Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest

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Objective: To see whether testicular sperm extraction could be used to perform intracytoplasmic sperm injection (ICSI) for men with nonobstructive azoospermia caused by maturation arrest.

Design: Uncontrolled prospective trial of an attempt to find occasional elongated spermatids or spermatozoa in testes of azoospermic patients with maturation arrest and to use these haploid cells for ICSI.

Setting: European university-based center for reproductive medicine and private American community hospital.

Patients: Thirty-eight azoospermic males without obstruction and with biopsy-documented maturation arrest, seven of whom elected, with their wives, to undergo scrotal exploration and testicular sperm extraction with ICSI in an attempt to become pregnant.

Interventions: Histologic evaluation of spermatid development in 38 patients with azoospermic maturation arrest. Testicular sperm extraction with ICSI in seven random volunteers from this group.

Main Outcome Measures: Presence or absence of mature spermatids in the testis biopsy specimen of patients with azoospermic maturation arrest. Fertilization, cleavage, and pregnancy after testicular sperm extraction and ICSI in patients with azoospermic maturation arrest.

Results: All seven patients with azoospermic maturation arrest had occasional sperm found with testicular sperm extraction. Five had sufficient numbers (between 6 and 30) for ICSI, and those five had ETs. In four, the partners became pregnant. In all 38 patients examined, the maturation defect was in meiosis rather than in spermigenesis.

Conclusion: Nonobstructive azoospermia caused by maturation arrest may be treated with testicular sperm extraction with ICSI apparently as successfully as Sertoli cell only.


Key Words: Testicular sperm extraction (TESE), intracytoplasmic sperm injection (ICSI), azoospermic maturation arrest

Until recently, it was thought that the major stumbling block to the treatment of infertile couples was the infertile male. However, with the development of intracytoplasmic sperm injection (ICSI), the presence of just a few, weakly twitching spermatozoa in the semen (i.e., severe oligoasthenozoospermia) is all that is required for normal fertilization and pregnancy (1, 2). Even men who appear to be azoospermic sometimes will be found to have very few numbers of spermatozoa noted after centrifugation of their ejaculate at 1,800 × g. In these cases also, normal fertilization and pregnancy can be achieved with ICSI (3). Thus, virtually all cases of oligoasthenoteratozoospermia now can be treated.

After this discovery, attention was turned to completely azoospermic patients. First, we developed the use of ICSI to treat obstructive azoospermia due to
congenital absence of the vas deferens and failed vasoepididymostomy or otherwise irreparable obstruction, using microsurgically retrieved epididymal sperm (4, 5). We coined this “MESA,” i.e., microsurgical epididymal sperm aspiration. Then we demonstrated the systematic use of ICSI with testicular sperm in cases in which there is either no epididymis or no motile sperm to be found in the epididymis (6, 7). Several months later, we demonstrated that ICSI using frozen thawed epididymal spermatozoa retrieved from a previous attempt at fresh microsurgical epididymal sperm aspiration was equally successful as using freshly retrieved sperm (8). The present state of the art appears to be that there are very few cases of obstructive azoospermia that cannot be treated successfully with ICSI so long as the wife has adequate eggs (9). This may involve the use of epididymal sperm or, if epididymal sperm cannot be retrieved, the use of testicular sperm. The only male infertility problems remaining to be solved are the cases of nonobstructive azoospermia, caused by “absent” or deficient spermatogenesis.

Applying the technique of testicular sperm extraction developed for obstructive azoospermia, it was found that even in azoospermic men with apparently absent spermatogenesis (diagnosed as “Sertoli cell only syndrome”), there very frequently is a tiny focus of sperm production still to be found somewhere in the testes (10–12). The original studies on quantification of spermatogenesis by histology demonstrated a puzzling number of completely azoospermic patients who had nonetheless demonstrated an average of one or two spermatids per seminiferous tubule (13–16). This went unaddressed in those early papers, but now it is apparent that an extremely diminished quantity of sperm production in the testes will result in absolute azoospermia in the ejaculate, even though there is some sperm being produced. A certain tiny threshold of sperm production is necessary before any sperm actually can appear in the ejaculate. Therefore, it was quite possible that very small, tiny numbers of spermatozoa might exist in the testes sufficient for an ICSI procedure, even in patients who are azoospermic apparently from “absence” of spermatogenesis. This observation led us to perform successful testicular exploration with sperm extraction for patients with azoospermia due to Sertoli cell only syndrome or cryptorchid testicular atrophy, who had high FSH levels, very small testes, apparently absent spermatogenesis, and no obstruction (11).

