CONTEMPORARY ENDOCRINOLOGY™

Office Andrology

Edited by

Phillip E. Patton, MD
David E. Battaglia, PhD

HUMANA PRESS

Office Andrology

Edited by

Phillip E. Patton, MD

and

David E. Battaglia, PhD

Department of Obstetrics and Gynecology,
Oregon Health and Science University,
University Fertility Consultants, Portland, OR

Humana Press
Totowa, New Jersey

15 Testis Biopsy and the Infertile Male

Sherman J. Silber, MD

CONTENTS

INTRODUCTION

This chapter begins with the use of diagnostic testis biopsy to evaluate azoospermia. It then describes the basics of spermatogenesis in oligospermic and normospermic men, and the use of therapeutic testis biopsy for sperm retrieval and intracytoplasmic sperm injection (ICSI). Finally, it discusses the differences in embryo quality, chromosomal abnormalities, and pregnancy rates with testis sperm versus ejaculated sperm.

In the modern era, the only clinical indication for diagnostic testis biopsy is azoospermia. However, invaluable information on the basis of spermatogenesis has been obtained by testis biopsies performed (in the past) on men with a wide range of sperm counts, from severe oligospermia to more than 100 million sperm per cc.

From: *Contemporary Endocrinology: Office Andrology*
Edited by: P. E. Patton and D. E. Battaglia

AZOOSPERMIA

Approximately 1 out of every 200 men in any population (even excluding those who have had a vasectomy) is azoospermic. Approximately 20% of couples in the United States are infertile *(1,2)*, and 25% of all infertile couples have a low-sperm count *(3)*. About 2% of infertile couples have azoospermia *(3)*. Thus, azoospermia represents approx 8% of the cases of male infertility.

We classify azoospermia as obstructive and nonobstructive. Obstructive azoospermia can be secondary to a vasectomy, congenital absence of the vas deferens (CBAVD), accidental surgical interruption of the vas or epididymis during a hernia or hydrocele operation, or primary epididymal blockage from previous infections. In all of these cases there is normal spermatogenesis in the testes. Most of these, with the exception of congenital absence of the vas (CAV), are amenable to microsurgical repair *(4–16)*. For obstructive azoospermia, ICSI simply adds a secondary alternative following failed reconstructive attempts or in cases of CAV (which is not reconstructable) *(17–19)*. 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 *(18)*.

Evaluation of the Azoospermic Man

The diagnosis of obstructive versus nonobstructive azoospermia should really be quite simple. However, it is sometimes approached in a confusing way that can lead to misjudgments, such as performing a vasoepididymostomy on a patient who has no obstruction or a sperm retrieval blindly for nonobstructive azoospermia when the problem is actually obstruction. If the diagnosis is obstructive azoospermia, the management is quite different than for nonobstructive azoospermia.

A few simple principles avoid these difficulties and allow a proper preoperative decision to be made: (1) If a testicle biopsy shows normal spermatogenesis (and azoospermic), then obstruction is the cause of the azoospermia. Everything else is superfluous. (2) If in addition, the vas deferens is palpable on physical examination, then the patient is a candidate for surgical exploration and probable vasoepididymostomy. All other data are irrelevant. (3) If the vas deferens is not palpable on physical examination, then the obstruction is nonreparable, and sperm retrieval with ICSI is required. In this case, no diagnostic testis biopsy is warranted.

A vasogram should be performed only as part of an 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 important, the test tells nothing about the epididymis, which is the location of the usual site of obstruction. If the diagnosis is obstruction, and a vas is present, then the most logical time to perform a vasogram is at the time of a planned scrotal exploration and vasoepididymostomy (to confirm that the vas empties distally into the ejaculatory duct and prostatic urethra). However, when the semen volume or fructose is normal, it is certain that the ejaculatory duct is not blocked.

A normal follicle-stimulating hormone (FSH) does not necessarily indicate normal spermatogenesis or obstruction. In fact, more commonly, a normal FSH indicates maturation arrest and nonobstructive azoospermia. The serum FSH level correlates most closely with the total number of spermatogonia, not with the number of mature spermatids or sperm count *(20–22)*. The most typical diagnosis for patients with azoospermia and a normal serum FSH level is maturation arrest, not obstruction. FSH is usually in the normal range in cases of nonobstructive azoospermia caused by maturation arrest because the total number of spermatogonia in these cases is normal. It is true that an elevated FSH level usually relates to reduced spermatogenesis because of an overall deficiency in the number of spermatogenic cells. This can be partial or complete Sertoli cell-only syndrome or can just be caused by a reduced number of seminiferous tubules. However, an elevated FSH can also be associated with only modest oligospermia and is definitely *not* predictive of whether an azoospermic man will or will not have sperm present in the testis or at a testicular sperm extraction procedure (TESE) *(23,24)*. Thus, endocrine evaluations are only modestly helpful in the diagnosis and management of azoospermia.

Semen volume and fructose are important to distinguish whether the seminal vesicles are present or whether the ejaculatory duct is blocked. A normal fructose or semen volume does *not* mean there is patency, but signifies that there is a seminal vesicle present with no ejaculatory duct blockage. Men with CAV usually have absent fructose and low-semen volume. However, this is only because in most cases, absence of the vas is accompanied also by absence of the seminal vesicle.

Physical examination of the epididymis and testes, along with a history (or lack thereof) of infection, can be very misleading. Testicles that produce a normal amount of sperm may be small, and those that produce no sperm (with maturation arrest) may often be quite large. Similarly, historical data can be confusing. At least half of our patients who were found to have epididymal obstruction from inflammatory causes had no prior history of clinical epididymitis. We 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 regarding whether or not the patient has obstruction. The physical examination is only relevant in that if a vas deferens is not palpable (i.e., CAV), and the semen volume is less than 1.0 cc, then no surgical anastomosis can be planned. Furthermore, normal spermatogenesis can be assumed. With that exception, the history and physical examination, serum FSH, leutinizing hormone (LH), testosterone levels, and vasography are of little use in diagnosis.

Diagnostic Testicle Biopsy

The open technique for diagnostic testicle biopsy (which we recommend) is very simple and should be a quick outpatient procedure under local anesthesia *(25)*. The spermatic cord is injected with about 6 mL of 0.5% marcaine (bupivacaine) via a 25-gage 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-cm incision is made down to the tunica albuginea. With this method, a small "window" is created through which the testis can be visualized. Then an incision is made in the tunica albuginea. A 1.5- to 1-cm long piece of testicular tissue is excised and placed in Zenker's (or Bouins) fixative with an atraumatic "no touch" technique. This clinical proceedure is completely painless (except for the initial injection of local anesthetic). The patient is able to get up and walk away immediately afterward with no greater pain than if he had had a vasectomy.

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 accomplish this unless performed multiple times, which is then ironically more invasive than the open biopsy technique.

The biopsy must be of adequate quality to determine (1) Does the patient have normal spermatogenesis, and therefore obstruction, which might be amenable to microsurgical repair? (2) If he has nonobstructive azoospermia, will the TESE have 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" that are not valid diagnoses *(21,26–28)*. Testicle biopsy has been used by most clinicians, including most pathologists, in a nonquantitative manner only. This has severely limited its usefulness and has led to many errors in interpretation *(29–32)*.

A simplified quantitative evaluation of the testicle biopsy is based on the normal histology and kinetics of spermatogenesis in humans *(33)*. The rate or speed of spermatogenesis in humans (or in any species) is constant for any variety of sperm counts, whether high or low. Reduced sperm production is

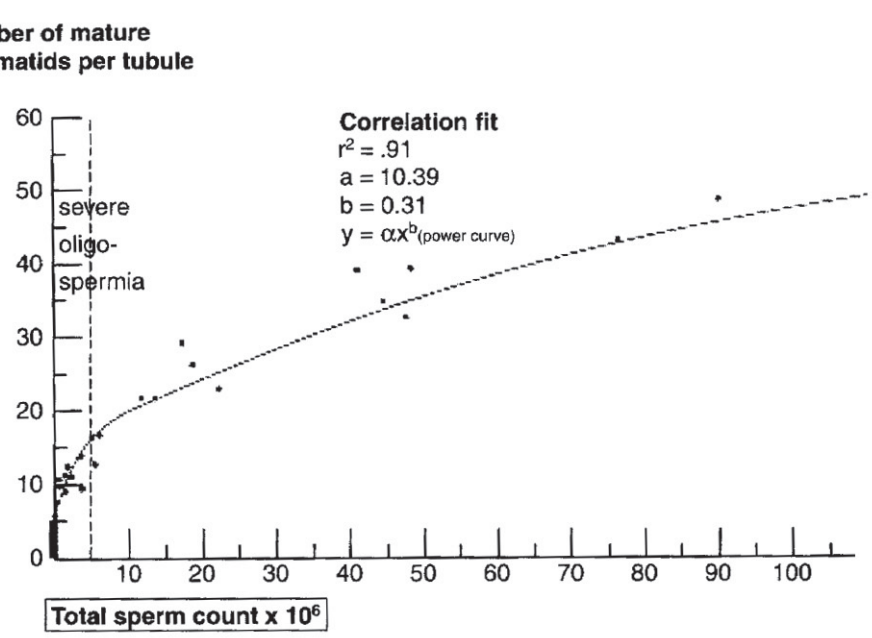

Fig. 1. An exponential curve that relates sperm count in the ejaculate to the average number of mature spermatids seen in each seminiferous tubule. A threshold of three to six mature spermatids per tubule has to be exceeded for sperm to appear in the ejaculate. (From ref. *21*.)

always caused by a lower number of sperm, not by a diminished rate of sperm production. Therefore, the daily sperm quantity being produced in 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 *(21,26–28,33–36)*. For patients who are severely oligospermic after a vasovasostomy, a quantitative testicle biopsy can thus clarify if partial blockage or just poor spermatogenesis is causing the oligospermia (Fig. 1).

