men who are not azoospermic represent a spectra of fertility (57,58,59).

#### Conventional Treatment of Male Infertility (Varicocelectomy, Clomid, Dietary Supplements, Etc.)

Devroey has argued that for the most part, treatment of male infertility, prior to IVF and ICSI, has been authority-based and not evidence-based. There are quite a few controlled studies which show no effect of varicocelectomy on male infertility. These papers are generally given much greater credence by infertility specialists other than urologists (2-6, 60,64-67). The only "controlled" studies that favored varicocelectomy were extremely flawed by obvious patient selection (68,69,70,2,3). It is highly doubtful whether the fertility of any male with oligospermia, or oligoasthenoteratospermia can be improved by any treatment, whatsoever, including anti-estrogens 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, which might be detected, even physical examination has no impact on therapeutic results for oligoasthenoteratospermia (62).

There is probably no subject that is more controversial in the area of male infertility than varicocele. Most non-urologist 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. Baker *et al* found that couples who underwent varicocelectomy, as well as couples who did not undergo varicocelectomy, had a conception rate (within one year)

of approximately 30%, and (by two 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 one year and a similar percent that had undergone varicocelectomy became pregnant within one year (Fig. 3). It is obviously not appropriate to advocate the effectiveness of any of the other popularly prescribed treatments for male infertility over the last 40 years without rigorously controlled studies (63,2,3,24).

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's Syndrome and hypopituitarism (61). Baker et al have shown that there is no difference either in pregnancy rate or in improvement of sperm count or motility between any of the conventional treatments for male infertility versus no treatment at all. 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 apply to the male, including vitamin C, erythromycin, or Proxceed, are actually having an impact because of the background 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 gone up, because careful longitudinal studies of semen analysis in untreated patients often appear to increase because of the phenomenon known as "regression toward the mean" (71-73,63,73). Whenever one measures a test result that is extremely variable, such as semen analysis, with the same patient performed at different times, the purely mathematical 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 time to have an improvement without any treatment at all. The same treatments applied toward a group of men with extremely high sperm counts will appear to result in a lowering of sperm count, once again because of "regression toward the mean." This phenomenon was recognized as early as the original study of McLeod and Gold in 1951 (16) and was mathematically elucidated with carefully controlled longitudinal trials by Baker in 1985, which serves as a model for evaluating ineffective treatments for male infertility that are mistakenly advocated with misguided enthusiasm (30,52-55,71-73,63,75,76,77,78-83).

## METHODS FOR SPERM RETRIEVAL IN AZOOSPERMIA

### Obstructive Azoospermia

Approximately 20% of couples in the United States are infertile (84,85,86), and approximately 25% of all infertile couples have a low sperm count. (86). About 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) are azoospermic. Approximately 5% of men who have previously been vasectomized (perhaps 10 million in the U.S. alone) become remarried and then wish to have children again (87). There is thus a huge population of infertile men who are azoospermic (88-100,101).

A normal FSH does not necessarily indicate normal spermatogenesis or obstruction. In fact, more commonly it indicates maturation arrest and non-obstructive 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. FSH is in the normal range because the total number of spermatogonia in these cases is normal. It is true that an elevated FSH level usually means inadequate spermatogenesis due to Sertoli cell only, but even this axiom is not always true. Thus, endocrine evaluations are only modestly helpful in the diagnosis of obstruction (103-123).

Since the first successful use of epididymal sperm aspiration and IVF for CBVAD was reported, ICSI has now made it possible for all azoospermic men with obstruction to have children (31,124,125,126). In fact, with ICSI, the pregnancy rate with epididymal sperm retrieval (MESA) is only related to female factors (31,78,79).

The men undergo a simple “window” scrotal exploration under local anesthesia immediately after their partners undergo oocyte aspiration. Under 10x to 40x 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/22mm; 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. 4). 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 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 over how to best collect epididymal or testicular sperm from azoospermic patients for ICSI. Most centers use only needle aspiration of either epididymis or testis often without even being clear about the diagnosis. The reader can decide what works best in his own particular setting, but our preference is as follows:

For obstructive azoospermia, there is usually some epididymis present no matter how severe the congenital defect. In these instances, we prefer MESA (Microsurgical Epididymal Sperm Aspiration). We do all sperm retrieval under 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 cc’s of 0.5% marcaine. This produces anesthesia of the testicle, but not of the scrotum. Then, several ml of 0.5% marcaine are used to infiltrate the anterior scrotal skin with a 25 gauge needle along a proposed 1-2 centimeter 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 fashion 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 a more painful than this microsurgical MESA procedure). For non-obstructive 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 non-obstructive azoospermia, an open testicular biopsy, performed under the microscope, can still be accomplished in the same fashion under the same type of local anesthetic with the patient wide awake and with minimum postoperative discomfort.

#### Testicular Sperm Extraction (TESE) For Non-Obstructive Azoospermia

Shortly 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 very 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 coined this procedure testicular sperm extraction (TESE). This approach was based on a quantitative study of spermatogenesis dating back to the late 1970's (105,106,126-129). Examination of the testicular histology of azoospermic, oligospermic and normospermic men shows 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 a few mature spermatids in their testis histology (Fig. 5). These studies of quantitative spermatogenesis, in the late 70's and early 80's, gave the theoretical basis for our efforts to extract sperm, however few, from men with azoospermia caused by Sertoli cell only or maturation arrest, and to use 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 is some sperm being produced in the testicle. There is simply a low threshold of sperm production that is necessary before any sperm can actually spill over into the ejaculate. There is some minute presence of spermatogenesis in 60% of azoospermic men (Fig. 6). The methods for retrieval of sperm from such patients are a subject of great controversy.

The initial approach to testicular sperm extraction (TESE) for non-obstructive azoospermia was very crude, often involving numerous extensive biopsies from multiple areas of the testis until sperm were located. Legitimate concerns were raised including: 1) how do you counsel the couple to be prepared for IVF and ICSI (with all that it entails for the wife) 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 won't, so as to better advise who should and shouldn't go through this procedure, 3) how do we assure the couple that they can go

through repeat procedures with successful sperm retrievals in future cycles?; and 4) is it possible to simply freeze the unused sperm derived from a TESE procedure without diminishing the results, and thereby avoid the necessity of having to time the wife's stimulation cycle to the husband's sperm retrieval?

While 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 difficult cases of non-obstructive azoospermia cannot always be reliably frozen and thawed with result equivalent to that of fresh. Therefore, two major goals of ours were to determine: 1) whether a prior diagnostic biopsy or any other test could predict the success or failure of testicle sperm extraction, and 2) whether or not a technique for TESE could be used which would be relatively painless and not compromise future attempts at fresh sperm retrieval?

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 (130,131). Some try to limit damage by using needle rather than open biopsy to obtain sperm for ICSI (132). However, control studies have shown that for difficult cases of non-obstructive azoospermia, where spermatogenesis is very meager, needle biopsy is much less likely to find the rare foci of spermatogenesis for ICSI than open biopsy (133,134). Yet some andrologists prefer not to risk future attempts at TESE with a massive open biopsy procedure.

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-shaped head that is observed histologically. Mature spermatids at TESE are no different in appearance than sperm. The solution to cases where there are no sperm to be seen on TESE is not to look for "round spermatids" (135,136). We never see round spermatids in the absence of mature spermatids, which at TESE, are what just appear to be sperm anyway (Fig. 7 and Fig. 8) (135,136,137).

All sperm retrieval cases can be, regardless of approach, performed under 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 under 16 to 40x magnification. After microdissection and evaluation of tubular dilation, often just a tiny microscopic removal of single dilated tubules can be employed 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 and no pressure atrophy. The tunica albuginea is closed with 9-0 nylon interrupted sutures, after meticulous hemostasis with micro-bipolar forceps (Fig. 9). This prevents any increase in intratesticular pressure, resulting in minimal pain and no subsequent atrophy.

Of the total cases subjected to microsurgical TESE for non-obstructive azoospermia, about 60% have sperm recovered. In Sertoli cell only, microsurgical dissection allows removal of only a very minuscule amount of testicular tissue to find this sperm, because normal tubules are 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 normal size, and the foci of

spermatogenesis are not easily discernible. Nonetheless, even in those microsurgical cases where 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 upon because of the closure with 9-0 nylon interrupted stitches, and consequently there is no increase in intra-testicular pressure. This results in no testicular damage, and minimal pain.

Our direct mapping gives evidence for a diffuse rather than regional distribution of spermatogenesis in non-obstructive azoospermia (105,106). 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 very small amounts of tissue blindly with a needle has a high success rate, but has a low success rate with non-obstructive azoospermia (Fig. 10).

Even in cases where the only solution is removal of a larger amount of testicular tissue, microsurgery still has a benefit, in that testicular deterioration that has been observed with overly aggressive TESE procedures (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) can be avoided. The tunica albuginea is a very non-flexible enclosure. A small degree of intratesticular bleeding causes a noticeable increase in intratesticular pressure. This can be readily observed by anybody doing conventional, multiple testicle biopsy samplings for TESE. Furthermore, the closure of open biopsies with the usual non-microsurgical suture, particularly in a running fashion with conventional TESE, further compromises the intratesticular volume and thereby adds to the increased pressure. Multiple blind needle biopsies to retrieve greater amounts of tissue can be just as damaging as open biopsies (for the same reason), and just as painful. That is why our solution is always microsurgical sperm retrieval under outpatient local anesthesia.

## ICSI AND THE FUTURE FERTILITY OF MALES IN OUR SOCIETY

Profoundly low sperm counts are thought to be mainly of genetic origin, such as microdeletions in the AZFc region on the Y chromosome, mutations on the X chromosome and even on the autosomes. With the advent of ICSI, these “sterile” men can now father children who inherit their genetic defects (138,140,141,142,143,144,145,146). This has stimulated speculation that ICSI may cause a declining fertility in the male population. Therefore, we have constructed mathematical models to try to forecast the impact of ICSI on the fertility of future generations of ICSI (143).

Up to 20% of couples worldwide are infertile, including some 2% of the male partners who are azoospermic (139). We have taken 1:100 as an extreme upper estimate of the overall occurrence of heritable defects of spermatogenesis in our current population and have used a simple model which assumes that a proportion,  $\square$ , of all these men are treated successfully with ICSI and that any sons they produce will inherit the condition. These assumptions probably overstate what could happen in practice, and so would represent a worst-case scenario. It is likely that most cases of severe oligospermia and azoospermia are genetic, and that one third are Y chromosomal, one third are X chromosomal, and one third are autosomal dominant (146a).

The simplest model predicts a generation by generation ( $i$  to  $i+1$ ) increase in the proportion of infertile males, according to:  $p_{i+1} = [(1-p_i) \times 0.01 + p_i \times \square] / [(1-p_i) + p_i \times \square]$ .

If half of all affected males were treated successfully with ICSI, the incidence of severe male infertility would double in seven generations, or after some 250 years (Fig. 11, Fig. 12, and Fig. 13). If 90% of men were treated, the incidence would double in one generation, and after another ten generations it would have risen seven-fold (see Tables 7 and 8). In theory, total male infertility in our society could be reached after 300 generations (about 10,000 years) if all male patients were given effective treatment. More complex models are now being generated which will more flexibly represent different scenarios of possible modes of inheritance male infertility. However, it is clear that ICSI will not significantly increase the burden of male infertility in human populations in the near future.