It remained then to tackle the problem of nonobstructive azoospermia caused by “maturation arrest.” Maturation arrest is characterized by normalized-sized testicles and normal early stages of spermatogenesis, but complete failure of reduction division, or meiosis, of pachytene spermatocytes to haploid spermatids (17). Maturation arrest is a common cause of nonobstructive azoospermia in sterile men with deficient spermatogenesis.

At first it had seemed logical to suppose that, in Sertoli cell only, there might be an occasional focus of spermatogenesis because of the prevalent theory that failure of germ cell migration to the primitive testes was the cause of Sertoli cell only syndrome and that presumably it would be possible that a few germ cells in such patients successfully made it to the primitive testes. Maturation arrest, however, appeared to be a genetic condition that, one would have surmised, should have affected the entire testicle with no focal areas of normal spermatid formation. However, it now seems likely that Sertoli cell only and maturation arrest both are mediated genetically, possibly by a common deletion in the same region of the Y chromosome (18). Therefore, we decided to perform extensive testicular explorations on men with nonobstructive azoospermia demonstrated to be caused by maturation arrest in an attempt to retrieve sperm for ICSI with the wife’s oocytes, in the same manner that originally was described by us for Sertoli cell only and postcryptorchidism testicular atrophy (11). A second purpose of this clinical study was to review in detail the histology of every case of azoospermic maturation arrest that we had seen between March 30, 1993 and August 2, 1995 in an effort to delineate accurately the nature of this deficient spermatogenesis in order to determine what would be the best approach for spermatozoa and spermatid retrieval with subsequent ICSI in these patients.

MATERIALS AND METHODS

Histologic Study

Thirty-eight azoospermic males with a diagnosis of maturation arrest were evaluated with detailed histology and Y chromosome mapping with the outlook toward eventually attempting to retrieve spermatids for subsequent ICSI. This histologic study was performed at the same time as 41 azoospermic males diagnosed as “Sertoli cell only syndrome” were studied with the same purpose in mind. This paper will concentrate on the patients with maturation arrest and then compare them with the Sertoli cell only group.

Azoospermia was defined as absence of spermatozoa in at least four ejaculates spun at 1,800 × g. Maturation arrest was defined as normal-sized testicles on physical examination, FSH within a normal range, demonstrated absence of any obstruction or ductal blockage, and testicular histology showing...
normal early stages of spermatogenesis, but an apparent absence of mature spermatids in the seminiferous tubules (Fig. 1). All maturation arrest patients were studied for the presence of various stages of spermatogonia, spermatocytes, immature round spermatids, and elongated mature spermatids on thin histologic sections fixed with either Zenker’s or Bouin’s solution.

**Intracytoplasmic Sperm Injection Cases**

Seven of these patients decided randomly by their own choice to enroll in our testicular sperm extraction-ICSI program and were the first patients with maturation arrest to attempt this procedure. There was no histologic or clinical difference between any of these seven patients and the others who have not yet enrolled in the program. All seven of these patients had azoospermia demonstrated by absence of spermatozoa on several centrifuged ejaculates, the presence of abundant spermatocytes on testicular biopsy, with the apparent absence of mature spermatids. At the same time, these seven patients with maturation arrest undergoing testicular sperm extraction-ICSI were compared with 18 patients with Sertoli cell only and 47 patients with irreparable obstructive azoospermia and normal spermatogenesis undergoing a similar procedure with their wives during the same intervals of time.

**Methodology for Testicular Sperm Retrieval**

**Testicular Sperm Extraction (TESE) and ICSI**

The surgical technique for testicular biopsy is extremely simple. A small 0.5-cm horizontal incision is made in the scrotal skin and carried through the peritoneal tunica vaginalis. A small incision is made in the tunica albuginea, and a small piece of extrud-

ing testicular tissue is excised and placed in a Petri dish with 3 mL of HEPES-buffered Earle’s medium (HEPES from Irvine Scientific, Santa Ana, CA). The tunica albuginea and scrotal skin are closed with several 3-0 vicryl stitches. After each biopsy, the tissue is prepared and examined in the adjacent IVF laboratory before any subsequent biopsies are made in other areas of the testicle or testicles. If no sperm are found after extensive search, then another biopsy is performed. For cases of nonobstructive azoospermia, up to four or five biopsies are performed if necessary on each testicle.