The quantitative testicle biopsy is evaluated as follows. At least 25 seminiferous tubules are included in the count from each testis. The mature spermatids (oval-shaped cells with dark, densely stained chromatin) and large pachytene spermatocytes are the easiest to count (Fig. 2A). These cells have the greatest correlation with sperm count and are the easiest to recognize. All steps of spermatogenesis—from spermatogonia to leptotene, zygotene, and pachytene spermatocytes, and to early spermatids—may be observed, but the only clinically important cells are the number of "mature spermatids" (i.e., condensed oval-shaped sperm heads) counted in a minimum of 25 tubules and divided by the number of tubules (Fig. 2A).

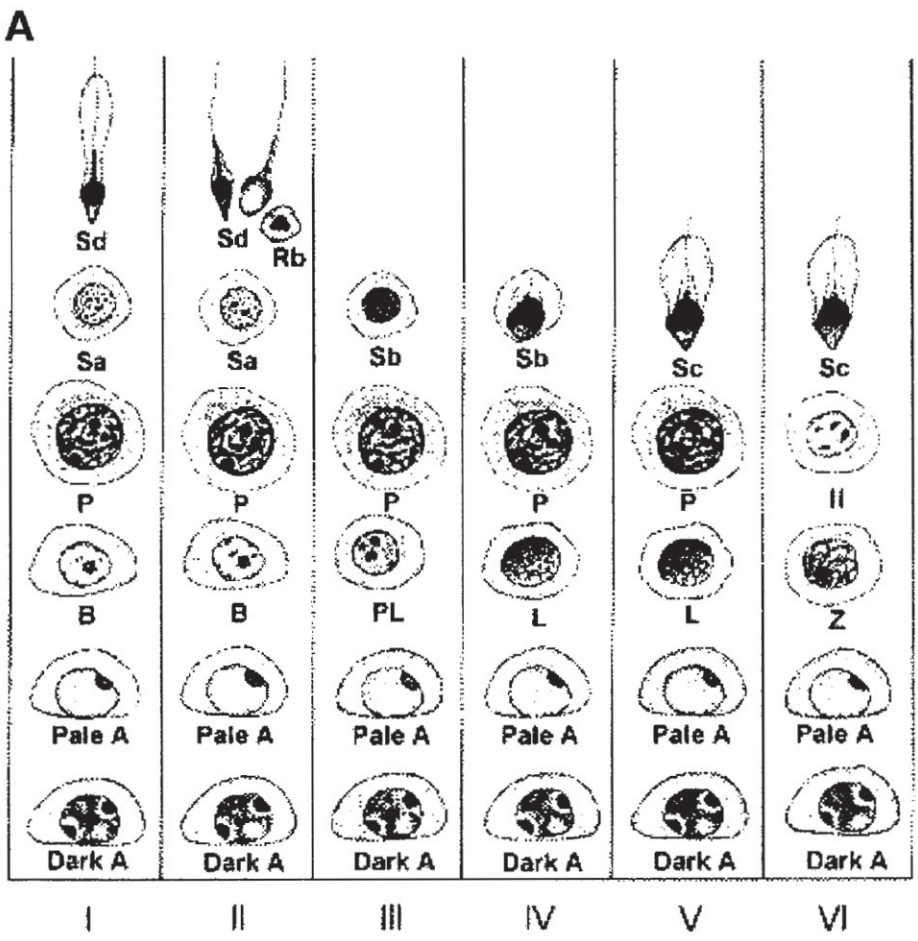

Fig. 2. (**A**) The six stages of spermatogenesis in the human testicle. (From ref. 7.)

Pachytene spermatocytes represent the final preparation stage of the chromosomes for the first meiotic division. This is equivalent to the germinal vesicle phase of the oocyte. Nearly all cases of maturation arrest in the testis are at this stage of spermatogenesis, just before the first meiotic division. If spermatogenesis goes beyond this, mature (elongated) spermatids will always develop.

Using an exponential curve (Fig. 1), 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, when the patient has 40 mature spermatids per tubule, the sperm count should be just under 60 million per cc; when there are 45 mature spermatids, the sperm count should be just over 85 million. The patient with a sperm count of more than 3 million would

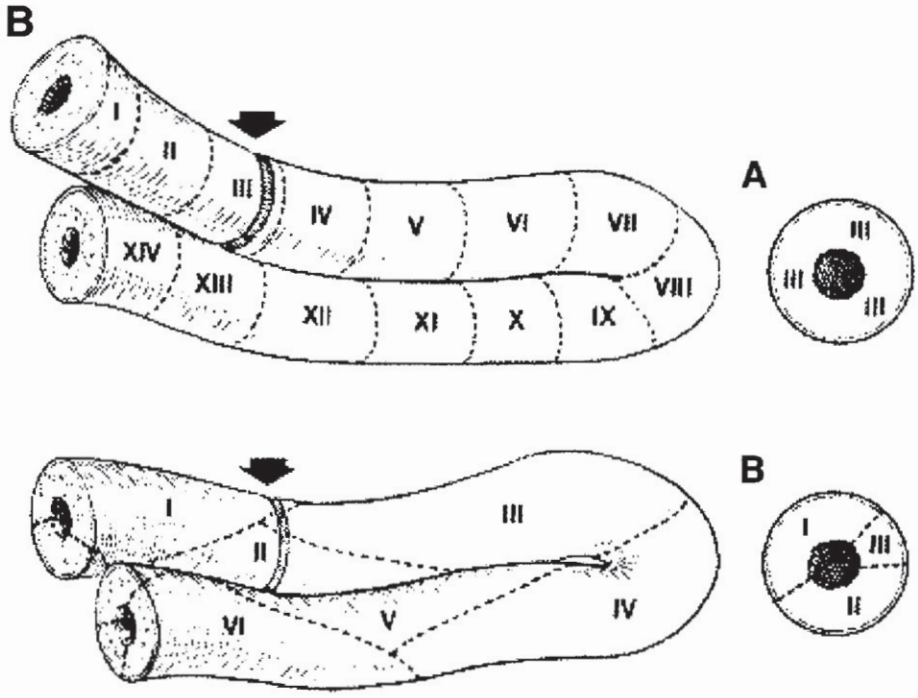

Fig. 2. (B) Drawings of the progression stages of spermatogenesis in the rat seminiferous tubule (a) and human seminiferous tubule (b). In most animals, there is a wave of spermatogenesis in an orderly manner down the seminiferous tubule. Howeverr in the human, there is a mosaic arrangement of the six stages of spermatogenesis. (From ref. 7.)

be expected to have only 6 to 10 mature spermatids per tubule. When there are less than three mature spermatids per tubule, the patient is virtually always azoospermic.

Frequently, patients undergo vasoepididymostomy inappropriately because the pathology report is incorrectly read as "normal spermatogenesis." Such readings are usually not quantitative, but are instead qualitative impressions that tubules are filled with spermatocytes and have some mature sperm. When the biopsy shows thick tubules with a large amount 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 owing to incomplete maturation arrest.

Considerable unnecessary confusion exists about the interpretation and counting of mature spermatids in the testis biopsy. The mature spermatid always has a tail, but it is rarely seen on histological section. This is because the sperm head is 4 μ wide and likely to be in the cut of the microtome's thin section, but the

sperm tail is less than 1 μ in thickness and is unlikely to be seen in this cut section. Therefore, when viewing a histologic section, the spermatids will appear to be without tails, despite the fact that with TESE, they will appear just like sperm.

Some clinicians have attempted to use the serum FSH level to monitor the amount of spermatogenesis. They might mistakenly assume that a normal FSH level in an azoospermic patient would indicate obstruction. Unfortunately, this correlation is very poor *(20)*. Patients with azoospermia casued by maturation arrest have a normal FSH level. The FSH level correlates more closely with total number of spermatogonia and testicular volume, not with the number of mature sperm.

Ironically, it is the scattered mosaic arrangement of the various spermatogenesis stages 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. 2B). In humans, a cut through any area of the testicle reveals a scattered array of all the stages. Thus, in humans (unlike most other animals), it requires only 25 seminiferous tubules from any location in the testis for a good statistical sample of the total range of spermatogenesis in the entire testicle.

SPERM RETRIEVAL AND INTRACYTOPLASMIC SPERM INJECTION FOR OBSTRUCTIVE AZOOSPERMIA

CBVAD occurs in about 1% of infertile couples *(37)*. Until the last 12 yr, it was a frustrating and dismal problem with very poor prognosis for treatment. Since the first successful use of epididymal sperm aspiration and in vitro fertilization (IVF) for CBVAD was reported, ICSI has now made it possible for all these men to have children *(18,37–42)*. In fact, with ICSI, the pregnancy rate with microsurgical epididymal sperm retrieval (MESA) is only related to female factors *(18,41,43)*.