### THE ROUND SPERMATID (ROSI) CONTROVERSY

We do not find round spermatids in human TESE (testicular sperm extraction) specimens in which we do not also find elongated sperm with tails (Fig. 14). We find many "round cells" in all TESE specimens, but in the absence of sperm, these are not round spermatids (147,148,149).

This observation is based on phase contrast evaluation of TESE microdroplets, and also on detailed stained histology of many hundreds of testis specimens, as well as from the previous literature on the histology and pathology of maturation arrest (150,151,152,153,80,103). The atlas of phase contrast wet preparation cells already published by Johnson et al. attests to the difficulty of distinguishing round spermatids from Leydig cells, Sertoli cell nuclei, and even from spermatogonia, without staining and whole tissue fixation (150,151). The only reason we concentrate on the albeit transient acrosomal vesicle and phase contrast is simply to provide an easy identification marker for wet microdroplet ICSI preparations (Fig. 16). The absence of round spermatids in wet TESE preparations based on phase contrast visualization of the acrosomal vesicle is merely confirmatory in the ICSI setting to what was already demonstrated in fixed tissue stained specimens over many years (Fig. 20 and Fig. 21).

The acrosomal vesicle forms very early after meiosis and, in fact, the Golgi apparatus is already present in the pachytene spermatocyte (Fig. 7 and Fig. 8). Holstein does not even draw in his atlas the very evanescent Golgi pre-vesicle phase (137). One could argue that round spermatids may be arrested at this very brief, pre-vesicle phase, i.e., before the acrosomal vesicle can be observed. But stained histologic sections (without any need to see the acrosomal vesicle) still reveal that maturation arrest in the human is apparently a block at meiosis. Thus, we still see no evidence that round spermatid arrest is a common event in human maturation arrest.

The notion that round spermatids can be readily distinguished from Sertoli cell nuclei, Leydig cells, spermatogonia, and other TESE components, with standard Hoffman optics, and without any need to identify an acrosomal vesicle, just by looking at size, is very challenging (149). The Sertoli cell nucleus has a diameter of about 10 microns on average, and red blood cells and round spermatids have an average diameter of about 8 microns. Spermatogonia average 9 microns, and pachytene spermatocytes are a much larger 12 microns on average. The intrinsic variability of several microns in many of these cells makes size alone a very unreliable way to distinguish (for human clinical ICSI) which cells to inject into the human egg (Fig. 16). The

efforts of Verheyen et al. (147) utilizing phase contrast with an inverted microscope represent a major effort toward positive identification of round spermatids for a viable ICSI setting.

A careful review of Holstein's E/M studies of fixed tissue specimens of normal spermatogenesis shows how similar the size and appearance of the round spermatid can be to the Sertoli cell nucleus (Fig. 8). With E/M, or with stained tissue sections, these distinctions are more easily seen. However, with wet preps the acrosomal vesicle seems to be the most reliable landmark. Nomarski differential contrast image of live human testicular cells does hold some promise of identifying various germ cells better with wet prep but it is still very difficult (Fig. 16).

A third issue is whether one can base his view of round spermatid injection (ROSI) on the well known CREM-mutant mouse model (154,155). According to the CREM model, the transcriptional activator, cyclic AMP-responsive element modulator (CREM) is required for germ cell specific genes that participate in sperm structuring. This means that in homozygous CREM knockout mice, there is azoospermia caused by maturation arrest at some stage after meiosis. Most of these post-meiotic haploid germ cells in the CREM deficient mice undergo apoptosis before an acrosomal vesicle can form. It is possible that looking at a wet TESE-ICSI prep with phase contrast in search of round spermatids might fail if spermatogenic arrest in the human occurs just before the formation of the acrosomal vesicle, as in the CREM deficient mouse.

Nonetheless, histologic sections (which are the gold standard even in the CREM literature), should still demonstrate early round spermatid arrest in azoospermic men if they were suffering from this problem. Most histologists fail to find this early round spermatid arrest in humans, and others find that it is very rare (0.9%) (134,152,153,80,103,156). Furthermore, even in the CREM deficient mouse, there actually were more advanced round spermatids (albeit small numbers, but they were not absent). Finally, what many ICSI programs determine to be viable early round spermatids are just apoptotic cells, as also demonstrated in the mouse CREM model (147).

How do we reconcile these observational differences and why bother with all this hair-splitting? Our reason for concern is that so many serious IVF centers are struggling to reproduce the clinical success reported by just a few authors (149,157,158,159,160,161,162). Some centers have experienced and reported consistently negative results with ROSI with desperate patients in whom no mature sperm or elongated spermatids could be found (163,164). Many other centers perform ROSI regularly but understandably fail to report their negative results. We have visited these centers and seen the confusion regarding which round cells to inject. We are aware of the very low success rate (1%) with round spermatid injection even in the successful mouse model with normal spermatogenesis (165,166,167). Furthermore, the mouse, whose centriole does not derive from the male, may be more favorable than primates for round spermatid fertilization.

Schulze et al. examined with exquisite histologic methodology the biopsies of 1,426 azoospermic men and found only 13 cases (0.9%) of spermatogenic arrest at the early ("round") spermatid stage, and concludes "that a complete maturation arrest at the stage of round spermatid is a rare phenomenon" (152). This agrees with early papers of Soderstrom and Suominen (153,168). Some IVF centers have treated dozens of patients with ROSI every month, and even

seem to find what they think are round spermatids in almost all non-obstructive azoospermic cases where they do not find elongated spermatids. Yet they do not have pregnancies.

Figure 14 demonstrates the typical appearance of Sertoli cell only tubules juxtaposed to tubules with normal spermatogenesis. The abundant round-appearing cells along the basement membrane of the tubule are simply Sertoli cell nuclei with their typical prominent nucleoli. These are the "round cells" typically seen in all cases of TESE (Fig. 15). We have only found actual round spermatids when elongated forms are also present. Thus, the solution to the problem of non-obstructive azoospermia is not at present to find immature sperm cells, but rather to find the occasional foci of spermatogenesis in testes otherwise devoid of sperm. The concept of "maturing" spermatocytes is still a problem for basic research.

#### BIGGEST QUESTION

ICSI versus conventional IVF for normospermic patients? See course narrative summary for Genetics of ICSI.

**POST-COURSE QUESTIONS**

1. Regression toward the mean:
  - a. Explains the successful results of ART programs which provide for a proper andrological workup of its male patients.
  - b. Describes the poor emotional development of ICSI offspring reported in New South Whales.
  - c. Implies that the favorable results of varicocelectomy or clomid therapy only last for one year, thereafter sperm counts regress, and so ART is only indicated after a one-year trial of specific treatment of the male.
  - d. Explains mathematically why low sperm counts tend to rise with time no matter what the treatment.
2. The risk of ICSI for the fertility of future generations:
  - a. Is minimal because most cases of low sperm counts are caused by correctable diagnoses such as varicoceles.
  - b. Poses grave risks.
  - c. Depends on whether Y chromosome deletions are detected.
  - d. Is independent of Y deletion testing results.

Answers:

1. D
2. D

**REFERENCES**

1. Devroey P, Vandervorst M, Nagy P, Van Steirteghem A. Do we treat the male or his gamete? *Hum Reprod.* 1998,13 (Suppl 1):178-185.
2. Nieschlag E, Hertle L, Fishedick A, Abshagen K, Behre HM. Update on treatment of varicocele: counselling as effective as occlusion of the vena spermatica. *Hum Reprod.* 1998,13:2147-2150.
3. Nieschlag E, Hertle L, Fishedick A, Behre HM. Treatment of varicocele: counselling as effective as occlusion of the vena spermatica. *Hum Reprod.* 1995,10:347-353.
4. Baker HWG, Burger HG, deKretser DM, Hudson B, Rennie GC, Straffon WGE. Testicular vein ligation and fertility in men with varicoceles. *Brit Med J.* 1985,291:1678-1680.
5. Rodriguez-Ragui LJ, Smith KD, Steinberger E. Relationship of varicocele to sperm output and fertility of male partners in infertile couples. *J Urol,* 1978,120:691-694.
6. Silber, S.J. (2000) Varicocele dilemma. *Hum Reprod Update,* 2001,7(1):70-77.
7. Silber SJ, Nagy Z, Liu J, Tournaye H, Lissens W, Ferec C, Liebaers I, Devroey P, Van Steirteghem A. The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. *Hum Reprod.* 1995,10:2031-2043.
8. Reijo R, Lee T, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S, Jaffe T, Straus D, Hovatta O, de la Capelle A, Silber S, Page DC. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat Genet,* 1995,10:383-393.
9. Silber SJ, Alagappan R, Brown LG, Page DC. Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. *Hum Reprod.* 1998,13:3332-3337.
10. Page DC, Silber S, Brown LG. Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. *Hum Reprod.* 1999,14:1722-1726.
11. Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, Smitz J, Wisanto A, Devroey P. High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod.* 1993,8: 1061-1066.
12. Sokol RZ and Sparkes R. Demonstrated paternity in spite of oligospermia. *Fertil Steril.* 1987,47 (2):356-358.
13. Smith KD, Rodriguez-Rigau LJ, Steinberger E. Relation between indices of semen analysis and pregnancy rate in infertile couples. *Fertil Steril.* 1977, 28:1314-1319.
14. World Health Organization. WHO laboratory manual for the examination of human semen and sperm cervical mucous interaction. 3<sup>rd</sup> Edition Cambridge University Press, Cambridge, 1992, pp 44-45.
15. Barratt CLR, Naeeni M, Clements S, Cooke, ID. Clinical value of sperm morphology for in-vivo fertility: comparison between World Health Organization criteria of 1987 and 1992. *Hum Reprod.* 1995,10:587-593.