The testicular tissue is minced finely in HEPES-buffered medium, and this effluent then is placed in a 5-mL Falcon tube (Becton Dickinson Labware, Lincoln Park, NJ) and centrifuged for 5 minutes at 300 × g (6, 7, 9, 19). The supernatant is removed and the pellet is suspended in medium. Percoll separation is not attempted. Therefore, one has no choice but to pick the individual sperm up out of the field of debris, red blood cells, and Sertoli cells for ICSI. The sperm suspension is kept at 37°C in the incubator (5% O₂, 5% CO₂, 90% N₂) in Earle’s medium until the time for the intracytoplasmic injection procedure to be performed on the oocytes.

The sperm droplet is not placed directly into the 10% polyvinylpyrrolidone (PVP; Sigma, St. Louis, MO) droplet as is done with most ICSI cases. Rather, the individual barely motile sperm is picked up out of the droplet of testicular concentrate and then placed into the PVP droplet, which usually then results in complete immotility. The tail of the sperm in all cases should be broken still, because these sperm may later exhibit increased motility. In cases of nonobstructive azoospermia, in which the numbers of sperm retrieved from the testis are often <25, as many as 20, 5-μL droplets of testicular suspension are placed in each ICSI dish, so that a substantial part of the testicular suspension can be searched for sperm.

**Oocyte Preparation for the Intracytoplasmic Injection Procedure**

Oocyte retrieval is carried out by vaginal ultrasound-guided puncture 36 hours after hCG. After the oocyte retrieval, the cells of the cumulus and corona radiata are removed by incubation for 30 to 90 seconds in HEPES-buffered Earle’s medium with 80 IU hyaluronidase/mL (Type VIII, specific activity 320 IU/mg; Sigma). The removal of the cumulus and corona cells is enhanced by aspiration of the complexes in and out of hand-drawn glass pipettes with serially smaller openings of approximately 300 to 150 μm. Afterward, the oocytes are rinsed several times in droplets of the same medium with 0.5%
bovine serum albumin (BSA) to clean off completely all of the granulosa cells. The oocytes then are placed in Menezo’s B2 medium (Fertility Technologies, Inc., Natick, ME) and carefully observed under the inverted microscope at ×200 magnification. This includes an assessment of the oocyte and the zona pel lucida, noting the presence or absence of a germinal vesicle or the first polar body. The oocytes then are incubated in 25-μL microdrops of B2 medium covered by lightweight paraffin oil (Sigma) at 37°C in an atmosphere of 5% O2, 5% CO2, and 90% N2. Just before ICSI, the oocytes are observed again to see whether more oocytes have extruded the first polar body. Intracytoplasmic sperm injection is carried out on all morphologically intact oocytes that have extruded the first polar body.

**Intracytoplasmic Sperm Injection Procedure**

For testicular sperm that are often barely motile, the set-up is different from epididymal or ejaculated sperm. A 3 to 5 μL sperm droplet is placed in the center of the Petri dish (Falcon type 1006) right next to a 5-μL PVP droplet and is surrounded by eight 5-μL droplets of HEPES-buffered medium with 0.5% BSA. These droplets are covered by approximately 3.5 mL of lightweight paraffin oil. In very difficult cases with very few sperm, 20 5-μL droplets of testicular suspension are placed in rows of five in the injection dish, with 1 5-μL droplet of PVP (reserved for collection and storage of sperm), and 3 to 8 5-μL droplets of HEPES-buffered medium are reserved for the eggs.