A simple "window" scrotal exploration under local anesthesia (just like for diagnostic testis biopsy) is performed on the same day that the female undergoes oocyte aspiration. Alternatively, MESA does not have to be coordinated with ICSI cycles. Frozen epididymal sperm provides results with ICSI no different than fresh. Under ×10 to ×40 magnification with an operating microscope, a 0.5-mm 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 micropipet (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.

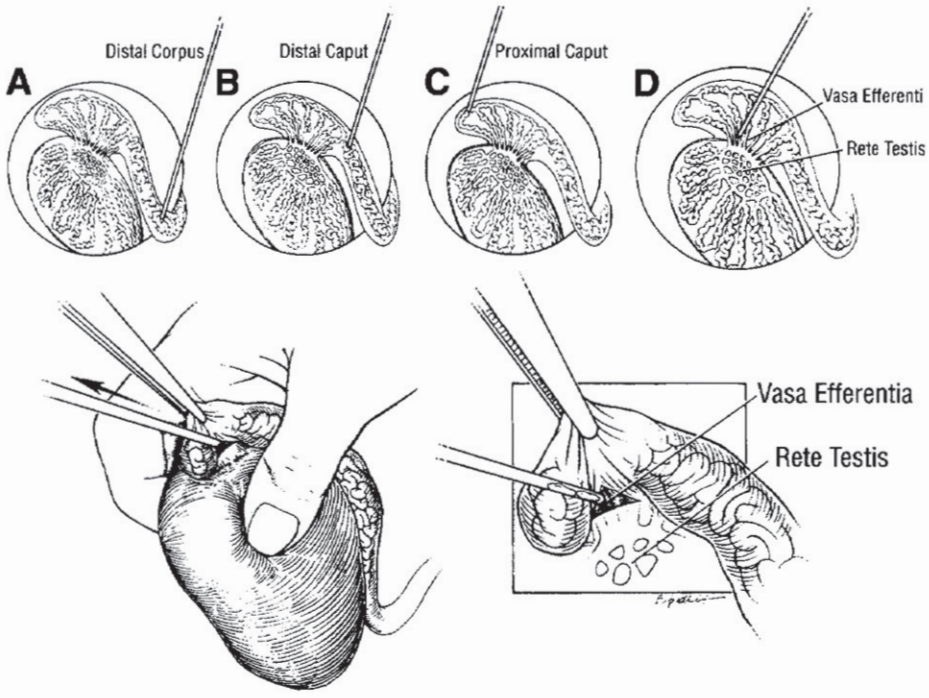

Fig. 3. A depiction of microsurgical epididymal sperm aspiration beginning at the distal corpus (A) and moving proximally to the distal caput, proximal caput, and the vasa efferentia (B–D). With obstructive azoospermia, there is an inversion of the usual physiological location of greatest and least sperm motility. With obstruction, the most motile sperm are always the most proximal. Because of senescence, distal sperm are the least motile. (From ref. *17*.)

If sperm motility is absent or poor, another 0.5-mm incision is made more proximally. Sperm are obtained from successively more proximal regions until progressive motility is found (Fig. 3).

Motile sperm are usually not obtained until the most proximal portion of the caput epididymis or vasa efferentia is reached, which is the opposite of what would be found in a normal nonobstructed epididymis. In the obstructed epididymis, the most recently produced sperm are the most proximal and are therefore the most viable and motile. The distal epididymal sperm are the most senescent and clearly nonviable. Once the area of motile sperm is found, an aliquot of epididymal fluid is used for ICSI, and the remainder is frozen.

There are virtually no cases of obstructive azoospermia that cannot be successfully treated with sperm retrieval methods and ICSI as long as the female does not have insurmountable problems. 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 easily and represents a simple, clean, easy, and indefinite supply of sperm for the laboratory without need for future invasive procedures.

There have been many trivial debates over how best to collect epididymal or testicular sperm from azoospermic patients for ICSI. What works best in the reader's own particular setting can be decided, but our preference is as follows. For obstructive azoospermia, there is typically some epididymis present regardless how severe the congenital defect. In these instances, we prefer MESA. All of our sperm retrievals are done 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 and, similarly to testis biopsy, the cord is then infiltrated with several cc of 0.5% marcaine. This produces anesthesia of the testicle and epididymis, but not the scrotum. Then, several cc of 0.5% marcaine are used to infiltrate the anterior scrotal skin with a 25-gage needle along a proposed 1- to 2-cm incision line. Once the tunica vaginalis is entered, the epididymis and testicle are exposed and brought into the field of an operating microscope. Indeed, the patient can watch the entire procedure on a video monitor and should be wide awake and comfortable. The advantage of epididymal sperm retrieval performed in this way is the large number of the most motile sperm that can be readily obtained from the most proximal duct and frozen for an unlimited amount of future ICSI cycles.

Often, there is 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).

An important warning is that for nonobstructive azoospermia: epididymal sperm can never be retrieved because the walls are collapsed. Nonetheless, for nonobstructive azoospermia, an open testicular biopsy performed under the microscope can still be accomplished in the same manner under the same type of local anesthetic with the patient wide awake and minimal postoperative discomfort.

TESTICULAR SPERM EXTRACTION FOR NONOBSTRUCTIVE 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 frequently a very minute number of sperm sparsely present in an extensive testicular biopsy, and these occasional testicular sperm could be used for ICSI *(19,21,22,42–44)*. We coined this procedure "testicular sperm extraction" or TESE. This approach was based

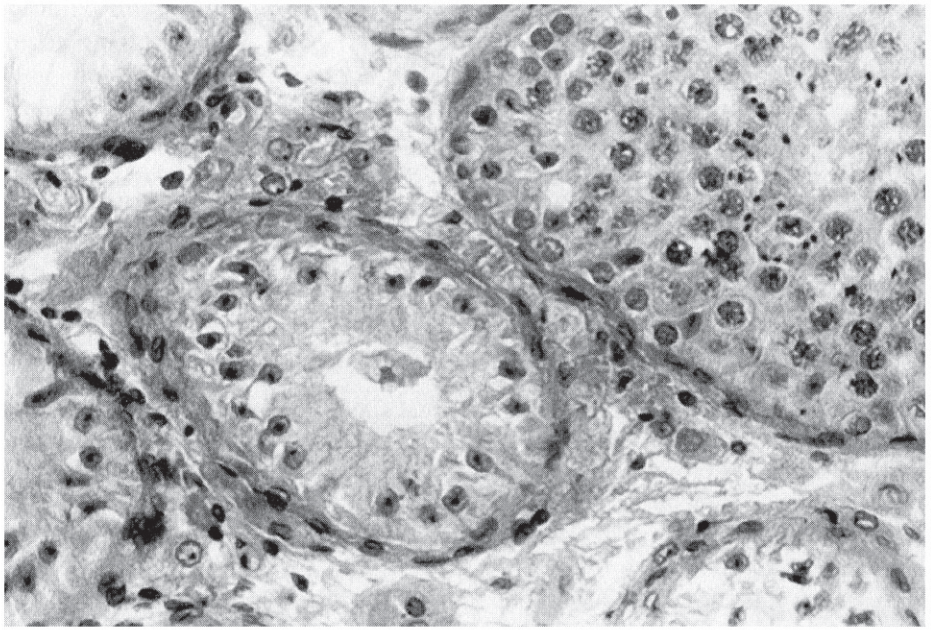

Fig. 4. A histologic section of testicle biopsy in a patient with Sertoli-cell only, elevated follicle-stimulating hormone, and occasional tubules with normal spermatogenesis. Upper right-hand tubule exhibits normal spermatogenesis, but all the other tubules are Sertoli cell-only (From ref. *50*.)

on quantitative studies of spermatogenesis dating back to the late 1970s *(27,28,34–36)*. 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 amount of tubules is predictive of the sperm count in the ejaculate. Intriguingly, the majority of patients with complete azoospermia have a few mature spermatids in their testis histology (Fig. 4).

These studies of quantitative spermatogenesis in the late 1970s and early 1980s 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 sperm for ICSI *(45)*. An extremely diminished quantity of sperm production in the testis will result in the absolute absence of sperm in the ejaculate, even though there is some sperm being produced in the testicle. A minimal threshold of sperm production is necessary before any sperm can actually spill over into the ejaculate. Thus, severe oligospermia, which is readily treated with

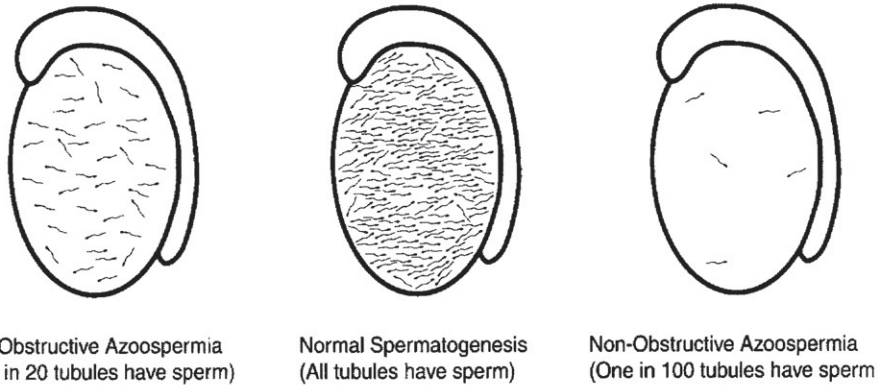

Fig. 5. Various degrees of azoospermia. Normal spermatogenesis (center drawing) is associated with obstructive azoospermia. With nonobstructive azoospermia, the testicular sperm extraction process may be as easy as in the drawing depicted on the left or very difficult as depicted in the drawing on the right.