16. MacLeod J and Gold RZ. The male factor in fertility and infertility. II. Sperm counts in 1000 men of known fertility and in 1000 cases of infertile marriage. *J Urol*. 1951,66:436.
17. Rehan N, Sobrero AJ, Fertig JW. The semen of fertile men: statistical analysis of 1300 men. *Fertil Steril*. 1975,26:492-502.
18. David G, Jonannet P, Martin-Boyce A, Spira A, Schwartz D. Sperm counts in fertile and infertile men. *Fertil Steril*. 1979, 31:453-455.
19. Nelson CM, Bunge RG. Semen analysis: evidence for changing parameters of male fertility potential. *Fertil Steril*. 1974,25:503-507.
20. Zukerman Z, Rodriguez-Rigau LJ, Smith KD, Steinberger E. Frequency distribution of sperm counts in fertile and infertile males. *Fertil Steril*. 1977,28:1310-1303.
21. Silber SJ. The relationship of abnormal semen parameters to male fertility. *Opinion. Hum Reprod*. 1989,4:947-953.
22. Silber SJ. Pregnancy after vasovasostomy for vasectomy reversal: a study of factors affecting long-term return of fertility in 282 patients followed for 10 years. *Hum Reprod*. 1989,4:318-322.
23. Jouannet P, Ducot B, Feneux D, Spira A. Male factors and the likelihood of pregnancy in infertile couples. I. Study of sperm characteristics. *Intl J Androl*. 1988,11:379-394.
24. Schoysman R, Gerris J. Twelve-year follow-up study of pregnancy rates in 1291 couples with idiopathically impathically impaired male fertility. *Acta Eur Fertil*. 1983,14:51-56.
25. Baker HWG, Burger HG. Male infertility in reproductive medicine. In: Steinberger E, Frajese G, Steinberger A. eds. *Reproductive Medicine*. New York, NY: Raven, 1986:187-197.
26. Kovacs GT, Leeton JF, Matthews CD, Steigrad SJ, Saunders DM, Jones WR, Lyneham R, McMaster R. The outcome of artificial donor insemination compared to the husband's fertility status. *Clin Reprod Fertil*. 1982,1:295-299.
27. Vessey,M, Doll R, Peto R, Johnson B, Wiggins P. A long-term follow-up study of women using different methods of contraception: an interim report. *J Biosoc Sci*. 1976,8:373-427.
28. MacLeod J, Gold RZ. The male factor in fertility and infertility. VI. Semen quality and other factors in relation to ease of conception. *Fertil Steril*. 1953,4:10-33.
29. Empeire JC, Gauzere-Soumireu E, Audebert AJ. Female fertility and donor insemination. *Fertil Steril*. 1982,37:90-93.
30. Hargreave TB, Elton RA. Is conventional sperm analysis of any use? *Brit J Urol*. 1983,55:774-779.
31. Silber SJ, Nagy Z, Devroey P, Camus M, Van Steirteghem AC. The effect of female age and ovarian reserve on pregnancy rate in male infertility: treatment of azoospermia with sperm retrieval and intracytoplasmic sperm injection. *Hum Reprod*. 1997,12:2693-2700.
32. Collins JA, Rowe TC. Age of the female partner is a prognostic factor in prolonged unexplained infertility. *Fertil Steril*. 1989,52:774-779.
33. Tournaye H, Devroey P, Camus M, Staessen C, Bollen N, Smits J, Van Steirteghem AC. Comparison of in-vitro fertilization in male and tubal infertility: a 3 year survey. *Hum Reprod*. 1992,7:218-222.

34. Talbert LM, Hammond MG, Halme J, O'Rand M, Fryer JG, Ekstrom RD. Semen parameters and fertilization of human oocytes in vitro. *Fertil Steril*. 1987,48:270-277.
35. Oehninger S, Kruger T. The diagnosis of male infertility by semen quality: clinical significance of sperm morphology assessment. *Hum Reprod*. 1995,10:1037-1038.
36. Grow DR, Oehninger S, Seltman HJ, Toner JP, Swanson RJ, Kruger TF, Muasher SJ. Sperm morphology is diagnosed by strict criteria: probing the impact of teratozoospermia on fertilization rate and pregnancy outcome in a large in vitro fertilization population. *Fertil Steril*. 1994,62:559-567.
37. Kruger TF, Menkveld R, Stander FSH, Lombard JP, van Zyl JA. Sperm morphologic features as a prognostic factor in in-vitro fertilization. *Fertil Steril*. 1986,46:1118-1123.
38. Comhair FH, deKretser D, Farley TMM, Rowe PJ. Towards more objectivity and diagnosis and management of male infertility. *Intl J Androl*. 1987,10:1-53.
39. Eliasson, R. Standards for investigation of human sperm. *Andrologie*. 1971,3:49-64.
40. Freund M. Standards for the rating of human sperm morphology: a cooperative study. *Intl J Fertil*. 1966,11:97-118.
41. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Veeck LL, Morshedi M, Brugo S. New method of evaluating sperm morphology with predictive value for human in vitro fertilization. *Urol*. 1987,30:248-251.
42. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertil Steril*. 1988,49:112-117.
43. Menkveld R, Stander FS, Kotze TJ, Kruger TF, van Zyl JA. The evaluation of morphology characteristics of human spermatozoa according to stricter criteria. *Hum Reprod*. 1990,5:586-592.
44. Davis RO, Gravance CG. Consistency of sperm morphology classification criteria. *J Androl*. 1993,15:88-91.
45. Liu DY, Baker HWG. Morphology in spermatozoa bound to the zona pellucida of human oocytes that failed to fertilize in vitro. *J Fertil Reprod*. 1992,94:71-84.
46. Liu DY, Baker HWG. Tests of human sperm function and fertilization in vitro. *Fertil Steril*. 1992,58:465-483.
47. Liu DY, Baker HWG. Sperm nuclear chromaton normality: relationship with sperm morphology, sperm-zona pellucida binding and fertilization rates in vitro. *Fertil Steril*. 1992,58:1178-1184.
48. Liu DY, Du Plessis YP, Nayudu PL, Johnston WIH, Baker HWG. The use of in vitro fertilization to evaluate putative tests of human sperm function. *Fertil Steril*. 1988,49:272-277.
49. Liu DY, Baker HWG. A simple method for assessment of the human acrosome reaction of spermatozoa bound to the zona pellucida: lack of relationship with ionophore A23187-induced acrosome reaction. *Hum Reprod*. 1996,11:551-557.
50. Liu DY, Baker HWG. A new test for the assessment of sperm zona pellucida penetration relationship with results of other sperm tests and fertilization in vitro. *Hum Reprod*. 1994,9:489-496.

51. Zaneveld LJD, Leyendran RS. Sperm function tests. *Infertil Reprod Med Clin North Am.* 1992,3:353-371.
52. Baker HWG, Liu DY, Bourne H, Lopata A. (1993) Diagnosis of sperm defects in selecting patients for assisted fertilization. *Human Reproduction*, 8:1779-1780.
53. Liu DY, Baker HWG. Tests of sperm function. *Fertil Steril.* 1993,59:698-699.
54. Baker HWG. Management of immunological infertility. And an approach to clinical andrology. Berger HG, Oshima H, eds. *Serona Symposia Reviews.* 1993,29:105-110.
55. Baker G. Editorial comment: The use of the semen analysis in predicting fertility outcome. *Australian and New Zealand Journal of Obstetrics and Gynecology*, 1992,32:154-155.
56. Clarke GN, Baker HWG. Detection of sperm antibodies using the immunobead test (IBT). *Aust J Med Sci.* 1988,9:66-70.
57. Vawda AI, Gumbly J, Younglai EV. Semen parameters as predictors of in vitro fertilization: the importance of strict criteria sperm morphology. *Hum Reprod.* 1996,11:1445-1450.
58. ESHRE Andrology Special Interest Group. Consensus workshop on advanced diagnostic andrology techniques. *Hum Reprod.* 1996,11:1463-1479.
59. Duncan WW, Flaherty S, Glew MJ, Wang XJ, Matthews CD. Prediction of in-vitro fertilization rates from semen variables. *Fertil Steril.* 1993,59:1233-1238.
60. Hargreave TB. Varicocele: a clinical enigma. Review article, *Brit J Urol.* 1993,72:401-408.
61. O'Donovan PA, Vandekerckhove P, Lilford RJ, Hughes E. Treatment of male infertility: is it effective? Review and Meta Analysis of Published Randomized Control Trials. *Hum Reprod.* 1993,8:1209-1222.
62. Dunphy BC, Kay R, Barratt CLR, Cooke ID. Is routine examination of the male partner of any prognostic value in routine assessment of couples who complain of involuntary infertility? *Fertil Steril.* 1989,52:454-456.
63. Baker HWG. Requirements for controlled therapeutic trials in male infertility. *Clin Reprod Fertil.* 1986,4:13-25.
64. Nilsson S, Edvinsson A, Nilsson B. Improvement of semen and pregnancy rate after ligation and division of the internal spermatic vein: fact or fiction? *Brit J Urol.* 1979,51:591-596.
65. Thomason M, Farris BL. The prevalence of varicocele in a group of healthy young men. *Milit Med*, 1979,144:181-186.
66. Uehling DT. Fertility in men with varicocele. *Intl J Fertil.* 1968,13:58-60.
67. Vermeulen A, Vandeweghe M, Deslypere JP. Prognosis of subfertility in men with corrected or uncorrected varicocele. *J Androl.* 1986,7:147-155.
68. Marmar JL, Kim Y. Subinguinal microsurgical varicocelectomy: A technical critique and statistical analysis of semen and pregnancy data. *J Urol.* 1994,152:1127-1132.
69. Girardi SK, Goldstein M. Varicocele. *Current Therapy in Endocrinology & Metabolism*, 1997,6:355-0358.
70. Madjar I, Weissenberg R, Lunenfeld B, Karasik A, Goldwasser B. Controlled trial of high spermatic vein ligation for varicocele in infertile men. *Fertil Steril.* 1995,63:120-124.

71. Baker HWG, Burger HG, de Kretser DM, Lording DW, McGowan P, Rennie GC. Factors affecting the variability of semen analysis results in infertile men. *Intl J Androl.* 1981,4:609-622.
72. Baker HWG, Straffon WGE, McGowan MP, Burger HG, de Kretser DM, Hudson B. A controlled trial of the use of erythromycin for men with asthenospermia. *Intl J Androl.* 1984,7:383-388.
73. Baker HWG, Kovacs GT. Spontaneous improvement in semen quality: regression towards the mean. *Intl J Androl.* 1985,8:421-426.
74. Devroey P. The relevance of semen analysis. Presented at Thirty-Second Annual Postgraduate Program of the American Society for Reproductive Medicine in Toronto, Canada in September 1999, 15-32.
75. Palermo G, Joris H, Devroey P, Van Steirteghem A. Pregnancies after intracytoplasmic injection of a single spermatozoa into an oocyte. *Lancet* 1992;3:17-18.
76. Silber SJ. Intracytoplasmic sperm injection (ICSI) today: a personal review. *Hum Reprod.* 1998,13:208-218.
77. Nagy ZP, Liu J, Joris H, Verheyen G, Tournaye H, Camus M, Derde MC, Devroey P, Van Steirteghem AC. The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters. *Hum Reprod.* 1995,10:1123-1129.
78. Silber SJ, Nagy ZP, Liu J, Godoy H, Devroey P, Van Steirteghem AC. Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. *Hum Reprod.* 1994,9:1705-1709.
79. Silber SJ, Van Steirteghem AC, Liu J, Nagy Z, Tournaye H, Devroey P. High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. *Hum Reprod.* 1995,10:148-152.
80. Silber SJ, Van Steirteghem A, Nagy Z, Liu J, Tournaye H, Devroey P. Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. *Fertil Steril.* 1996,66:110-117.
81. Devroey P, Liu J, Nagy Z, Tournaye H, Silber SJ, Van Steirteghem AC. Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection. *Fertil Steril.* 1994,62:639-641.
82. Devroey P, Silber S, Nagy Z, Liu J, Tournaye H, Joris H, Verheyen G, Van Steirteghem A. Ongoing pregnancies and birth after intracytoplasmic sperm injection with frozen—thawed epididymal spermatozoa. *Hum Reprod.* 1995,10:903-906.
83. Devroey P, Liu J, Nagy Z, Goossens A, Tournaye H, Camus M, Van Steirteghem A, Silber S. Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum Reprod.* 1995,10:1457-1460.
84. Mosher W. Infertility: why business is booming. *Am. Demograph.* July 1987:42-43.
85. Mosher WD. Fecundity and infertility in the United States 1965-1982. *Adv. Data.* 1985, 1:1.
86. Hull MGR, Glazener CMA, Kelly MJ, Conway DI, Foster PA, Hinton RA, Coulsom C, Lambert PA, Watt EM, Desai KM. Population study of causes treatment and outcome of infertility. *Brit Med J.* 1985,291:1693-1697.