For testicular sperm, a single weakly motile spermatozoon, wherever found, is retrieved from a sperm droplet by tail-first aspiration into the tip of the injection pipette. The spermatozoon then is placed in the separate 5-μL droplet of 10% PVP in HEPES-buffered Earle’s medium for storage. The tail is broken with the tip of the injection pipette to immobilize the sperm. Each 5-μL droplet with testis suspension is searched carefully until a spermatozoon is located, retrieved, and then placed in the PVP droplet for storage. Once eight or more spermatozoa (depending on the number of eggs available) have been found and stored, eggs are placed in each of the eight HEPES-buffered Earle’s droplets for injection. The Petri dish then is placed in a position to visualize an oocyte in one of these droplets. The oocyte is immobilized by slight negative pressure exerted on the holding pipette. The polar body is held at six o’clock and the micropipette with an aspirated sperm in a head-first position is pushed through the zona pellucida and the oolemma into the ooplasm at three o’clock. A single spermatozoon is injected into the ooplasm with approximately 1 to 2 pL of medium.

The injection pipette then is withdrawn gently and the injected oocyte is released from the holding pipette. The aspiration of a single spermatozoon and injection into the ooplasm thus is repeated until all metaphase II oocytes are injected. The injected oocytes then are washed in B2 medium and transferred into 25-μL droplets of B2 medium covered by lightweight paraffin oil. The Petri dishes with the oocytes then are incubated (Heraeus, B5060 EK/O2; Van der Heyden, Brussels, Belgium) at 37°C, 5% CO2, 5% O2, and 90% N2.

**Assessment of Fertilization, Embryo Cleavage, and Pregnancy**

Further handling of the injected oocytes is similar to our standard IVF procedure. Approximately 16 to 18 hours after microinjection, the oocytes are observed under the inverted microscope (×200 or ×400 magnification) for any sign of damage that may have been due to the microinjection and for the presence of pronuclei and polar bodies. Fertilization is considered normal when two clearly distinct pronuclei containing nucleoli are present. The presence of one pronucleus or three pronuclei is noted together with the presence of one, two, or fragmented polar bodies. If a single pronucleus is observed, a second evaluation is carried out approximately 4 hours later to see whether the pronuclear status had changed. The embryo cleavage of the 2 pronuclear oocytes is evaluated after further 24 hours of in vitro culture. The embryos are scored according to the equality of size of the blastomeres and the number of anucleate fragments. Cleaved embryos with <50% of their volume filled with anucleate fragments are eligible for transfer. Up to three and occasionally more embryos (depending on the age of the wife and the embryo quality) are loaded in a few microliters of Earle’s medium into a Frydman catheter (LG 4.5; Prodimed, Neuillyen-Thelle, France) and transferred into the uterine cavity. Embryo replacement usually is done approximately 48 hours after the microinjection procedure.

Pregnancy is confirmed by detecting increasing serum hCG concentrations on at least two occasions ≥12 days after embryo replacement. Clinical pregnancy is determined by observing a gestational sac by means of echographic screening at 7 weeks of pregnancy. Patients are asked to provide detailed information of the evolution of the pregnancy and the outcome of the delivery.

**Evaluation of Testicular Histology**

The histologic sections were prepared very carefully from the beginning by excising the seminiferous tubules with a wet, sharp micro Iris scissors using a “no touch” method allowing the specimen to
Table 1  Testicular Sperm ICSI

<table>
<thead>
<tr>
<th></th>
<th>Maturation arrest</th>
<th>Sertoli cell only</th>
<th>Normal spermatogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>7</td>
<td>18</td>
<td>47</td>
</tr>
<tr>
<td>No. of cycles with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>insufficient sperm for ICSI</td>
<td>2 (29)</td>
<td>7 (39)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>No. of cycles with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>transfer</td>
<td>5 (71)</td>
<td>10 (56)</td>
<td>42 (89)</td>
</tr>
<tr>
<td>No. of clinical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pregnancies</td>
<td>4</td>
<td>5</td>
<td>17</td>
</tr>
</tbody>
</table>

*Values in parentheses are percentages.

fall into Zenker’s solution without being handled. The specimens were stained with hematoxylin and eosin and cut into thin sections. The biopsy was not considered valid if ≥20 seminiferous tubules could not be evaluated. All of the steps of spermatogenesis from spermatogonia through resting spermatocyte, leptotene spermatocyte, zygotene spermatocyte, pachytene spermatocyte, round early spermatids (S-A and S-B), and late mature spermatids (S-C and S-D) were evaluated. The number of Sertoli cells were not counted routinely because previous studies have demonstrated great variability in Sertoli cell population, which does not serve as a useful guide. Sertoli cell only was defined as a biopsy showing predominantly seminiferous tubules with only Sertoli cells and no spermatogenesis whatsoever. A single, isolated tubule with spermatogenesis in a field of tubules that were strictly Sertoli cell only still were defined as Sertoli cell only but with focal spermatogenesis. Maturation arrest was defined as an apparent absence of any mature spermatids despite normal early stages of spermatogenesis. The presence of a rare spermatid in an entire slide of otherwise total maturation arrest did not change the diagnosis.