ICSI, is just a quantitative variant of azoospermia where more than three mature spermatids per tubule are found in the testis. There is some minute presence of spermatogenesis is in 60% of azoospermic men (Fig. 5). However, the amount of spermatogenesis present in these men is below the threshold (three mature spermatids per tubule) necessary for these few sperm to spill over into the ejaculate *(21)*.

The initial approach to TESE for nonobstructive azoospermia was very crude, often involving numerous extensive biopsies from multiple areas of the testis until sperm were located. Legitimate concerns were raised. (1) How is the couple to be counseled for IVF and ICSI (with all that it entails for the female) when there is only a 55 to 60% chance that any sperm will be found? (2) Can the success or failure of sperm retrieval be predicted? (3) In cases of severely compromised testes, should the couple be assured that multiple repeat procedures will result in successful sperm retrievals in future cycles? (4) Is it possible simply to freeze unused sperm derived from a TESE procedure without diminishing the results, thereby avoiding the necessity of timing the female's stimulation cycle to the male's sperm retrieval?

Although it is clear that effective results can often be obtained with thawed testicular sperm for cases of obstructive azoospermia, frozen sperm from the testicle in the most severe nonobstructive azoospermia cases will not give a result equivalent to that of fresh sperm. Therefore, our two major goals were to determine (1) whether a prior diagnostic biopsy or any other test could predict the success or failure of a future TESE, and (2) whether or not a TESE technique could be used that would be harmless and relatively painless, so as not to com-

promise future attempts at fresh sperm retrieval? In fact, a small prior diagnostic testis biopsy is quite predictive of the likelihood of finding sperm in a TESE procedure in 90% of cases *(21)*. But in 10% of cases, prior diagnostic testis biopsy is not predictive. Our solution to this dilemma is a microsurgical approach to testicular sperm extraction (micro-TESE).

MICROSURGICAL TESTICULAR SPERM EXTRACTION

When extensive multiple biopsies from every area of the testis are performed to find sufficient sperm for TESE, much testicular damage can result and may limit "successful" patients to only one attempt *(23–24)*. An effort to limit damage by using multiple needle sticks rather than open biopsy to obtain sperm for ICSI is just as invasive and quite risky as well *(46)*. Furthermore, controlled studies have shown that for difficult cases of nonobstructive azoospermia where spermatogenesis is very meager, needle biopsy is much less likely to find the rare foci of spermatogenesis than open biopsy *(47,48)*.

We studied the distribution of spermatogenesis in azoospermic men and have outlined a *microsurgical* approach to TESE that minimizes tissue loss and pain and maximizes the chance of finding sperm. Knowledge of the distribution of spermatogenesis and microsurgical technique helps to prevent testicular damage and postoperative pain, making multiple repeat TESE procedures (if needed) safe and reliable *(21,26)*.

Unnecessary confusion exists with testicular sperm, mature spermatids, and round spermatids. Sperm tails are seldom seen on histology, and only the thicker sperm head shows up in thin sections, and usually only an oval-shaped head is observed. Mature spermatids at TESE are no different in appearance than sperm. The solution in cases with no sperm seen on TESE is *not* to look for "round spermatids" *(49,50)*. We never see round spermatids in the absence of mature spermatids, which at TESE are what appear to be sperm (Fig. 6A–C) *(49–51)*. The solution is to search for the few sperm that are sparsely and diffusely present.

Technique

All microsurgical TESE cases are performed under local anesthesia. Just like for MESA or for diagnostic testis biopsy, the procedure is truly painless. The tunica vaginalis is opened and the testicle is exteriorized. The operating microscope is then used under ×16 to ×40 magnification. After microdissection and evaluation of tubular dilation, a tiny microscopic removal of single dilated tubules can often 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 hemo-

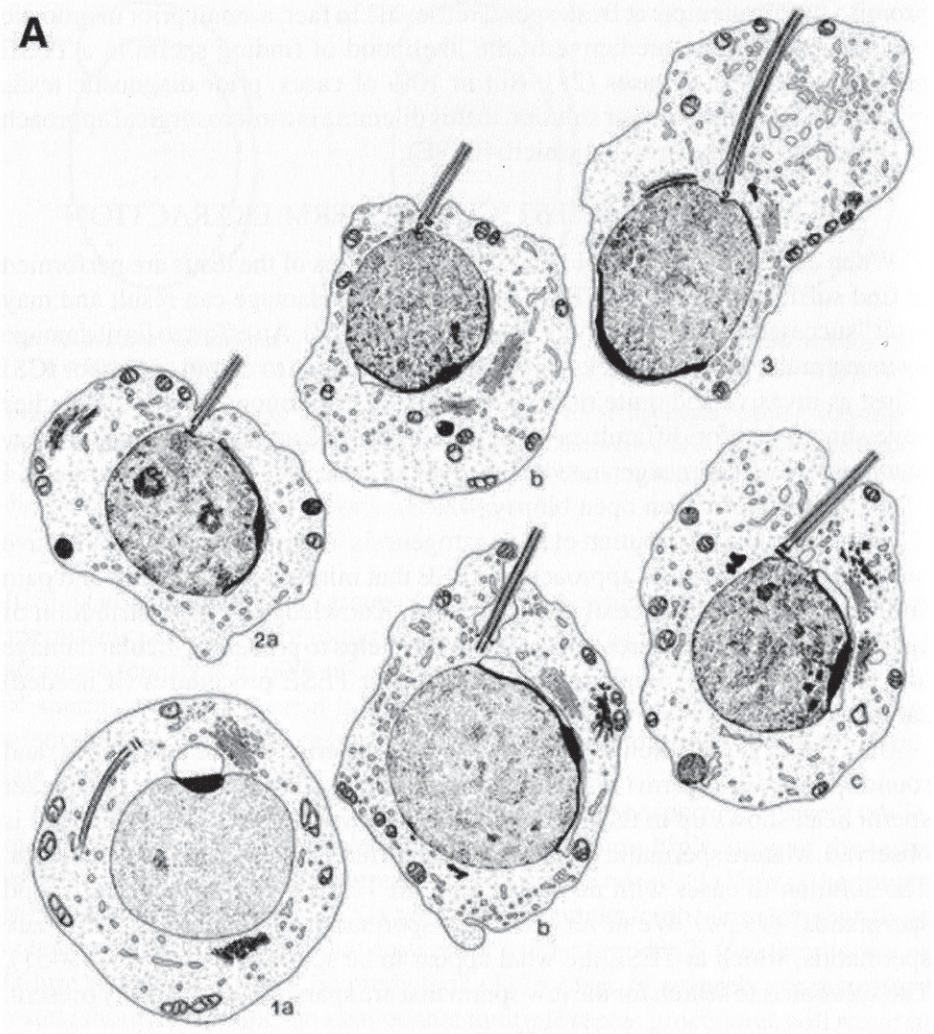

Fig. 6. (**A**) Drawings of the stages of spermiogenesis after the second meiotic division has occurred. Prior to the formation of the tail, the round spermatid can always be recognized by the prominent acrosomal vesicle (1a). As the acrosomal vesicle recedes, the tail begins to form. (From ref. *51*.)

stasis with microbipolar forceps (Figs. 7 and 8). This prevents any increase in intratesticular pressure, resulting in minimal pain and absence of subsequent atrophy.