87. Silber SJ. Vasectomy. In: Knobil E, ed. *Encyclopedia of Reproduction*. San Diego/London/Boston/New York/Sydney/Tokyo/Toronto: Academic Press. 1999,4:977-985.
88. Silber SJ. Pregnancy after vasovasostomy for vasectomy reversal: a study of factors affecting long-term return of fertility in 282 patients followed for 10 years. *Hum Reprod*. 1989,4:318-322.
89. Silber SJ. Results of microsurgical vasoepididymostomy: role of epididymis in sperm maturation. *Hum Reprod*. 1989,4:298-303.
90. Silber SJ. *Microsurgery*. Baltimore, MD: The Williams & Wilkins Company, Waverly Press, Inc., 1979.
91. Silber SJ. *Reproductive Infertility Microsurgery in the Male and Female*. Baltimore, MD: Williams & Wilkins, Waverly Press, Inc., 1984.
92. Silber SJ. Microscopic vasoepididymostomy: specific microanastomosis to the epididymal tubule. *Fertil Steril*. 1978,30:565-571.
93. Silber SJ. Microscopic technique for reversal of vasectomy. *Surgery, Gynecology & Obstetrics*, 1976,143:630-631.
94. Silber SJ. Perfect anatomical reconstruction of vas deferens with a new microscopic surgical technique. *Fertil Steril*. 1977,28:72-77.
95. Silber SJ. Microscopic vasectomy reversal. *Fertil Steril*. 1977,28:1191-1202.
96. Silber SJ. Vasectomy and vasectomy reversal. *Modern Trends*. 1978,29:125-140.
97. Silber SJ. Sperm granuloma and reversibility of vasectomy. *The Lancet*. 1977:588-589.
98. Silber SJ. Vasoepididymostomy to the head of the epididymis: recovery of normal spermatozoal motility. *Fertil Steril*. 1980,34:149-154.
99. Silber SJ. Epididymal extravasation following vasectomy as a cause for failure of vasectomy reversal. *Fertil Steril*. 1979,31:309-316.
100. Silber SJ. Ejaculatory duct obstruction. *J Urol*. 1980,124:294-297.
101. Silber SJ. Congenital absence of the vas deferens. *N Engl J Med*. 1990,323:1788-1792.
102. DeKretser DM, Burger HG, Hudson B. The relationship between germinal cells and serum FSH levels in males with infertility. *J Clin Endocrinol Metab*. 1974,38:787.
103. Silber SJ, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC. 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.
104. Silber SJ. Microsurgical testicular sperm extraction and the distribution of spermatogenesis in non-obstructive azoospermia. *Hum Reprod*. 2000,15:2278-2284.
105. Silber SJ, Rodriguez-Rigau LJ. Quantitative analysis of testicle biopsy: determination of partial obstruction and prediction of sperm count after surgery for obstruction. *Fertil Steril*. 1981,36:480-485.
106. Silber SJ, Patrizio P, Asch RH. Quantitative evaluation of spermatogenesis by testicular histology in men with congenital absence of the vas deferens undergoing epididymal sperm aspiration. *Hum Reprod*. 1990,5:89-93.
107. Charny CW. Testicular biopsy: Its value in male sterility. *JAMA*. 1940,115:1429.
108. Nelson WO. Interpretation of testicular biopsy. *JAMA*. 1953,151:1449.

109. Mannion RA, Cottrell TLC. Correlation between testicular biopsy and sperm count. *J Urol.* 1961,85:953.
110. Albert A. The mammalian testis. In: Young WC. ed. *Sex and Secretions*, ed 3. Baltimore, MD: Williams & Wilkins, 1961:305-365.
111. Heller CG, Clermont Y. Kinetics of the germinal epithelium in man. *Recent Prog Horm Res.* 1964,20:545.
112. Silber SJ. Vasectomy and its microsurgical reversal. *Urol Clin N Amer*, 1978,5:573-584.
113. Shapiro EI, Silber SJ. Open-ended vasectomy, sperm granuloma, and postvasectomy orchialgia. *Fertil Steril.* 1979,32:546-550.
114. Silber SJ, Devroey P, Tournaye H, Van Steirteghem AC. Fertilizing capacity of epididymal and testicular sperm using intracytoplasmic injection (ICSI). *Reprod Fertil Dev*, 1995,7:281-293.
115. Silber SJ, Galle J, Friend D. Microscopic vasovasostomy and spermatogenesis. *J Urol.* 1977,117:299.
116. Silber SJ, Crudop J. Kidney transplantation in inbred rats. *Am J Surg*, 1973,125:551.
117. Silber SJ, Crudop J. A three kidney rat model. *Invest Urol*, 1974,11:466.
118. Silber SJ, Malvin RL. Compensatory and obligatory renal growth in rats. *Am J Physiol*, 1974,226:114.
119. Silber SJ. Growth of baby kidneys transplanted into adults. *Arch Surg.* 1976,111:75.
120. Silber SJ. Transplantation of rat kidneys with acute tubular necrosis into salt-loaded and normal recipients. *Surgery.* 1975,77:487.
121. Silber SJ. Successful autotransplantation of an intra-abdominal testicle to the scrotum using microvascular anastomosis. *J Urol.* 1976,115:452.
122. Silber SJ. Compensatory and obligatory renal growth in babies and adults. *Aust N Z J Surg*, 1974,44:421.
123. Silber SJ. Reversal of vasectomy in the treatment of male infertility. *J Androl.* 1980,1:261.
124. Silber SJ, Nagy Z, Liu J, Tournaye H, Lissens W, Ferec C, Liebaers I, Devroey P and Van Steirteghem AC. The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. *Hum Reprod* 1995,10:2031-2043.
125. Silber SJ. Reversal of vasectomy in the treatment of male infertility: Role of microsurgery, vasoeptidiymostomy, and pressure-induced changes of vasectomy. *Urol Clin N Am.* 1981,8:53.
126. Tournaye H, Devroey P, Liu J, Nagy Z, Lissens W, Van Steirteghem A. Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. *Fertil Steril.* 1994,61:1045-1051.
127. Steinberger E, Tjioe DY. A method for quantitative analysis of human seminiferous epithelium. *Hum Rreprod.* 1968,19:960-970.
128. Zukerman Z, Rodriguez-Rigau L, Weiss DB, Chowdhury LJ, Smith KD, Steinberger E. Quantitative analysis of the seminiferous epithelium in human testicle biopsies and the relation to spermatogenesis to sperm density. *Fertil Steril.* 30:448-455.

129. Clermont Y. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycles in spermatogonial renewal. *Physiological Reviews*. 52:198-236.
130. Tournaye H, Liu J, Nagy PZ, Camus M, Goossens A, Silber S, Van Steirteghem AC, Devroey P. Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa. *Hum Reprod*. 1996,11:127-132.
131. Tournaye H, Verheyen G, Nagy P, Ubaldi F, Goossens A, Silber S, Van Steirteghem AC, Devroey P. Are there any predictive factors for successful testicular sperm recovery in azoospermic patients? *Hum Reprod*. 1997,12:80-86.
132. Craft I, Tsirigotis M, Courtauld E, Farrer-Brown G. Testicular needle aspiration as an alternative to biopsy for the assessment of spermatogenesis. *Hum Reprod*. 1997,12:1483-1487.
133. Friedler S, Raziel A, Strassburger D, Soffer Y, Komarovsky D, Ron-el R. Testicular sperm retrieval by percutaneous fine needle sperm aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. *Hum Reprod*. 1997,12:1488-1491.
134. Rosenlund B, Kvist U, Ploen L, Rozell BL, Sjoblom P, Hillensjo T. A comparison between open and percutaneous needle biopsies in men with azoospermia. *Hum Reprod*. 1998,13:1266-1271.
135. Silber SJ, Johnson L. Are spermatid injections of any clinical value? ROSNI and ROSI revisited. *Hum Reprod*. 1998,13:509-523.
136. Silber SJ, Johnson L, Verheyen G, Van Steirteghem A. Round spermatid injection. *Fertil Steril*. 2000,73:897-900.
137. Holstein AF, Roosen-Runge ED, editors. *Atlas of human spermatogenesis*. Berlin: Grosse Verlag, 1981.
138. Chang PL, Sauer MV, Brown S. Y chromosome microdeletion in a father and his infertile sons. *Hum Reprod* 1999, 14:2689-2694.
139. Hull MGR, Glazener CMA, Kelly MJ et al. Population study of causes, treatment and outcome of infertility. *Brit Med J* 1985, 291:193-197.
140. Page DC, Silber S, Brown LG. Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. *Hum Reprod* 1999, 14:1722-1726.
141. Silber SJ. Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. *Hum Reprod* 2000, 15:2278-2284.
142. Wang PJ, McCarrey JR, Yang F and Page DC. An abundance of X-linked genes expressed in spermatogonia. *Nature Genetics* 2001; in press.
143. Faddy MJ, Silber SJ and Gosden RG. Intra-cytoplasmic sperm injection and infertility. *Nature Genetics* 2001, 29:131.
144. Mosher W. Infertility: why business is booming. *Am Demograph*, July 1987, 42-43.
145. Mosher WD. Fecundity and infertility in the United States 1965-1982. *Adv. Data*, 1985, 1:1.
146. Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Silber S, Oates R, Rozen S and Page DC. The AZFc region of the Y

- chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nature Genetics* 2001, 29,279-286.
147. Silber SJ, Repping S. Transmission of male infertility to future generations: lessons from the Y chromosome. *Hum Reprod*. In press.
  148. Verheyen G, Crabbe E, Joris H and Van Steirteghem A. Simple and reliable identification of the human round spermatid by inverted phase contrast microscopy. *Hum Reprod* 1998, 13:1570-1577.
  149. Silber SJ, Verheyen G and Van Steirteghem AC. Letter to the Editor: Spermatid conception. *Hum Reprod* 1998, 13:2976-77.
  150. Tesarik J. Letter to the Editor: Spermatid conception. *Hum Reprod* 1998, 13:2977-2979.
  151. Johnson L, Petty CS and Neaves W. A new approach to quantification of spermatogenesis and its application to germinal attrition during human spermiogenesis. *Biol Reprod* 1981, 25:217-226.
  152. Johnson L, Chaturvedi P and Williams JD. Missing generations of spermatocytes and spermatids in seminiferous epithelium contribute to low efficiency of spermatogenesis in humans. *Biol Reprod* 1992, 47:1091-1098.
  153. Schulze W, Hohenberg H and Knuth UA. Cryopreservation of testicular tissue: a highly effective method to provide sperm for successful TESE/ICSI procedures. *Fertil and Reprod Med* 1998, 621-626.
  154. Soderstrom K-O and Suominen J. Histopathology and ultrastructure of meiotic arrest in human spermatogenesis. *Arch Pathol Lab Med* 1980, 104:476-482.
  155. Nantel F, Monaco L, Foulkes NS, Masquillier D, LeMuer M, Henriksen K et al. Spermiogenesis deficiency and germ-cell apoptosis in CREM-mutant mice. *Nature* 1996, 380:159-165.
  156. Blendy JA, Kaestner KH, Weinbauer GF, Nieschlag E and Schutz G. Severe impairment of spermatogenesis in mice lacking the CREM gene. *Nature* 1996, 380:162-165.
  157. Brandell RA, Mielnik A, Liotta D, Ye Z, Veeck LL, Palermo GD et al. AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test. *Hum Reprod* 1998, 13:2812-2815.
  158. Antinori S, Versaci C, Dani G, Antinori M, Possa L and Selman HA. Fertilization with human testicular spermatids: four successful pregnancies. *Hum Reprod* 1997, 12:286-291.
  159. Barak Y, Kogosowski A, Goldman S, Soffer Y, Gonen Y and Tesarik J. Pregnancy and birth after transfer of embryos that developed from single-nucleated zygotes obtained by injection of round spermatids into oocytes. *Fertil Steril* 1998, 70:67-70.
  160. Fishel S, Green S, Bishop M, Thorton S, Hunter S, Fleming S et al. Pregnancy after intracytoplasmic injection of spermatids. *Lancet* 1995, 345:1641-1642.
  161. Tesarik J, Mendoza C, Testart J. Viable embryos from injection of round spermatids into oocytes. *N Engl J Med* 1995, 333:525.
  162. Tesarik J, Greco E and Mendoza C. ROSI, instructions for use: 1997 update. *Hum Reprod* 1998, 13:519-523.
  163. Amer M, Soliman E, El-Sadek M, Mendoza C and Tesarik J. Is complete spermiogenesis failure a good indication for spermatid conception? *Lancet* 1997, 350:116.

164. Ghazzawi I, Taher M and Sousa S. Pregnancies after round spermatid injection; a virtual reality! *Hum Reprod* 1998, 13, Abstract Book 1 (O-180), 46-47.
165. Ghunaim S, Shaban MA, Dakkak A, Tell B, Keilani S and Keilani Z. The outcome of round spermatid injection in assisted reproduction. *Hum Reprod* 1998, 13, Abstract Book 1 (O-094), 90-91.
166. Ogura A and Yanagimachi R. Round spermatid nuclei injected into hamster oocytes form pronuclei and participate in syngamy. *Biol Reprod* 1993, 48:219-225.
167. Ogura A, Yanagimachi R and Usui N. Behaviour of hamster and mouse round spermatid nuclei incorporated into mature oocytes by electrofusion. *Zygote* 1993, 1:1-8.
168. Ogura A, Matsuda J and Yanagimachi R. Birth of normal young after electrofusion of mouse oocytes with round spermatids. *Proc Natl Acad Sci USA* 1994, 91:7460-7462.
169. Pousette A, Leijonhufvud P, Arver S, Kvist U, Pelttari J and Hoog C. Presence of synaptonemal complex protein 1 transversal filament-like protein in human primary spermatocytes. *Hum Reprod* 1997, 12:2414-2417.

**TABLE 1.** Frequency distribution of sperm counts in 1000 fertile men and 1000 infertile men (MacLeod and Gold, 1951)

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

**TABLE 2.** Frequency distribution of sperm counts in 190 fertile men and in 2889 infertile men (David *et al.*, 1979)

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

**TABLE 3.** Frequency distribution of motile sperm count and pregnancy rates following vasovasostomy in men whose wives did nor did not become pregnant (10-year follow/up) (Silber, 1989)

Total motile sperm count (10 <sup>6</sup> /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%

**TABLE 4.** Pregnancy rates in 1291 oligozoospermic men (Schoysman and Gerris, 1983)

Motile sperm count (10 <sup>6</sup> /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

**TABLE 5.** Percentage chance of conception for the next year (Wife with normal investigation results) (Hargreave and Elton, 1983)

	<i>Motile density</i>	<i>Duration of infertility (months)</i>			
		12	24	48	96
Azoospermia		0	0	0	0
Sperm present	0	0	0	0	0
(millions of motile sperm/ml)	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

**TABLE 6.** Results of intracytoplasmic sperm injection (ICSI) using ejaculated spermatozoa. Results are categorized according to sperm quality

	No. of cycles	2 PN (%)	Transfer (%)	Clinical pregnancies (%)
<b>Sperm count (total)</b>				
'0'	57	58	86	25
>0 to 1 x 10 <sup>6</sup>	97	64	96	26
>1 to 5 x 10 <sup>6</sup>	128	70	96	22
>5 x 10 <sup>6</sup>	684	71	93	30
<b>Motility (%)</b>				
0 <sup>a</sup>	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
<b>Morphology</b>				
0	48	68	88	31
>1 to 3	125	70	96	33
>4 to 13	307	71	94	26
>14	203	75	95	29

<sup>a</sup>Nagy *et al.* (1995).

2 PN = oocytes that had two pronuclei..

**TABLE 7.** Transmission of severe male infertility to future generations via ICSI - mathematical assumptions

---

1.	theta = 0.5:	after 10 generations there will be 2% infertile (limit)	
2.	theta = 0.75:	after 2 generations there will be 2% infertile	
		4	3%
		20	4% (limit)
3.	theta = 0.9:	after 2 generations there will be 2.5% infertile	
		6	5%
		13	7.5%
		80	10% (limit)

Faddy MJ, Silber SJ and Gosden RG (2001)



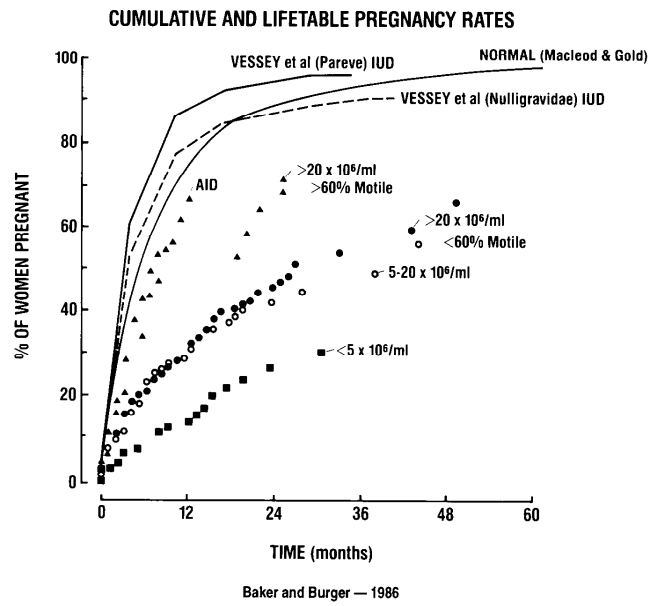
## FIGURES

- Figure 1 Cumulative and lifetable pregnancy rates in couples with varying sperm counts. (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).
- Figure 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, pregnancies occurred. Although some patients were followed up for longer than five years (those in before ligation group for maximum of 92 months, after ligation group for 108 months), 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 British Medical Journal. 1985,291:1678-1680).
- Figure 3 Life table analysis of pregnancy rate in patients with varicocele who undergo counseling only, and who undergo varicocelectomy, and showing no difference in pregnancy rate. (From Nieschlag E, Hertle L, Fishedick A, Abshagen K, Behre HM. Update on treatment of varicocele: counselling as effective as occlusion of the vena spermatica. Hum Reprod. 1998,13:2147-2150; Nieschlag E, Hertle L, Fishedick A, Behre HM. Treatment of varicocele: counselling as effective as occlusion of the vena spermatica. Hum Reprod. 1995,10:347-353).
- Figure 4 A depiction of microsurgical epididymal sperm aspiration (MESA) 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 physiological 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).
- Figure 5 This is a histologic section of testicle biopsy in a patient with Sertoli cell only, elevated FSH, and occasional tubules with normal spermatogenesis. Upper right-hand tubule exhibits normal spermatogenesis, but all of the other tubules are Sertolicell only (From Silber SJ, Johnson L, Verheyen G, Van Steirteghem A. Round spermatid injection. Fertil Steril. 2000,73:897-900).

- Figure 6 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 in order for sperm to appear in the ejaculate. (From Silber SJ, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC. 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).leakage and no stricturing.) (From Silber SJ. Microscopic technique for reversal of vasectomy. *Surgery, Gynecology & Obstetrics*, 1976,143:630-631).
- Figure 7 Drawings of the stages of spermiogenesis after the second meiotic division has occurred. Prior to 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. (From Holstein AF, Roosen-Runge ED, editors. *Atlas of human spermatogenesis*. Berlin: Grosse Verlag, 1981).
- Figure 8 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. (From Holstein AF, Roosen-Runge ED, editors. *Atlas of human spermatogenesis*. Berlin: Grosse Verlag, 1981).
- Figure 9 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 testicular sperm extraction and the distribution of spermatogenesis in non-obstructive azoospermia. *Hum Reprod.* 2000, 15:2278-2284).
- Figure 10 Various “degrees” of azoospermia. Normal spermatogenesis (center drawing) is associated with obstructive azoospermia. With non-obstructive azoospermia, TESE may be easy as in the drawing depicted on the left, or very difficult as depicted in the drawing on the right.
- Figure 11 Predicted incidence of male infertility in future years (by generation) after successfully treating varying percentages of patients with ICSI to overcome severe oligospermia or azoospermia. (From Faddy MJ, Silber SJ and Gosden RG. Intra-cytoplasmic sperm injection and infertility. *Nature Genetics* 2001, 29:131).
- Figure 12 Predicted future male infertility if 50% are successfully treated. (From Faddy MJ, Silber SJ and Gosden RG. Intra-cytoplasmic sperm injection and infertility. *Nature Genetics* 2001, 29:131).

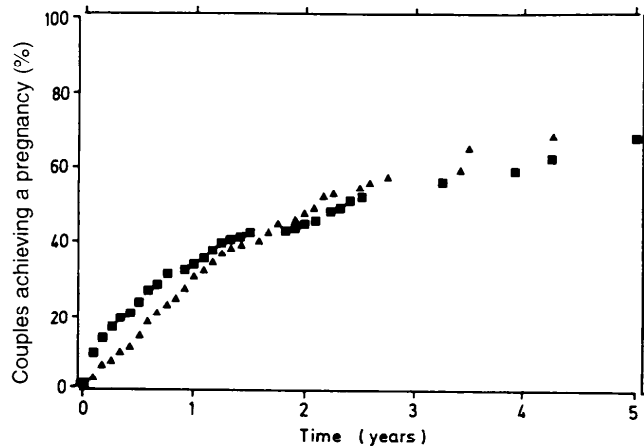
- Figure 13 Predicted future male infertility if 100% are successfully treated. (From Faddy MJ, Silber SJ and Gosden RG. Intra-cytoplasmic sperm injection and infertility. *Nature Genetics* 2001, 29:131).
- Figure 14 A tubule with normal spermatogenesis within a testes that is otherwise Sertoli cell only at 400x, hematoxylin and eosin (H&E). Note that each Sertoli cell nucleus at the base of the tubule is the same "cell" that appears like a "round cell" on the TESE wet prep. (From Silber SJ, Johnson L, Verheyen G, Van Steirteghem A. Round spermatid injection. *Fertil Steril*. 2000,73:897-900.)
- Figure 15 Sertoli cell only viewed under Hoffman optics. Arrows point to Sertoli cell nucleus, not a round spermatid. (From Silber SJ, Verheyen G and Van Steirteghem AC. Letter to the Editor: Spermatid conception. *Hum Reprod* 1998, 13:2976-77.)
- Figure 16 Dispersed, live, human testicular cells as observed by Nomarski optics. Sertoli cell nuclei (SC) have a non-spherical shape although some profiles appear to be spherical. Sertoli cell cytoplasm is granular in appearance (open arrow). Germ cells with spherical nuclei include primary spermatocytes (PS) and round spermatids (RS). Testicular sperm (TS) are detached from other cells. Occasionally, degenerating germ cells (primary spermatocytes; DP) are observed in culture. Bar length equals 10  $\mu$ m. (From Johnson, L., Texas A & M University). (From Silber SJ, Johnson L, Verheyen G, Van Steirteghem A. Round spermatid injection. *Fertil Steril*. 2000,73:897-900.)