RESULTS

Intracytoplasmic Sperm Injection, Fertilization, and Pregnancy Rates

Of seven patients with azoospermia due to maturation arrest, a very few number of spermatozoa and spermatids (<30) were found in all seven. However, in two cases, only one or two spermatids were found for ICSI after a very intensive search and, in these cases, there was no fertilization. Thus, two of seven cases did not have sufficient sperm or spermatids retrievable for successful ICSI. However, five of seven did. Table 1 demonstrates that five of seven patients with “complete” maturation arrest causing azoospermia had ET, and four have developed healthy, ongoing pregnancies. The two pronuclei fertilization rate was 42%, and the cleavage rate was 80%, which is not significantly different from Sertoli cell only cases or obstructive azoospermia with normal spermatogenesis (Table 2). The implantation rate for maturation arrest patients was 24%, which was similar to the implantation rate with Sertoli cell only patients of 26%, and compared favorably to an implantation rate reported in our studies with obstructive azoospermia and testicular sperm of 18% (7, 9). The pregnancy rates with testicular sperm extraction and ICSI were 57% for maturation arrest (only 7 cases), 28% for Sertoli cell only (only 18 cases), and, as has been reported already, 36% for obstructive azoospermia with normal spermatogenesis (7). Thus, there was no apparent decrease in implantation rate or pregnancy rate in patients with testicular-derived sperm who had maturation arrest or Sertoli cell only compared with those with normal spermatogenesis.

The incidence of insufficient sperm being retrievable for ICSI on testicular exploration in our azoospermic Sertoli cell only population was 39%, and our incidence of insufficient sperm in the azoospermic maturation arrest population was 29%. In the population of patients with obstructive azoospermia and normal spermatogenesis, all patients had sufficient sperm recoverable from testicle biopsy for ICSI.

In many instances, the elongated spermatids had to be dissected out of the Sertoli cell with the ICSI pipette, because of the absence of available free sperm in the testicular effluent. This appeared to cause no discernable difference in results. Finding enough sperm to inject into the available eggs from the wife required many hours of examination of every single microdroplet prepared from the entire specimen of centrifuged testicular effluent. It is entirely possible that, in the cases in which insufficient sperm were recoverable, if we had looked longer and harder or if we had taken more samples of testicular tissue, perhaps more sperm would have been recoverable. A subjective aspect of the procedure was that,

Table 2  Testicular Sperm ICSI

<table>
<thead>
<tr>
<th></th>
<th>Maturation arrest</th>
<th>Sertoli cell only</th>
<th>Normal spermatogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of metaphase II oocytes</td>
<td>72</td>
<td>151</td>
<td>570</td>
</tr>
<tr>
<td>No. of two pronuclei fertilization</td>
<td>30 (42)</td>
<td>57 (38)</td>
<td>269 (47)</td>
</tr>
<tr>
<td>No. of cleaved embryos (&lt;50% fragmentation)</td>
<td>24 (80)</td>
<td>35 (61)</td>
<td>163 (61)</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>21</td>
<td>31</td>
<td>143</td>
</tr>
<tr>
<td>No. of fetal sacs</td>
<td>5 (24)</td>
<td>8 (26)</td>
<td>26 (18)</td>
</tr>
</tbody>
</table>

*Values in parentheses are percentages.
after many hours, the procedure would be terminated even though, of course, all of the testicular tissue obviously could not be evaluated. There was no real apparent difference in any of the technical issues or problems encountered by the laboratory in dealing with maturation arrest as opposed to Sertoli cell only cases.

