We perform and, in fact, are pioneers in sperm aspiration here at The New York Presbyterian Hospital-Cornell Medical Center. Sperm aspiration involves extraction of sperm from either the ducts leading out from the testicle (vas deferens or epididymis) or from the testicle itself. Sperm obtained by aspiration can only be used for in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). This is because aspirated sperm in men who are blocked do not swim well and will not fertilize the egg unless the sperm is injected directly into the egg (ICSI). For men undergoing microsurgical vaso-vasostomy on at least one side, we usually do not recommend aspiration of sperm at the time of the surgery because our success rate for return of sperm in the semen after vaso-vasostomy is 99.5%. If a vaso-epididymostomy is necessary on both sides however, the success rate drops to 80% for return of sperm. Therefore in all men undergoing only vaso-epididymostomy, we recommend the aspiration and freezing of sperm in the operating room at the time of surgery. This will allow sperm to be available for future IVF in the event that the surgery to repair the blockage is not successful.

Sperm aspiration may be performed instead of vasectomy reversal. We usually do not recommend this as the primary treatment, because it also requires the wife to go through an IVF procedure. This requires daily hormone injections for 30 days, at the end of which time the wife undergoes sonograms on a daily basis to determine when the follicles are ripe. When the follicles are ripe, the wife is brought into the hospital and either put to sleep or given heavy sedation. The eggs are then removed through the vagina with long needles. The sperm that was aspirated are injected directly into the egg. If the eggs fertilize, three or five days later they are transferred back into the wife with a plastic catheter. Currently the cost of the IVF with ICSI is $15,000 for each attempt. This does not include the cost of aspirating sperm from the man. A single attempt yields approximately a 45% chance of taking home a baby if the wife is under 35. This procedure must be repeated for each attempt at pregnancy and for each future pregnancy.

Because the reversal of vasectomy yields pregnancy rates between 50% and 70% with natural intercourse, we usually recommend sperm aspiration as a secondary procedure. That is, if sperm are present in the semen after the reversal operation, but the wife is not getting pregnant, then IVF with ICSI can be employed using sperm from the semen of the man without him requiring another operation. By performing a vasectomy reversal and if necessary, sperm aspiration simultaneously, all bets are covered.
For those who desire only sperm aspiration, this may be done in two ways. Under general anesthesia, the ducts of the epididymis are exposed under a microscope, the epididymis is punctured, the sperm is then sucked into glass pipettes and placed into a special medium for freezing. Currently the pregnancy rate with IVF and ICSI using frozen sperm is virtually identical to that with fresh sperm. Enough sperm can be extracted and frozen at a single operation for at least 4 or 5 attempts at in-vitro fertilization. This operation is less extensive than the reversal operation, but does require a general anesthetic and does require a cut into the scrotum. This operation will take approximately 1-2 hours to perform.

A second way to obtain sperm is by putting a needle directly into the testicle under either a local or light general anesthetic. This is called a non-surgical sperm aspiration technique. We perform these here at Cornell as well. Because sperm obtained this way are much fewer in number, and cannot be frozen, this procedure must be done simultaneously with the attempt at IVF. With this procedure it is necessary for your wife to be seen at Cornell’s IVF program so that we can coordinate the aspiration of testicular sperm with the IVF cycle. Currently the pregnancy rates with testicular sperm are lower than the pregnancy rates using aspirated epididymal or vasal sperm obtained surgically or after reversal of the vasectomy. The complications of blind needle aspiration of sperm (non-surgical sperm aspiration technique) are injury to the blood vessels of the testicle, which have, on rare occasion, resulted in atrophy (disappearance of, or diminished in size) of the affected testicle.

I would be happy to discuss all of these options for fertility after vasectomy with you at the time of our consultation or over the phone.
Information Regarding Financial Arrangement, Cost and Insurance for Sperm Aspiration

1. The surgeon’s fee, including the post-operative visit, is estimated at $12,650. Most insurance companies are paying large portions of this fee. Because of the elective nature of this surgery, a **$6,325 deposit is required four weeks in advance of your surgery.** The balance is due one month after the surgery. We will accept payment with a credit card, cash, money order or a personal check made payable to Dr. Marc Goldstein. This fee is refundable in full if my office is notified of your intent to cancel at least two weeks prior to your surgery date.

2. You will be billed separately for anesthesia fees, which we estimate at approximately $4,500. This must be paid directly to your anesthesiologist.

3. Hospitalization and laboratory costs tend to vary, but the basic cost for the operating room and hospital room averages $14,000 to $16,000. Some insurance companies are paying these hospitalization costs.