FIGURE 1



**FIGURE 2**

Before						
ligation	611	132	61	25	14	7
After						
ligation	283	133	53	23	14	9



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, pregnancies occurred.<sup>12</sup> Although some patients were followed up for longer than five years (those in before ligation group for maximum of 92 months, after ligation group for 108 months), the longest duration of follow up to pregnancy was 60 months. There was no significant difference between the two curves by log rank test.

FIGURE 3

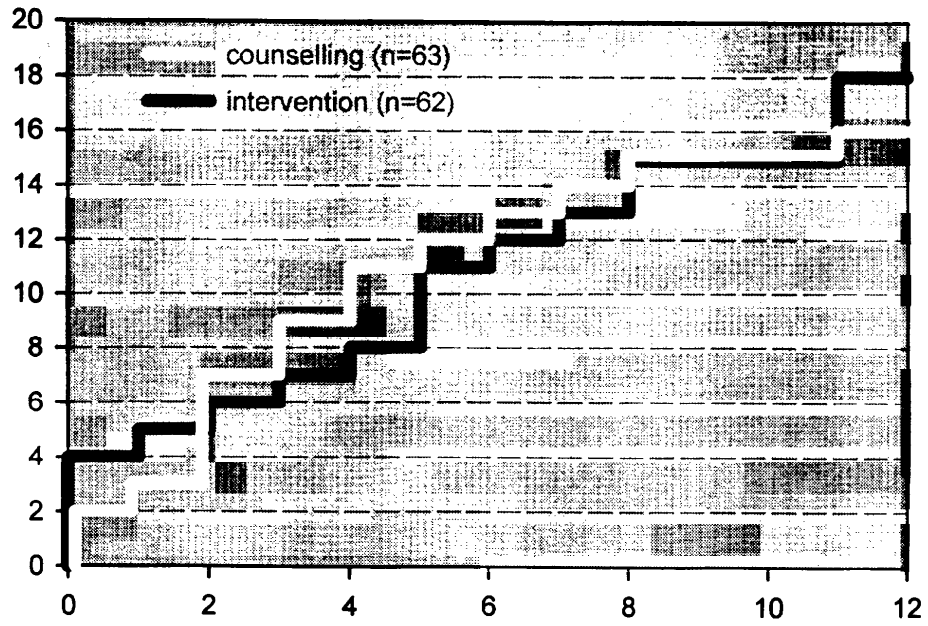


FIGURE 4

**Quantitative Testicle Biopsy and Sperm Count**

**Number of mature spermatids per tubule**

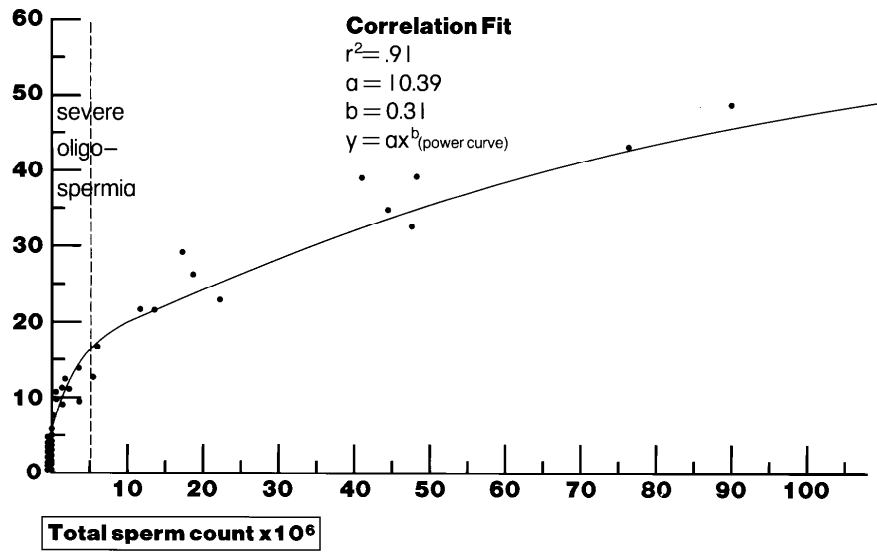
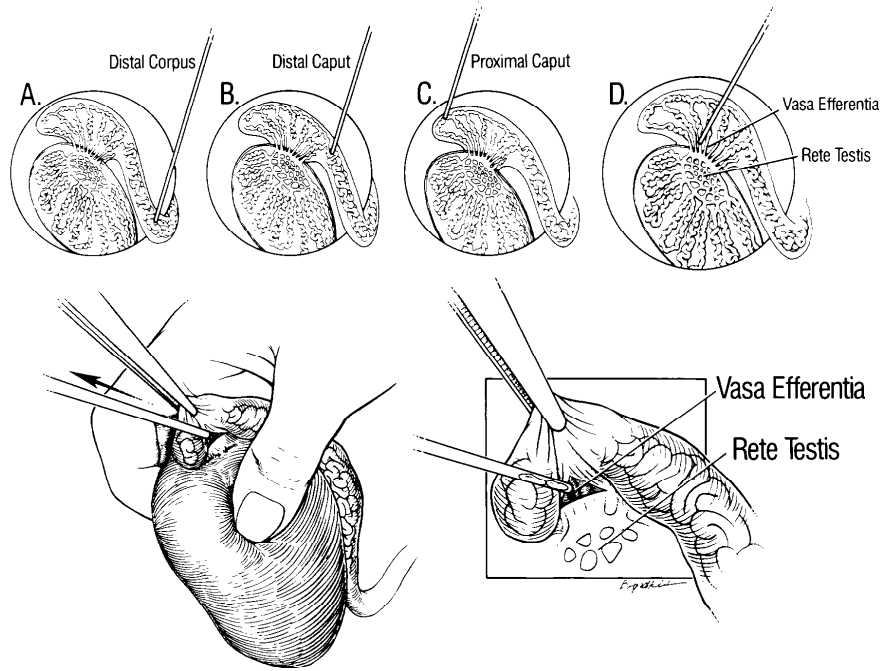


FIGURE 5



**FIGURE 6**

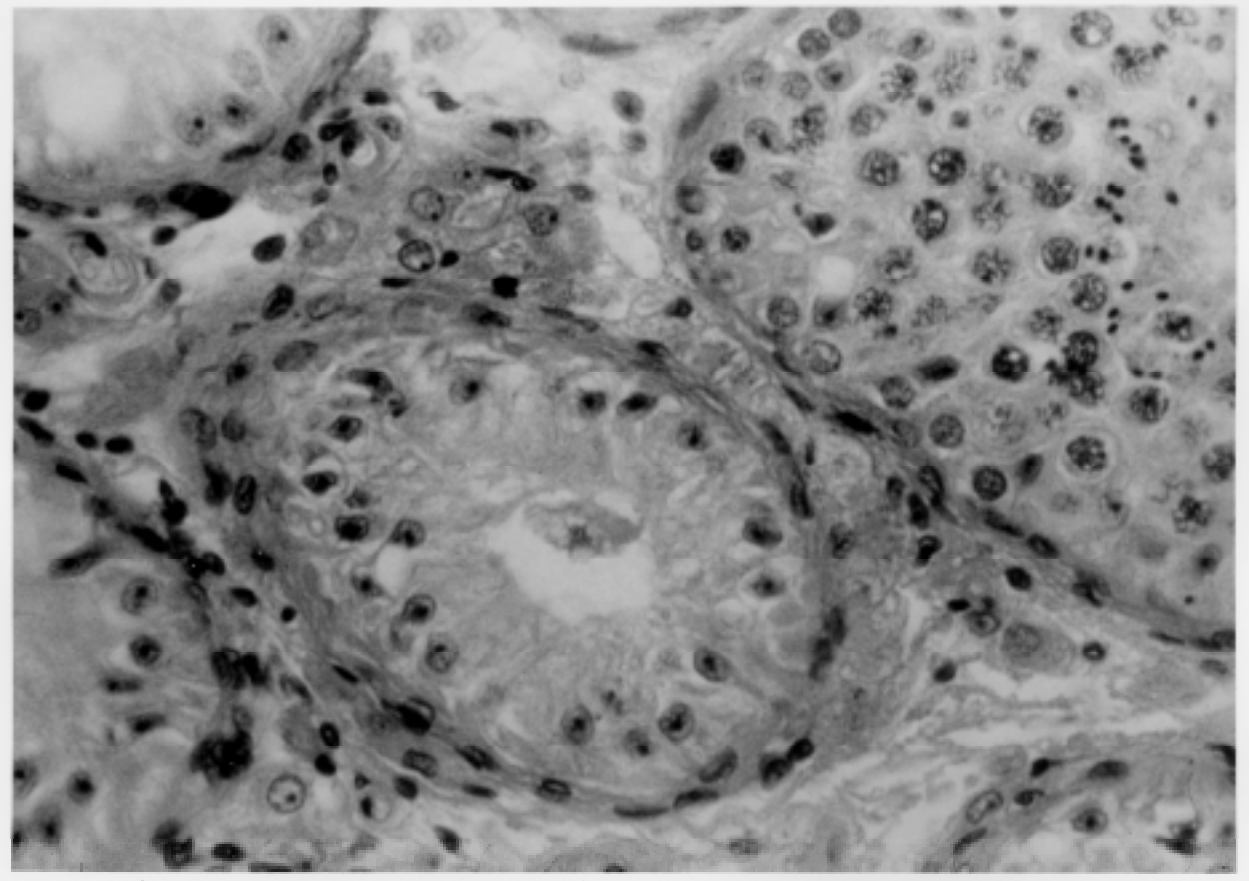
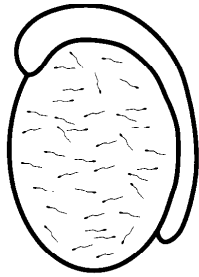
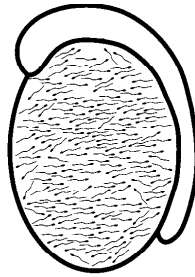