Of the total cases subjected to microsurgical TESE for nonobstructive azoospermia, about 60% yield sperm sufficient for ICSI. In Sertoli cell-only, microsurgical dissection often (but not always) allows the removal of only a

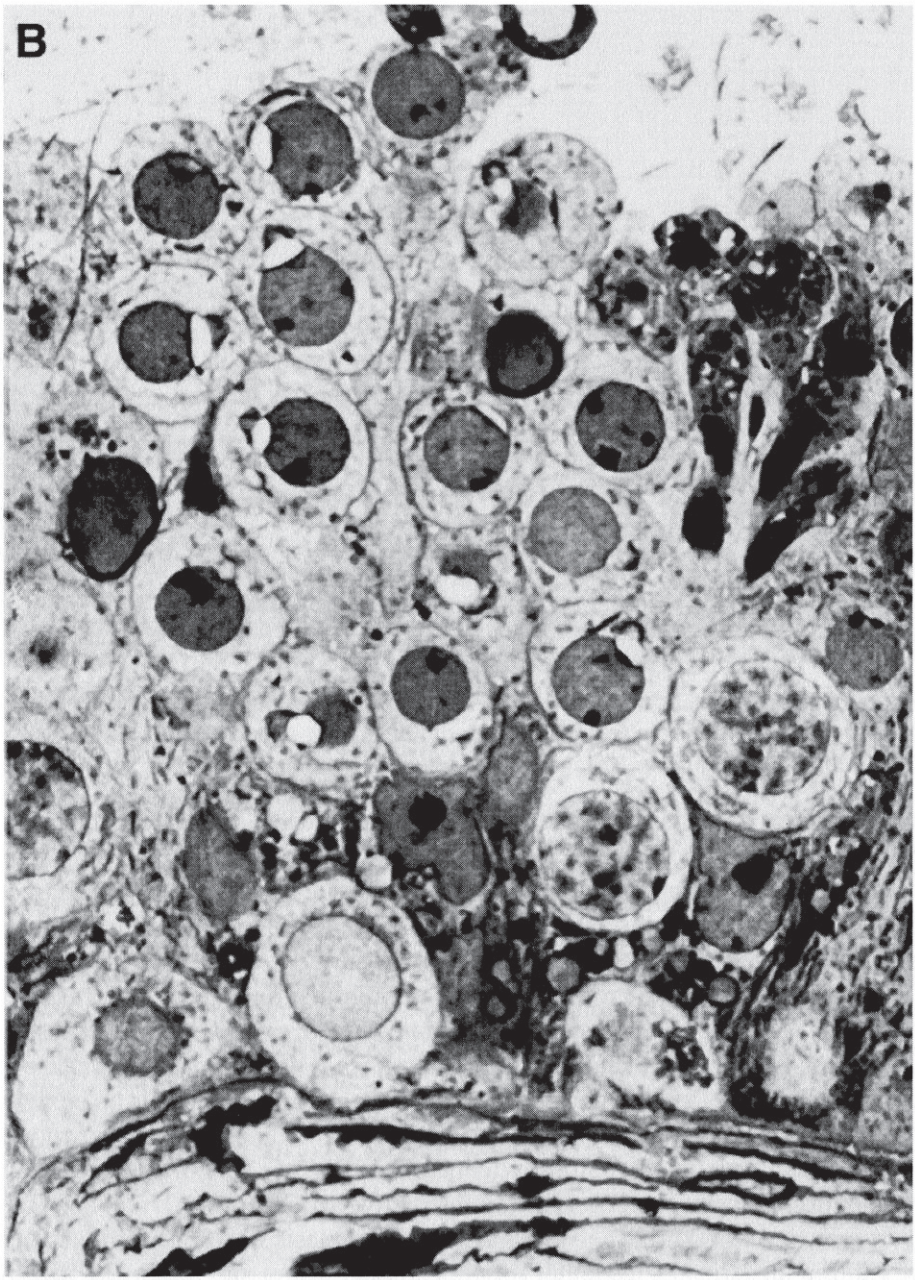

Fig. 6. (B) Electron micrograph of a section of human spermatogenesis demonstrating pale "type A" spermatogonia, Sertoli cell nuclei, pachytene spermatocytes, early round spermatids with acrosomal vesicle, and mature spermatids with an oval dark-staining head. (From ref. *51*.)

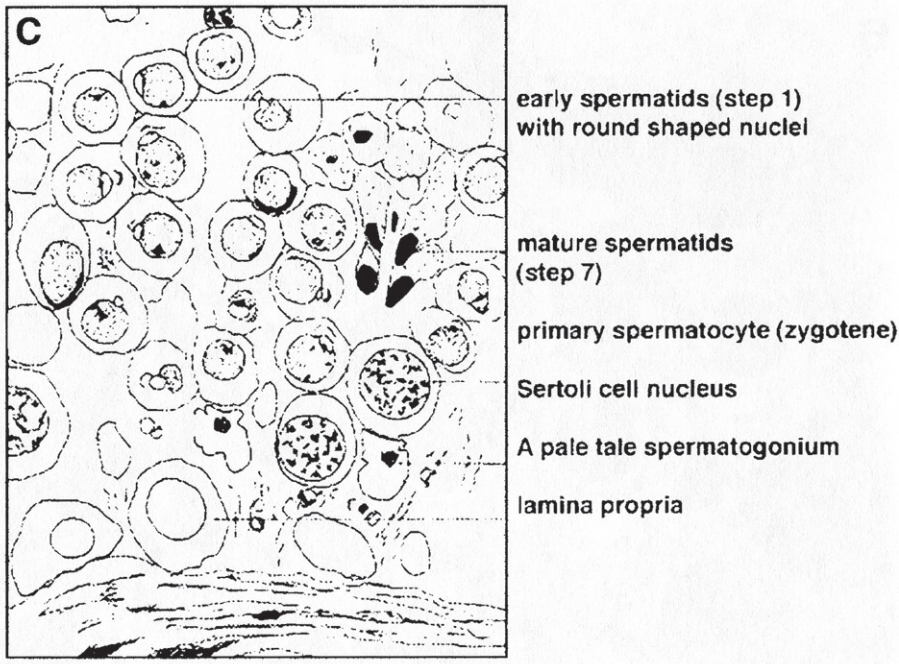

Fig. 6. (C) Diagrammatic depiction of Fig. 6B with labeling of the specific cells involved in spermatogenesis. (From ref. *51*.)

small amount of testicular tissue to find this sperm because normal tubules are full in thickness, and Sertoli cell-only tubules are usually thin and empty. In maturation arrest, a larger amount of testicular tissue usually 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 with micro-TESE because blood supply is not interrupted; microscopic bleeders are meticulously coagulated; and the tunica albuginea is not encroached because of the closure with 9-0 nylon interrupted stitches. Consequently, there is no increase in intratesticular pressure, no testicular damage, and minimal pain (Figs. 7 and 8).

Our direct mapping provides evidence for a diffuse, rather than regional, distribution of spermatogenesis in nonobstructive azoospermia *(21,26–28)*. 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. Also explained is how, removal of small amounts of tissue blindly with a needle, yeilds sperm in most cases with obstructive azoospermia, but does not work for nonobstructive azoospermia (Fig. 5). Microsurgery under the operating microscope trivializes any testicular damage.

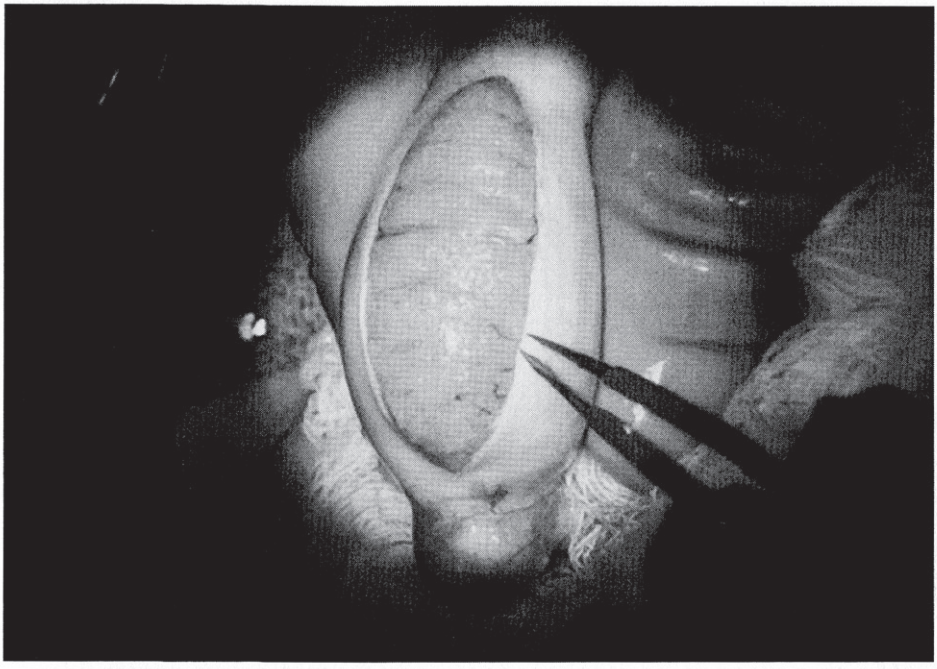

Fig. 7. A single large testicular incision for a testicular sperm extraction procedure under the operating microscope results in minimal to no testicular damage, minimal-to-no postoperative pain, and an ability to analyze each specific seminiferous tubule for the presence of spermatogenesis. (From ref. *26*.)

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 because of minor amounts of bleeding within the enclosed tunica albuginea. The tunica albuginea is a very nonflexible enclosure. A small degree of intratesticular bleeding causes a noticeable increase in intratesticular pressure, which can be readily observed by those doing conventional, multiple-testicle biopsy samplings for TESE. Furthermore, the closure of open biopsies with the usual nonmicrosurgical suture, particularly in a running manner with conventional TESE, further compromises the intratesticular volume and thereby adds to the increased pressure (Figs. 7,8).