Testicular Histology in Maturation Arrest

In all of the cases of maturation arrest examined in this study, as defined by azoospermia, absence of obstruction, and the presence of only the early stages of spermatogenesis throughout the testicle biopsy, with normal-sized testes and a normal FSH level, the block in spermatogenesis was at the pachytene spermatocyte (Fig. 2A and B). The first meiotic division was not being completed in the vast majority of tubules in any of these patients. However, a thorough search often revealed very occasional round and elongated spermatids. We saw no evidence of round spermatids failing to develop via “spermiogen-

esis” into elongated, mature spermatids and spermatozoa. Whenever occasional round spermatids were observed in a focal area of a maturation arrest slide, mature elongated spermatids also were observed. Thus, the phenomenon of maturation arrest appears to occur just before the completion of the first meiotic division at the pachytene spermatocyte level. There did not appear to be a problem in the development of the haploid cell from round spermatid to elongated spermatid. The only problem with “maturation” was the failure of meiosis.

This histologic finding correlated with our observation that “elongated” spermatozoa were recovered in all seven patients in whom exhaustive testicular exploration was performed in an effort to retrieve spermatozoa for an ICSI procedure with the patient’s wife. We did not find round spermatids in the absence of elongated spermatids. The average number of mature spermatids per tubule (determined by adding up the total number of mature spermatids in the histologic section and dividing by the number of tubules evaluated) was less than one in both groups of patients. With obstructive azoospermia and normal spermatogenesis by contrast, the average number of mature spermatids per tubule is consistently >15 (16). It should be clarified that a spermatid is defined as any haploid germ cell still contained within the Sertoli cell. Elongated spermatids obviously have the appearance of being no different from spermatozoa, except that they have not yet been released. Released sperm and the tails of sperm and spermatids usually are not seen on testicular histology.

DISCUSSION

Male infertility can be divided into oligoasthenoteratozoospermia and azoospermia. Virtually all degrees of the most severe cases of oligoasthenoteratozoospermia now can be treated successfully with ICSI so long as metaphase II oocytes are retrievable from the female partner (3). In fact, the only obstacle to successful pregnancy in the most severe cases of oligoasthenoteratozoospermia is the age and ovarian reserve of the female partner. The goals of this present paper were first to review the evolution of our attempts at managing azoospermia in general and then to concentrate on our experience with azoospermia caused by maturation arrest.

Obstructive azoospermia can be treated quite successfully either with microsurgery, or in irreparable cases such as congenital absence of the vas, with either sperm retrieval from the epididymis or extraction of sperm from a small piece of testicular tissue. It is clear now that there are no cases of obstructive azoospermia that cannot be treated successfully.
Furthermore, it has become apparent from testicular explorations in patients with azoospermia caused by Sertoli cell only syndrome and postcryptorchidism testicular atrophy, as well as some as of yet unpublished cases with postchemotherapy azoospermia and even Klinefelter’s Syndrome, that in a majority of such seemingly hopeless patients, there is some tiny, minimal degree of spermatogenesis taking place somewhere in the testicles (7, 9, 10–12). If >10 sperm can be retrieved from such patients and if the wife has sufficient oocytes, it is no longer controversial that normal fertilization and pregnancy can be achieved (11). It has not been addressed yet whether, in those patients with Sertoli cell only in whom we do not find sufficient sperm for ICSI (39%), there is any benefit to prior diagnostic testicle biopsy or whether vasectomy and epididymal sperm retrieval could help locate the very few sperm that eluded detection with testicular sperm extraction.

The focus of this study is whether results comparable to Sertoli cell only can be achieved in patients with azoospermia due to maturation arrest. It is very clear that the two pronuclear fertilization rate using ICSI is somewhat less for testicular and epididymal sperm than has been reported for ejaculated sperm. This appears to be the case regardless of whether the patient has normal spermatogenesis or severely deficient spermatogenesis and whether the diagnosis is Sertoli cell only or maturation arrest (19). There is no difference between the results with maturation arrest, Sertoli cell only, or normal spermatogenesis. The cleavage rate and implantation rate is also no different in any of these groups. Even when less than five sperm are retrievable from the testes, the two pronuclear fertilization rate is no different than with normal spermatogenesis in which millions of testicular sperm are retrieved. The pregnancy rate and implantation rate also seem comparable to that already reported with ejaculated sperm and with testicular sperm in cases of normal spermatogenesis.