FIGURE 7

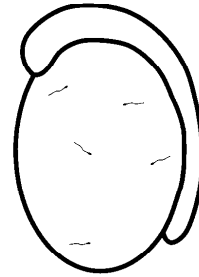
## Degrees of Azoospermia



Non-Obstructive Azoospermia  
(One in 20 tubules have sperm)

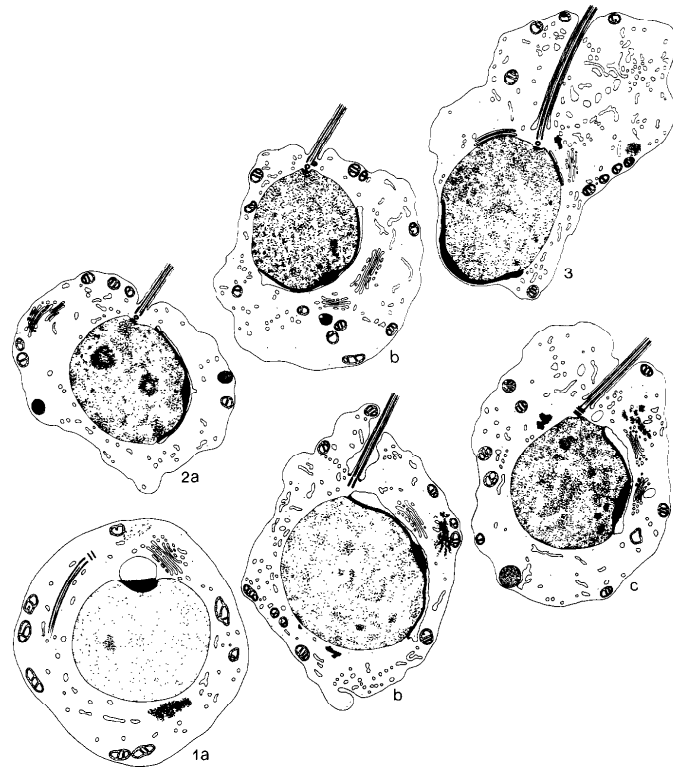


Normal Spermatogenesis  
(All tubules have sperm)

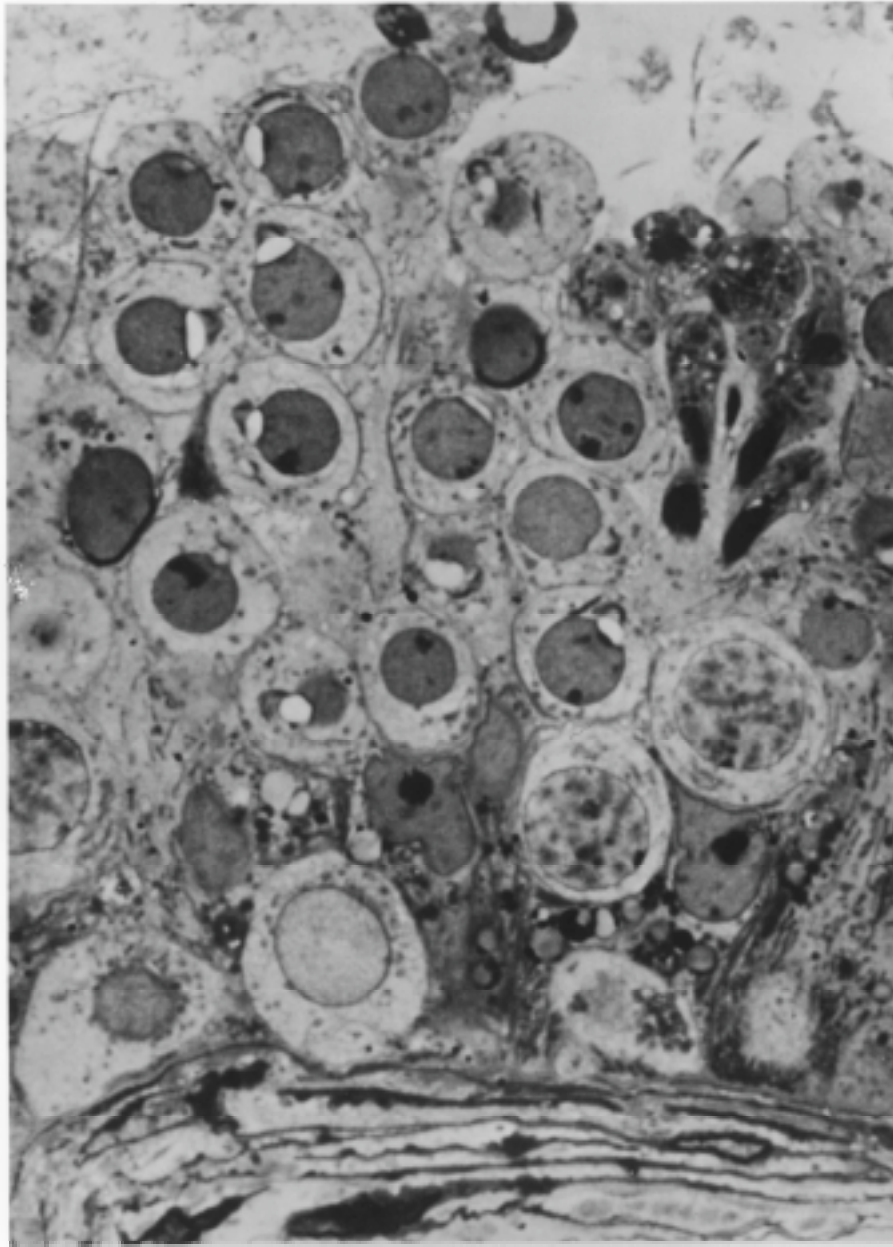


Non-Obstructive Azoospermia  
(One in 100 tubules have sperm)