CHROMOSOMAL ERRORS, EMBRYO QUALITY, AND PREGNANCY RATES FOR INTRACYTOPLASMIC SPERM INJECTION WITH TESTICULAR SPERM

Early studies demonstrated that the major determinant of success with ICSI was not the quality or origin of the sperm, but instead the age and fertility of the

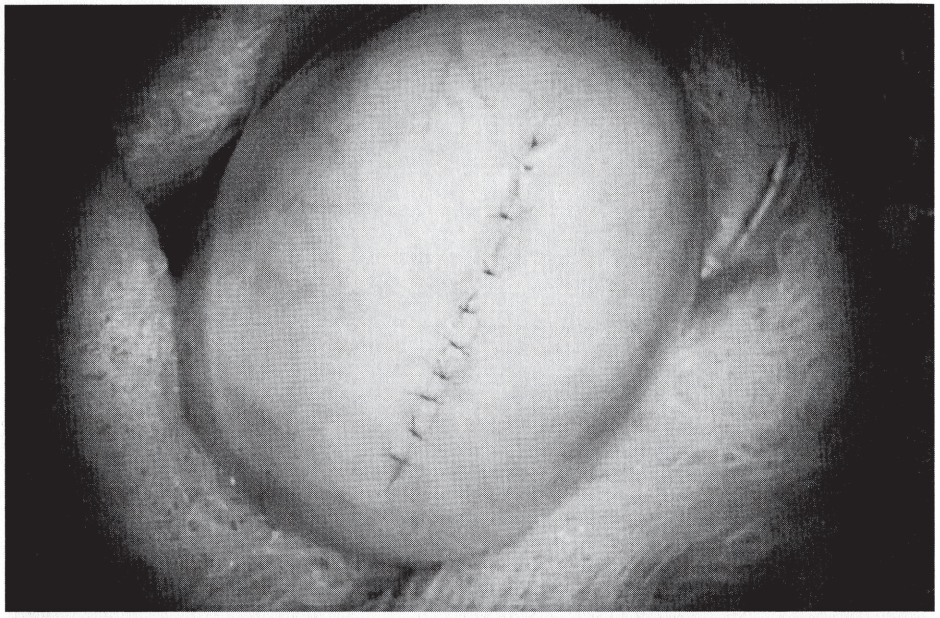

Fig. 8. Microsurgical closure of the tunica albigunea of the testes after a microsurgical testicular sperm extraction procedure results in no increase in intratesticular pressure and subsequently no loss of testicular function. (From ref. *26*.)

wife *(18,19,41–43,52,53)*. However, a detailed review of delivery rates with ICSI in couples with varying degrees of severity of spermatogenic defect, as well as fluoresence *in situ* hybridization (FISH) studies of both sperm and embryos derived from this sperm, indicates that sperm may also have an impact on ICSI results.

FISH analysis of embryos from ICSI cycles for nonobstructive azoospermia (requiring TESE) vs ICSI cycles for oligospermia demonstrates that embryos derived from TESE have a significantly higher rate of chromosome mosaicism *(54)*. In our initial research describing this phenomenon, ICSI cycles with ejaculated sperm produced 830 embryos: 41.8% were normal, 26.2% were aneuploid, and 26.5% were mosaic. In contrast, ICSI cycles with TESE for nonobstructive azoospermia produced 100 embryos: only 22% were normal, 17% were aneuploid, and 53% were mosaic. The difference in mosaicism rates between the two groups was highly significant ($p < 0.001$). Most mosaic embryos were chaotic. We continue to find a higher number of chromosomally abnormal embryos derived from TESE cases with severe spermatogenic deficiency. Sperm derived from TESE for nonobstructive azoospermia probably have a higher rate of comprised or immature centrosome structures, and this may be the cause of increased rates of chaotic chromosome errors in the subsequently derived embryos. None-

theless, for oligospermia, the rate of chromosome abnormalities found in embryos produced by ICSI is similar to that found with conventional IVF. Thus, ICSI itself does not appear to be a teratogenic *(55,56)*. It is only the severity of spermatogenic defect, not the ICSI procedure itself, that causes this increase in chromosomal errors.

A slightly lower clinical pregnancy rate has been found for testicular sperm derived from men with nonobstructive vs obstructive azoospermia *(57)*. Also, a slightly increased incidence of chromosomal anomalies is found in ICSI offspring when compared to a normal newborn population *(58–63)*. Additionally, there is an increased incidence of abnormalities found in peripheral lymphocytes of males who require ICSI *(64–69)*. Even if the infertile male is chromosomally normal in his peripheral lymphocytes, meiotic disruption still generates higher rates of sperm chromosome abnormalities *(70)*. Thus, these few sperm in TESE cases have a higher rate of chromosomal errors.

In fact, we have observed a higher percentage of aneuploidy in testicular sperm from men with nonobstructive azoospermia (21%) than in ejaculated sperm from men with oligospermia (11%). However, this increased aneuploidy in the testicular sperm of azoospermic men cannot easily explain the dramatic increase in chaotic chromosomal abnormalities of TESE embryos. For this reason, we suspect other sperm abnormalities may be the cause. Most FISH studies on sperm of infertile males have found higher rates of aneuploidy than in fertile males *(71)*. However, with the exception of the most severe defects in spermatogenesis requiring TESE, the increase in sperm sex chromosomal abnormalities was very small and not correlated with an increase in spontaneous abortions of neonatal abnormalities *(72)*. However, it does likely explain the slight, but definite, increase in sex chromosomal anomalies indicated in ICSI offspring *(61)*.

Although most chromosomal studies of the sperm of infertile men had focused on aneuploidy, chromosomal abnormalities in human embryos are not limited to aneuploidy *(73)*. In younger women, the most common abnormality in cleavage-stage embryos is mosaicism, not aneuploidy *(74,75)*. These mosaic embryos can reach blastocyst stage, but still do not result in viable offspring *(73,76,77)*. Different mosaic types have been described in cleavage-stage embryos, and possible mechanisms that produce mosaicism have been proposed *(56,78–80)*. Occasionally, an infertile male in multiple IVF cycles produces mostly chaotic mosaics, but when donor sperm is used, produces normal embryos *(81)*. Significantly, chaotic mosaic embryos are more likely to be of poor quality in appearance than aneuploid embryos, which usually look quite normal. Therefore, the increase in chromosomal abnormalities (chaotic) in TESE embryos results in a higher incidence of poor-quality embryo appearance. This is different from the simple aneuploidy found to be increased in embryos from older women, and which can

appear quite deceptively normal. Therefore, the chromosomal abnormalities associated with TESE sperm can be deleted with standard embryo morphology assessment, and do not lead to birth defects or increased miscarriages but do lead to slightly lower pregnancy implantation rates.

Early chromosomal studies of embryos obtained after conventional IVF vs ICSI (in cases of moderate oligospermia) have shown no difference in the incidence of chromosomal abnormalities *(55,56)*. However, there have been growing concerns regarding possible chromosomal anomalies in ICSI offspring of men with the most severe spermatogenic defects. Consistently, there has been a 0.8% to 1% incidence of sex chromosomal anomalies in ICSI offspring in comparison to a population norm of 0.14% to 0.2% *(59,61–66)*. These newborns would appear normal at birth, and the sex chromosomal anomaly (most frequently Klinefelter's) would not be identified without a prenatal karyotype. Autosomal aneuploidies in this population were no different than what would be expected based on maternal age in a non-ICSI population *(61)*. Perhaps a more alarming problem in ICSI-produced neonatal karyotypes was the 0.36% incidence of *de novo*-balanced translocations in comparison to the normal newborn population of 0.07% *(61)*. Additionally, there was a 0.92% incidence of inherited translocations transmitted via ICSI from the father. Of those inherited translocations, 10% were unbalanced. Thus, the total incidence of chromosomal aberrations in the ICSI population was 2.5%.

It is the sperm of infertile men that is the source of this low, but definite increase in chromosomal abnormalities of ICSI offspring, instead of the ICSI procedure itself *(55)*. Since 1994, many studies have been reported on the chromosomal analysis of spermatozoa by FISH *(73,74,85–94)*. A great deal of controversy was generated by these studies about the percentages of aneuploid sperm in infertile men. Although there seems to be a mathematically and statistically significant increase in sperm aneuploidy from infertile men, these differences were so slight to subsequently negate a major biological impact *(95)*. However, there have been several conflicting studies (Levron et al., Martin et al., and Palermo et al.) of the sperm found in the testes of men with nonobstructive azoospermia *(91–94)*. Our data confirm no significant increase in sperm aneuploidy with oligospermia, but about twice the rate of sperm aneuploidy in nonobstructive azoospermia.

It is known that aneuploidy of embryos is not closely associated or correlated with embryo morphology. As women age and the rates of aneuploidy increase, abnormalities in embryo morphology do not increase *(56)*. However, mosaicism, chaotic mosaicism, and polyploidy are associated with an increase in morphologic abnormalities in the embryos and do not increase with age. Aneuploidy appears to rise with maternal age and is related to defects in the egg, but mosaicism and chaotic mosaicism may be linked more to defects in the sperm and can

result in a high percentage of chaotic mosaic embryos derived from ICSI with nonobstructive azoospermia.