Histology and detailed Y chromosomal mapping indicates that maturation arrest and Sertoli cell only simply may be different expressions of the same genetic problem (18). Our findings in this study indicate that the same success rate is to be expected with nonobstructive azoospermia caused by maturation arrest as with Sertoli cell only syndrome and cryptorchid testicular atrophy. This is consistent with the view that most cases of spermatogenic arrest are probably incomplete and that, if you look very hard, you usually can find one or two spermatids in what would appear otherwise to be complete maturation arrest.

There has, however, been very little modern, quantitative histologic evaluation reported on maturation arrest. In the current study, there was no case of so-called “spermiogenic” arrest, i.e., arrest of spermatid maturation from the round to the elongated stage. The present experience would indicate that maturation arrest appears to be a problem in meiosis. If that is true, then the solution for most such cases would not depend necessarily upon trying to find round spermatids, although that certainly is something that could be attempted. Ogura and Yanagimachi (20) and Sofikitis et al. (21) have demonstrated the feasibility of such an approach. Another approach, as demonstrated herein, is to collect and inject the few elongated spermatids or released spermatozoa produced somewhere in such testicles and only to resort to round spermatids when there are not sufficient elongated spermatids to inject. What we thought to be complete maturation arrest was in most instances incomplete. This surprising finding is consistent with the notion that Sertoli cell only and maturation arrest may be coexisting conditions related to similar genetic causes.

The classic studies on quantification of spermatogenesis seem to be confirmed by this study. Clermont (13) first brought light into the details of spermatogenesis and demonstrated that it is “a long, elaborate process by which spermatogonial stem cells produce highly differentiated haploid cells, the spermatozoa: the first phase during which spermatocytes are produced as a result of spermatogonial mitotic divisions, the second phase during which spermatids are produced as a consequence of the meiotic divisions of the spermatocytes, and the third phase during which spermatozoa are formed as a result of metamorphosis of spermatids.” Clermont (13) noted that for a given species each step of spermatogenesis has a constant duration and, therefore, the germ cells differentiate and unfold in a regulated, rigidly timed program. Poor spermatogenesis is never related to a slowing of the rate of spermatogenesis, but only to a breakdown of the transition from early cell type to more advanced cell type. The seminiferous epithelium is composed of five or six generations of germ cells that are not arranged randomly but form specific cellular associations. Because of the precise and regular timing of the steps of spermatogenesis, spermatids at a given step always are associated with spermatocytes and spermatogonia at given steps in their development. These cellular groupings Clermont (13) defined as the cycle of the seminiferous epithelium.

Heller and Clermont (22) determined the specific time interval of these cellular associations and found that the duration of both the cycle of the seminiferous epithelium and the cycle of a single generation of spermatogenesis was 16 days and that the whole of spermatogenesis extends over a duration of 4.6 cycles, thus requiring approximately 74 days.
mone stimulation had no affect whatsoever on this pattern (22). It is at meiosis that the problem of maturation arrest appears to be concentrated (23).

It may seem puzzling at first as to why most of the patients with spermatogenic maturation arrest had FSH levels within the normal range. There is often an expectation that serum FSH level is related inversely to the sperm count or sperm production rate. However, it has been known for >20 years that the serum FSH really is not influenced directly by the sperm count or the number of mature or, for that matter, immature spermatids (24). Rather, the FSH level is related inversely to the total number of spermatogonia and the only relationship between FSH and the more advanced stages of spermatogenesis is a very indirect one, depending strictly on the primary inverse relationship between FSH and spermatogonia. Therefore, patients with maturation arrest could be expected to have normal FSH levels, whereas patients with Sertoli cell only would be expected to have elevated FSH levels (25).

What can we expect from the offspring of these previously sterile men? One can only speculate at this moment, but the growing assumption that spermatogenesis is affected by defects in the Y chromosome would have us speculate that most likely the male offspring will grow up to have the same spermatogenic defect as their father. Hopefully, however, this condition will be as readily treatable in the offspring when they grow up as it has been in their father. When this possibility was presented to these patients, they accepted it and thus far have expressed no reservation about attempting the procedure. However, future study will be necessary on a larger cohort of children and caution must be exercised.

REFERENCES