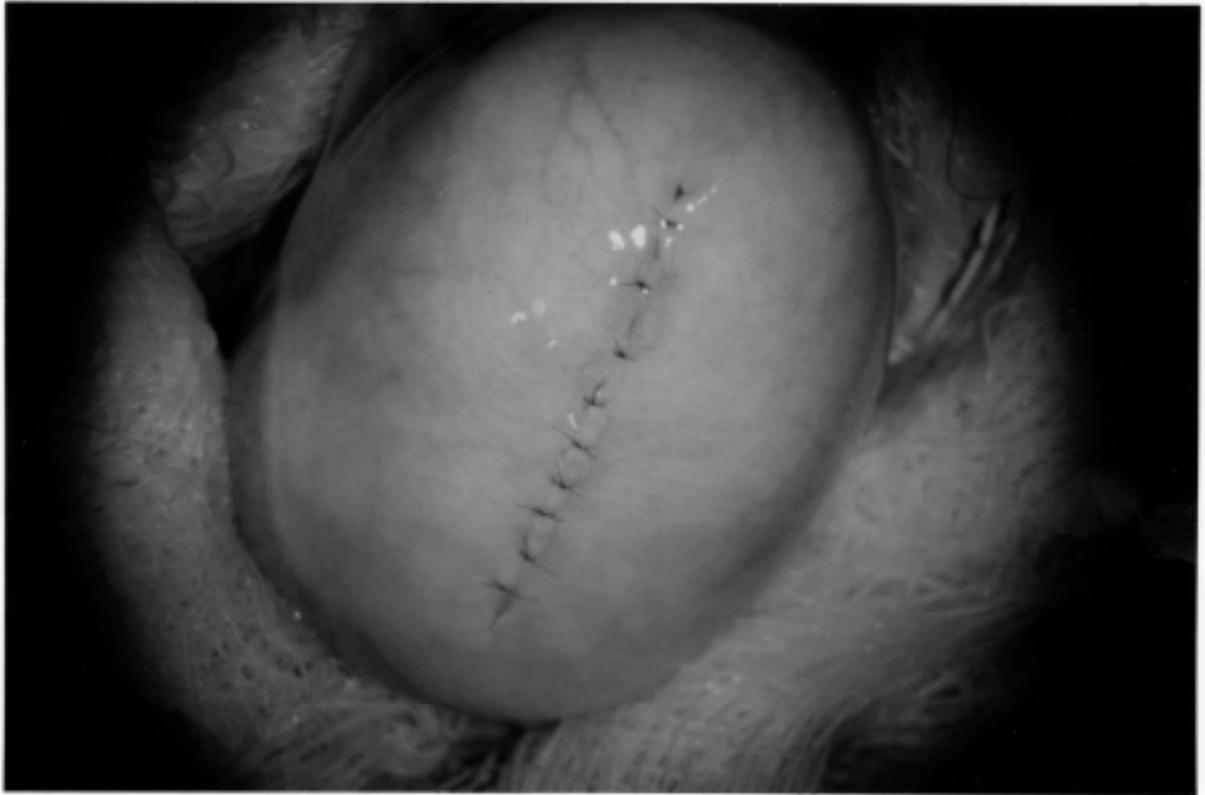
FIGURE 8



**FIGURE 9**

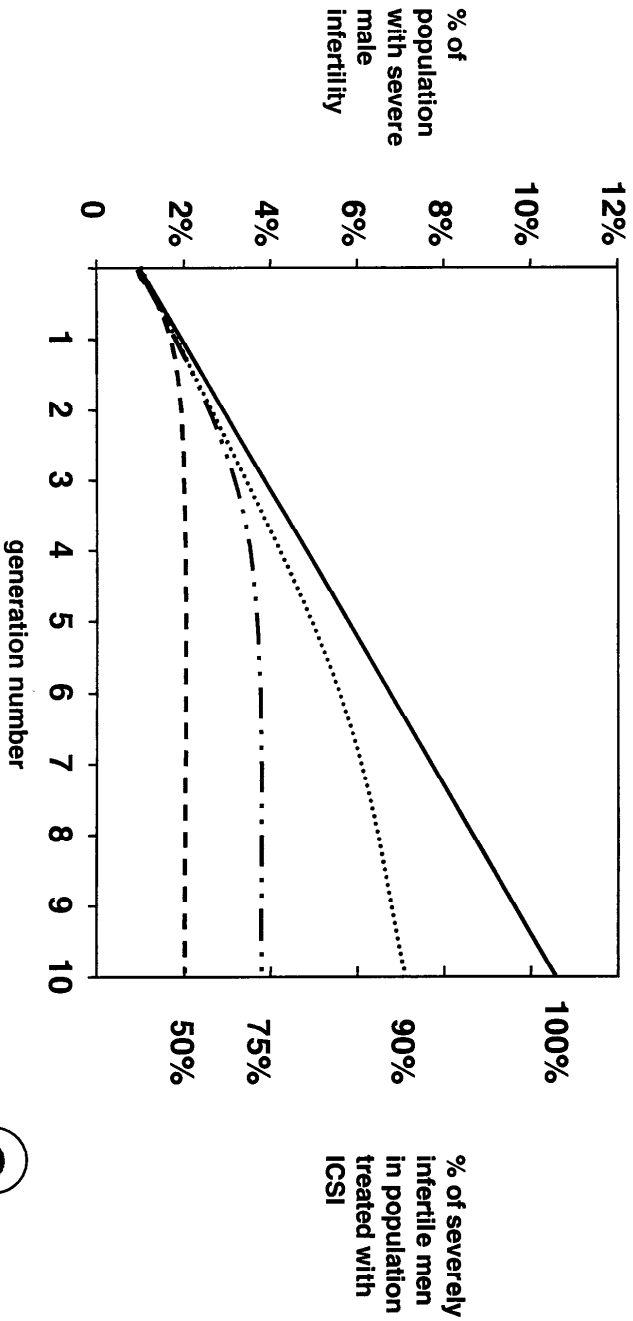


**FIGURE 10**



# Transmission of Severe Male Infertility to Future Generations via ICSI

FIGURE 11



# Transmission of Severe Male Infertility to Future Generations via ICSI

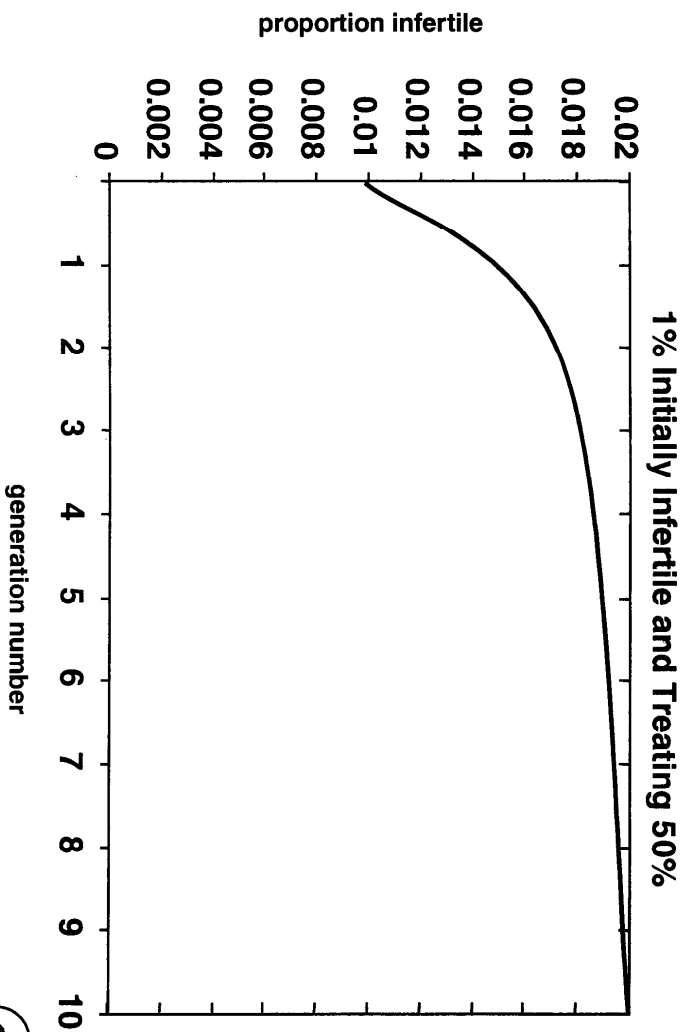
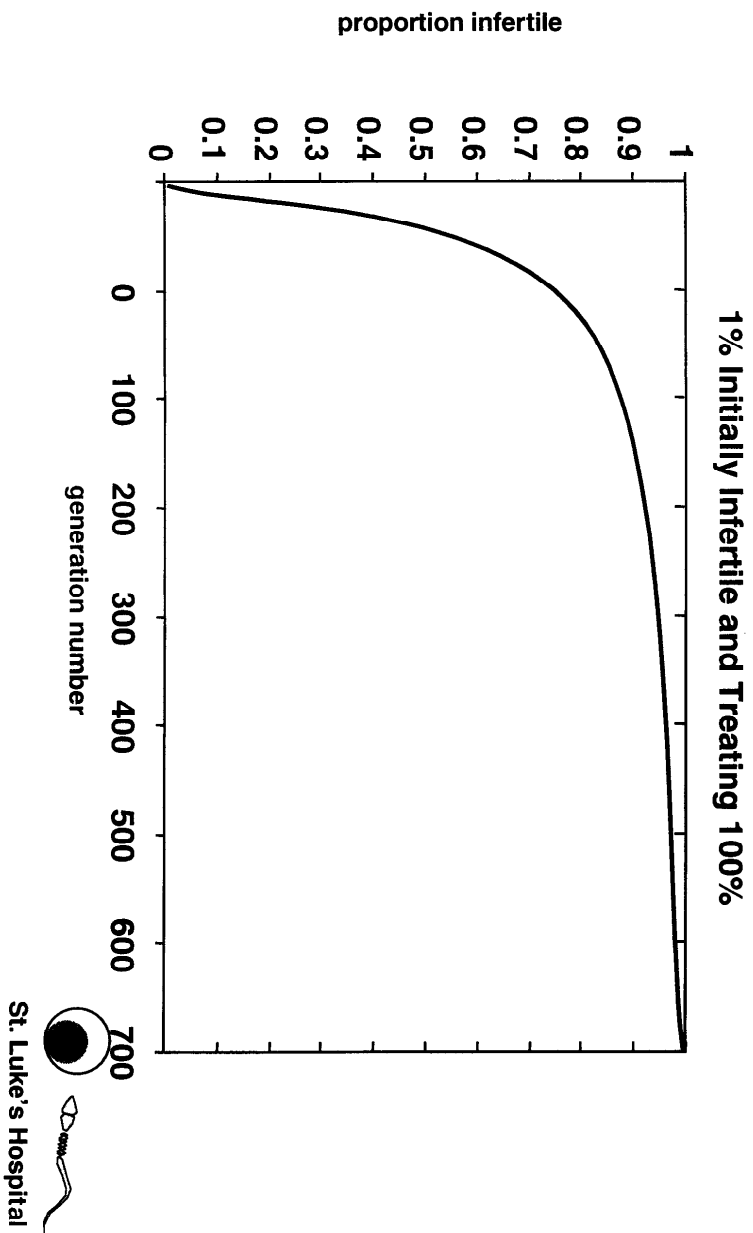


FIGURE 12



# Transmission of Severe Male Infertility to Future Generations via ICSI

FIGURE 13



**FIGURE 14**

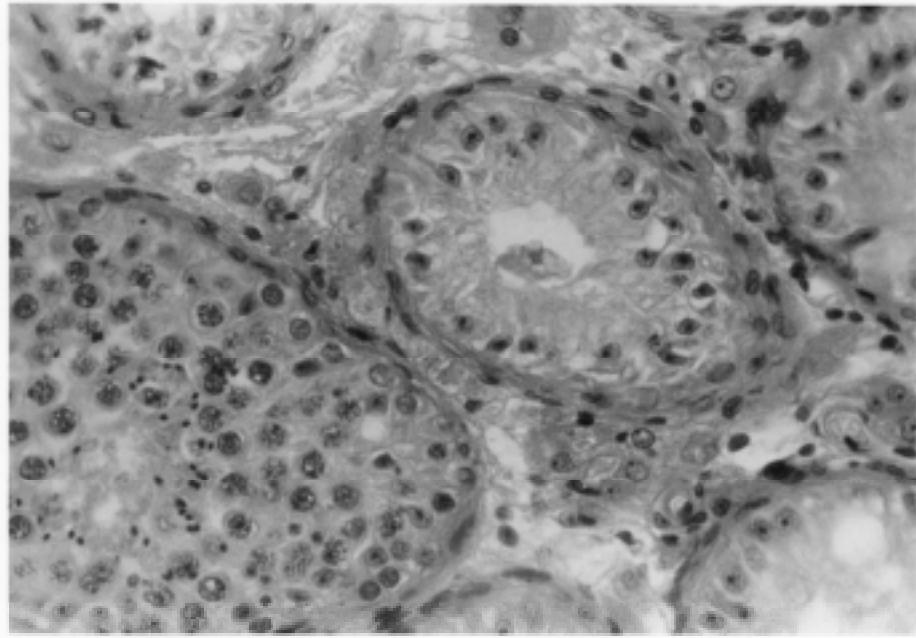


FIGURE 15

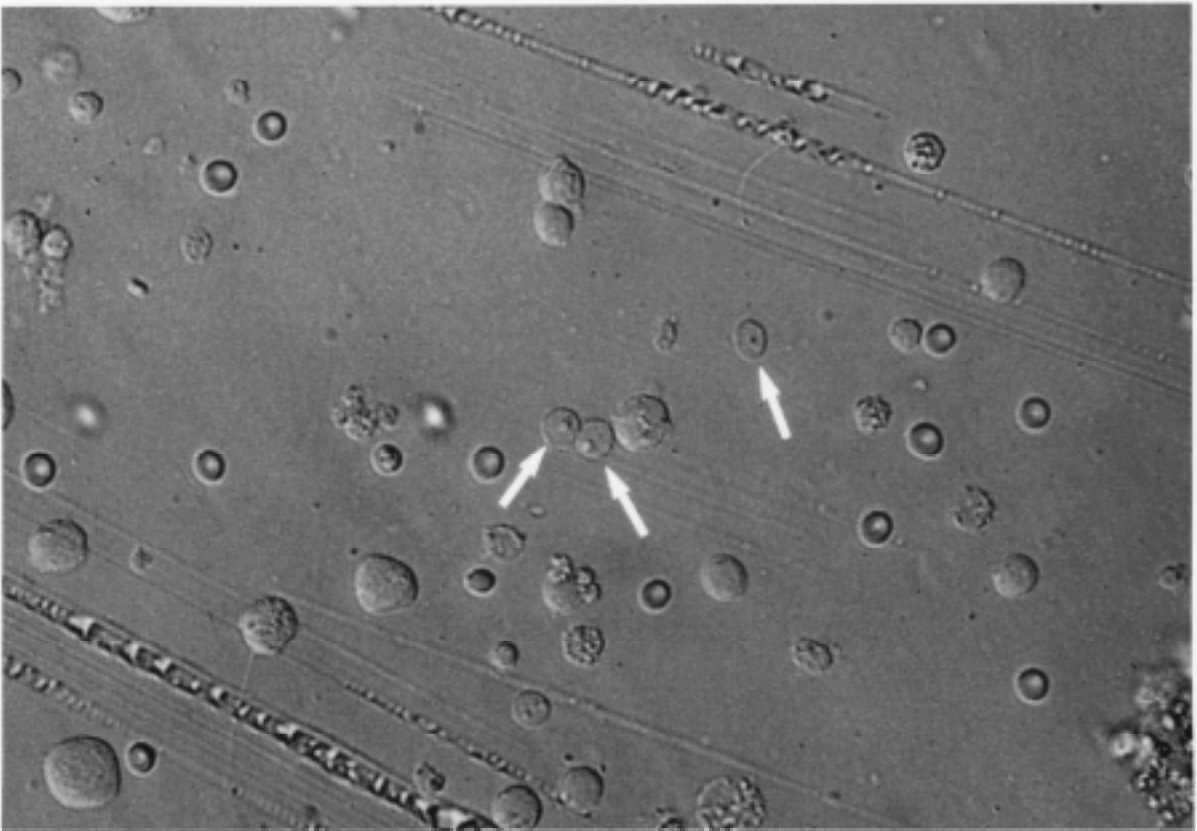


FIGURE 16