The high rate of mosaic embryos observed as a result of TESE-ICSI may be more related to defects in the sperm centriole than to a higher incidence of numerical chromosome abnormalities. Our TESE-ICSI-derived embryos had no greater incidence of aneuploidy than ICSI with ejaculated sperm from men with higher sperm-production rates. However, a dramatically increased rate of mosaic errors was found in these embryos because of abnormal mitosis, which could be related to defects in the sperm centriole *(96)*. Similarly, an early report on MESA-ICSI for obstructive azoospermia in which distal (senescent) epididymal sperm were utilized demonstrated an inexplicably high miscarriage rate despite the young age of the female partners *(97)*. This phenomenon might also be explained by defects in embryo cleavage associated with centriole dysfunction *(98,99)*. Thus, the most severe degrees of spermatogenic defect, resulting in nonobstructive azoospermia and requiring TESE, or even senescent nonmotile sperm from distal epididymis, may result in a higher frequency of chromosomal abnormalities. But those abnormalities may be more related to errors in mitosis during early cleavage of the embryo than to sperm aneuploidy.

Present data points to a male origin of chaotic embryos. Because the first mitotic divisions are controlled by the spermatozoon centrosome *(100)*, this may result in abnormal chromosome distribution among sister cells. For instance, dispermic embryos have high rates of first mitotic mosaicism that appear as chaotic mosaics, and they are produced by an abnormal number of male centrioles (no haploids and two polyspermics) or suboptimal centriole function *(101–103)*. Sperm integrity is clearly necessary for normal mitotic division and early embryonic development *(104)*.

Alternatively, another possible explanation is that the higher incidence of autosomal aneuploidy in these TESE-derived sperm might possibly result in initially aneuploid zygotes. Then, the chaotic mosaicism in the subsequent blastomere could be a result of a cellular attempt during early embryo cleavage for mitotic correction of the initial aneuploidy. However, that would not explain the absence of this phenomenon in aging aneuploid eggs.

Severe spermatogenic defects, as in nonobstructive azoospermia, may result in a higher percentage of mosaic and chaotic mosaic embryos, causing less efficient implantation and live birth rates. The live birth rate for TESE with nonobstructive azoospermia is slightly lower than for obstructive azoospermia (normal spermatogenesis). ICSI for nonobstructive azoospermia is also associated with poorer embryo quality (corresponding to the increased incidence of chromosomal mosaicism) and lower live birth rates. Thus, despite the dramatic success of TESE-ICSI for azoospermic men with severe spermatogenic defects, the results are nonetheless adversely affected by sperm factors in these severe cases.

However, the negative impact of sperm factors is only modest (causing mosaicism) when compared to the negative impact of the female's age (causing aneuploidy).

REFERENCES

1. Mosher W. Infertility: why business is booming. Am Demograph 1987;42,43.
2. Mosher WD. Fecundity and infertility in the United States 1965-1982. Adv Data 1985;1:1.
3. Hull MGR, Glazener CMA, Kelly MJ, et al. Population study of causes treatment and outcome of infertility. Brit Med J 1985;291:1693–1697.
4. 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.
5. Silber SJ. Results of microsurgical vasoepididymostomy: role of epididymis in sperm maturation. Hum Reprod 1989;4:298–303.
6. Silber SJ. Microsurgery. The Williams & Wilkins Company, Waverly Press, Baltimore, MD, 1979.
7. Silber SJ. Reproductive Infertility Microsurgery in the Male and Female. Williams & Wilkins, Waverly Press, Baltimore, MD, 1984.
8. Silber SJ. Microscopic vasoepididymostomy: specific microanastomosis to the epididymal tubule. Fertil Steril 1978;30:565–571.
9. Silber SJ. Microscopic technique for reversal of vasectomy. Surg Gynecol Obst 1976;143: 630,631.
10. Silber SJ. Perfect anatomical reconstruction of vas deferens with a new microscopic surgical technique. Fertil Steril 1977;28:72–77.
11. Silber SJ. Microscopic vasectomy reversal. Fertil Steril 1977;28:1191–1202.
12. Silber SJ. Vasectomy and vasectomy reversal. Modern Trends 1978;29:125–140.
13. Silber SJ. Sperm granuloma and reversibility of vasectomy. Lancet 1977;2(8038):588–589.
14. Silber SJ. Vasoepididymostomy to the head of the epididymis: recovery of normal spermatozoal motility. Fertil Steril 1980;34:149–154.
15. Silber SJ. Epididymal extravasation following vasectomy as a cause for failure of vasectomy reversal. Fertil Steril 1979;31:309–316.
16. Silber SJ. Ejaculatory duct obstruction. J Urol 1980;124:294–297.
17. Silber SJ. Congenital absence of the vas deferens. N Engl J Med 1990;323:1788–1792.
18. Silber SJ, Nagy Z, Devroey P, et al. 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.
19. Silber SJ. Intracytoplasmic sperm injection (ICSI) today: a personal review. Hum Reprod 1998;13:208–218.
20. DeKretser DM, Burger HG, Hudson B. The relationship between germinal cells and serum FSH levels in males with infertility. J Clin Endocrinol Metab 1974;38:787–793.
21. 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.
22. Silber SJ, Van Steirteghem A, Nagy Z, et al. Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. Fertil Steril 1996;66:110–117.
23. Tournaye H, Liu J, Nagy PZ, et al. Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa. Hum Reprod 1996; 11:127–132.

24. Tournaye H, Verheyen G, Nagy P, et al. Are there any predictive factors for successful testicular sperm recovery in azoospermic patients? Hum Reprod 1997;12:80–86.
25. Silber SJ. Evaluation and treatment of male infertility. In: Blanco, JD, Keye, RK, eds. Clinical Obstetrics and Gynecology. 2000;43:854–888.
26. Silber SJ. Microsurgical testicular sperm extraction and the distribution of spermatogenesis in non-obstructive azoospermia. Hum Reprod 2000;15:2278–2284.
27. 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.
28. 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.
29. Charny CW. Testicular biopsy: its value in male sterility. JAMA 1940;115:1429–1432.
30. Nelson WO. Interpretation of testicular biopsy. JAMA 1953;151:1449.
31. Mannion RA, Cottrell TLC. Correlation between testicular biopsy and sperm count. J Urol 1961,85:953.
32. Albert A. The mammalian testis. In: Young WC, ed. Sex and Secretions, 3rd ed. Williams & Wilkins, Baltimore, MD, 1961, pp. 305–365.
33. Heller CG, Clermont Y. Kinetics of the germinal epithelium in man. Recent Prog Horm Res 1964;20:545.
34. Steinberger E, Tjioe DY. A method for quantitative analysis of human seminiferous epithelium. Hum Rreprod 1968;19:960–970.
35. Zukerman Z, Rodriguez-Rigau L, Weiss DB, et al. Quantitative analysis of the seminiferous epithelium in human testicle biopsies and the relation to spermatogenesis to sperm density. Fertil Steril 1978;30:448–455.
36. Clermont Y. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycles in spermatogonial renewal. Physiolog Rev 1978;52:198–236.
37. Silber SJ, Ord T, Balmaceda J, et al. Congenital absence of the vas deferens. The fertilizing capacity of human epididymal sperm. New Eng J Med 1990;323:1788–1792.
38. Silber SJ. New treatment for infertility due to congenital absence of vas deferens. Lancet 1987;850–851.
39. Silber SJ, Balmaceda J, Borero C, et al. Pregnancy with sperm aspiration from the proximal head of the epididymis: a new treatment for congenital absence of the vas deferens. Fertil Steril 1988;50:525–528.
40. Tournaye H, Devroey P, Liu J, et al. 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.
41. Silber SJ, Nagy ZP, Liu J, et al. Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. Hum Reprod 1994;9:1705–1709.
42. Silber SJ, Van Steirteghem AC, Liu J, et al. High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. Hum Reprod 1995;10:148–152.
43. Silber SJ, Nagy Z, Liu J, et al. The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. Hum Reprod 1995;10:2031–2043.
44. Devroey P, Liu J, Nagy Z, et al. Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. Hum Reprod 1995;10:1457–1460.

45. Silber SJ. Sertoli cell only revisited. Hum Reprod 1995;10:1031,1032.
46. 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.
47. Friedler S, Raziel A, Strassburger D, et al. Testicular sperm retrieval by percutaneous five needle sperm aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. Hum Reprod 1997;12:1488–1491.
48. Rosenlund B, Kvist U, Ploen L, et al. A comparison between open and percutaneous needle biopsies in men with azoospermia. Hum Reprod 1998;13:1266–1271.
49. Silber SJ, Johnson L. Are spermatid injections of any clinical value? ROSNI and ROSI revisited. Hum Reprod 1998;13:509–523.
50. Silber SJ, Johnson L, Verheyen G, Van Steirteghem A. Round spermatid injection. Fertil Steril 2000;73:897–900.
51. Holstein AF, Roosen-Runge ED, eds. Atlas of human spermatogenesis. Grosse Verlag, Berlin, 1981.
52. Nagy ZP, Liu J, Joris H, et al. The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters. Hum Reprod 1995;10:1123–1129.
53. Silber SJ, Devroey P, Tournaye H, et al. Fertilizing capacity of epididymal and testicular sperm using intracytoplasmic injection (ICSI). Reprod Fertil Dev 1995;7:281–293.
54. Silber S, Escudero T, Sadowy S, et al. Chromosomal abnormalities in embryos derived from testicular sperm extraction. Fertil Steril 2003;79:30–38.
55. Munne S, Marquez C, Reing A, et al. Chromosome abnormalities in embryos obtained after conventional in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril 1998a; 69:904–908.
56. Munne S, Cohen J. chromosome abnormalities in human embryos. Hum Reprod Update 1998b;4:842–855.
57. Silber SJ, Colls P, Zheng X, et al. Spermatogenic deficiency and chromosomal abnormalities. ESHRE 2004, Abstract.
58. Bonduelle M, Wilikens J, Buysse A, et al. Prospective study of 877 children born after intracytoplasmic sperm injection with ejaculated, epididymal, and testicular spermatozoa, and after replacement of cryopreserved embryos obtained after ICSI. Hum Reprod 1996;11:131–159.
59. Bonduelle M, Aytoz A, Wilikens A, et al. Prospective follow-up study of 1,987 children born after intracytoplasmic sperm injection (ICSI). In: Filicori, M, Flamigni C, eds. Treatment of Infertility: the New Frontiers. Communications Media for Education, Princeton, NJ, 1998, pp. 445–461.
60. Palermo GD, Schleel PN, Hariprashad JJ, et al. Fertilization and pregnancy outcome with intracytoplasmic sperm injection for azoospermic men. Hum Reprod 1999;14:741–748.
61. Bonduelle M, Aytoz A, Van Assche E, et al. Incidence of chromosomal aberrations in children born after assisted reproduction through intracytoplasmic sperm injection. Hum Reprod 1998b;13:781,782.
62. Bonduelle M, Camus M, De Vos A, et al. Seven years of intracytoplasmic sperm injection and follow-up of 1,987 subsequent children. Hum Reprod 1999;14:243–264.
63. Bonduelle M, Ponjaert I, Van Steirteghem A, et al. Developmental outcome at 2 years of age for children born after ICSI compared with children born after IVF. Hum Reprod 2003;18:342–350.
64. Vernaeve V, Bonduelle M, Tournaye H, et al. Pregnancy outcome and neonatal data of children born after ICSI using testicular sperm in obstructive and non-obstructive azoospermia. Hum Reprod 2003;18:2093–2097.
65. Bonduelle M, Van Assche E, Joris H, et al. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. Hum Reprod 2002;17(10):2600–2614.

66. Aboulghar H, Aboulghar M, Mansour R, et al. A prospective controlled study of karyotyping for 430 consecutive babies conceived through intracytoplasmic sperm injection. Fertil Steril 2001;76:249–253.
67. Abyholm T, Stray-Pedersen S. Hypospermiogenesis and chromosomal aberrations. A clinical study of azoospermic and oligospermic with normal and abnormal karyotype. Int J Androl 1981;4:546–558.
68. Egozcue J, Templado C, Vidal F, et al. Meiotic studies in a series of 1100 infertile and sterile males. Hum Genet 1983;65:185–188.
69. Pandiyan N, Jequier AM. Mitotic chromosomal anomalies among 1210 infertile men. Hum Reprod 1996;11:2604–2608.
70. Testart J, Gautier E, Brami C, et al. Intracytoplasmic sperm injection in infertile patients with structural chromosome abnormalities. Hum Reprod 1996;11:2609–2612.
71. Van Assche EV, Bonduelle M, Tournaye H, et al. Cytogenetics of infertile men. Hum Reprod 1996;11:1–26.
72. Yoshida A, Miura K, Shirai M. Chromosome abnormalities and male infertility. Assisted Reprod Rev 1996;6:93–99.
73. Huang WJ, Lamb DJ, Kim ED, et al. Germ-cell nondisjunction in testes biopsies of men with idiopathic infertility. Am J Hum Genet 1999;64:1638–1645.
74. Vegetti W, Van Assche E, Frias A, et al. Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence in situ hybridization in infertile men. Hum Reprod 2000;15:351–365.
75. Colombero LT, Hariprashad JJ, Tsai MC, et al. Incidence of sperm aneuploidy in relation to semen characteristics and assisted reproductive outcome. Fertil Steril 1999;72:90–96.
76. Verlinsky Y, Cieslak J, Ivakhnenko V, et al. Birth of healthy children after preimplantation diagnosis of common aneuploidies by polar body fluorescent in situ hybridization analysis. Fertil Steril 1996;66:126–129.
77. Munné S, Alikani M, Tomkin G, et al. Embryo morphology developmental rates and maternal age are correlated with chromosome abnormalities. Fertil Steril 1995b;64:382–391.
78. Marquez C, Sandalinas M, Bahce M, et al. Chromosome abnormalities in 1255 cleavage-stage embryos. Reprod Biomed Online 2000;1:17–27.
79. Evsikov S, Verlinsky Y. Mosaicism in the inner cell mass of human blastocysts. Hum Reprod 1998b;11:3151–3155.
80. Liu J, Nagy Z, Joris H, et al. Intracytoplasmic sperm injection does not require a special treatment of the spermatozoa. Hum Reprod 1994;9:1127–1130.
81. Munné S, Weier HUG, Grifo J, Cohen J. Chromosome mosaicism in human embryos. Biol Reprod 1994c;51:373–379.
82. Harper JC, Coonen E, Handyside AH, et al. Mosaicism of autosomes and sex chromosomes in morphologically normal, monospermic preimplantation human embryos. Prenatal Diagn 1995a;15:41–49.
83. Delhanty JDA, Harper JC, Ao A, et al. Multicolour FISH detects frequent chromosomal mosaicism and chaotic division in normal preimplantation embryos from fertile patients. Hum Genetics 1997;99:755–760.
84. Obasaju M, Kadam A, Sultan K, et al. Sperm quality may adversely affect the chromosome constitution of embryos that result form intracytoplasmic sperm injection. Fertil Steril 1999; 72:1113–1115.
85. Miharu N, Best RG, Young SR. Numerical chromosome abnormalities in spermatozoa in fertile and infertile men detected by fluorescence in situ hybridization. Hum Genet 1994; 93:502–506.

86. Moosani N, Pattinson HA, Carter MD, et al. Chromosomal analysis of sperm from men with idiopathic infertility using sperm karyotyping and fluorescence in situ hybridization. Fertil Steril 1995;64:81–817.
87. Bernardini I, Martini E, Geruedts PM, et al. Comparison of gonosomal aneuploidy in spermatozoa of normal fertile men and those with severe male factor detected by in situ hybridization. Mol Hum Reprod 1997;3:431–438.
88. Rives N, Mazurier S, Sibert L, et al. Incidence of aneuploidy in sperm nuclei of infertile men. Hum Reprod 1998;13(Suppl);Abstract O-245, 126,127.
89. Rives N, Mazurier S, Bellet D, et al. Assessment of autosome and gonosome disomy in human sperm nuclei by chromosome painting. Hum Genets 1998;102:616–623.
90. Pang MG, Hoegerman SF, Cuticchin AJ, et al. Detection of aneuploidy for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y by fluorescence in situ hybridization in spermatozoa from nine patients with oligoasthenoteratospermia undergoing intracytoplasmic sperm injection. Hum Reprod 1999;14:1266–1273.
91. Martin RH, Greene C, Rademaker A, et al. Chromosome analysis of spermatozoa extracted from men with non-obstructive azoospermia. Hum Reprod 2000;15:1121–1124.
92. Palermo GD, Colombero LT, Hariprashad JJ, et al. Chromosome analysis of epididymal and testicular sperm in azoospermic patients undergoing ICSI. Hum Reprod 2002;17:570–575.
93. Yogev L, Paz G, Yavetz H. Chromosome analysis of spermatozoa extracted from testes of men with non-obstructive azoospermia. Hum Reprod 2000;15:2685–2688.
94. Levron J, Aviram-Goldring A, Madjar I, et al. Sperm chromosomal abnormalities in men with severe male factor infertility who are undergoing in vitro fertilization with intracytoplasmic sperm injection. Fertil Steril 2001;63:479–484.
95. McInness B, Rademaker A, Greene CA, et al. Abnormalities for chromosomes 13 and 21 detected in spermatozoa from infertile men. Hum Reprod 1998;13:2787–2790.
96. Sathananthan AH, Ratnam SS, Ng SC, et al. The sperm centriole: Its inheritance, replication and perpetuation in early human embryos. Hum Reprod 1996;11:345–356.
97. Tournaye H, Devrowy P, Liu J, et al. 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.
98. Schatten G. The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. Dev Biol 1994;155:299–335.
99. Schatten H, Schatten G, Mazia D, et al. Behavior of centrosomes during fertilization and cell division in mouse oocytes and in sea urchin eggs. Proc Natl Acad Sci USA 1986;83: 105–109.
100. Palermo G, Munné S, Cohen J. The human zygote inherits its mitotic potential from the male gamete. Hum Reprod 1994;9:1220–1225.
101. Zhou H, Kuang J, Zhong L, et al. Tumor amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nature Genet 1998;20:189–193.
102. Doxsey S. The centrosome - a tiny organelle with big potential. Nature Genet 1998;20:104–106.
103. Sathasivam K, Woodman B, Mahal A, et al. Centrosome disorganization in fibroblast cultures derived from R6/2 Huntington's disease (HD) transgenic mice and HD patients. Human Molec Genet 2001;21:2425–2435.
104. Moomjy M, Colombero LT, Veeck L, et al. Sperm integrity is critical for normal mitotic division and early embryonic development. Molec Hum Reprod 1999;5(9):836–844.