4. Your insurance company is more likely to pay for your surgery if your policy does not specifically exclude fertility treatment. Please be advised that if fertility treatment is an excluded service from your policy, you may not be reimbursed. Unfortunately, we have no way of determining the extent of your insurance coverage until the claim is filed. To maximize your insurance reimbursement for microsurgical vasovasostomy, vasoepididymostomy, aspiration of sperm from the ducts (vas deferens or epididymis) or testis your diagnosis will be recorded as: Bilateral Epididymitis with Obstruction (ICD 604.90), Other Unspecified Anomalies of the Genital Organs (ICD 752.89), Stricture of the Genital Ducts (ICD 608.85). The operation is: Microsurgical epididymal sperm aspiration (CPT 54865-22): $8,000; Scrotal exploration (CPT 55110): $3,000; Sperm identification from aspiration (CPT 89257): $550; Cytopathology evaluation of aspirate (CPT 88172): $550; Insertion Urinary Catheterization (CPT 51701): $550; Testis Biopsy (CPT 54505-50-22) $6,500.

5. Our office will help you to obtain maximum reimbursement from your insurance company. After your surgery, we will send an insurance claim form and a bill to you for submittance to your insurance company. You may also sign the bottom portion of the surgery cancellation policy form and we will submit your claim to the insurance company on your behalf. Reimbursement can be sent directly to you or to our office.

If you wish further information on any matters, please feel free to call or write.
A SIMPLIFIED METHOD OF EPIDIDYMAL SPERM ASPIRATION

Gerald J. Matthews, and Marc Goldstein

ABSTRACT
We present a simple technique of epididymal sperm aspiration that uses inexpensive and readily available materials. Men undergoing epididymal reconstruction with vasoepididymostomy or autogenous sperm reservoir had sperm aspiration for cryopreservation. Mean total and total motile sperm per aspirate recovered from 25 men have been $25.1 \pm 4.8 \times 10^6$ and $4.0 \pm 1.4 \times 10^6$, respectively. Two ongoing pregnancies have been achieved with intracytoplasmic sperm injection using thawed epididymal sperm. Sperm aspiration and cryopreservation maximize a couple's fertility potential with a single procedure and provide a viable fertility alternative to a second surgical procedure in the event of a primary reconstructive failure. UROLOGY* 47: 123-125, 1996.

Men undergoing a microsurgical vasoepididymostomy (VE) or an autogenous sperm reservoir (ASR) procedure face a significant possibility of reconstructive failure. We have previously reported that the likelihood of a durable response, as measured by the recovery of motile sperm either in the ejaculate following a VE or from a percutaneous aspiration of an ASR is 52% and 47%, respectively. For men who fail initial reconstructive attempts, the only alternative available for those desiring a pregnancy is a repeat surgical procedure. However, if sperm were recovered and cryopreserved at the time of the initial surgery, an alternative to a second surgical procedure can be considered.

We have attempted to maximize ultimate success in a single procedure by using a simple and atraumatic method of sperm recovery at the time of epididymal reconstruction. This method uses inexpensive materials, readily available in most operating theaters. We currently offer all men with epididymal obstruction the option for simultaneous epididymal sperm aspiration and cryopreservation.

TECHNIQUE
Our technique for the construction of an ASR and for microsurgical VE has previously been reported. Prior to reconstruction and epididymal sperm recovery, communication with the sperm bank or in vitro fertilization (IVF) laboratory is made to ensure the timely processing and cryopreservation of the aspirate. Using an operating microscope, epididymal tubule dissection and aspiration are performed using \( \times 15 \) to \( \times 32 \) magnification. The epididymal tubule and tunic are prepared for either VE or an ASR. Meticulous hemostasis is obtained with micro-bipolar forceps prior to aspiration, as the presence of erythrocytes and leukocytes has been demonstrated to diminish sperm function.

The most distal tubule containing clear or opalescent fluid should be selected. Tubules containing yellow inspissated material should be avoided. A 0.5- to 1.5-mm buttonhole is made in the epididymal tubule with fine blunt-tipped microscissors. Alternatively, the epididymal tubule may be sharply incised with a microknife. After opening the epididymal tubule, a 5-\( \mu \)L micropipette with a 0.5-mm internal diameter, 0.9-mm outer diameter, and scored at 1-\( \mu \)L intervals (Drummond Scientific Co., Broomall, Pa.) is placed adjacent to the effluxing epididymal tubule. A standard hematocrit pipette is also satisfactory and readily available.

Sperm are drawn into the micropipette by simple capillary action. Negative pressure, as is generated by action of an in-line syringe, should not be applied during sperm recovery, as this may damage the delicate epididymal mucosa. For this reason, we do not recommend a syringe and angiocatheter technique. The micropipette/capillary action technique provides a direct visual confirmation and quantification of epididymal fluid recovery (Fig. 1). Air drawn into a syringe during the negative pressure aspiration of epididymal fluid results in less precise volume quantification and a more difficult transfer into buffer. Multiple micropipettes may be used simultaneously to increase speed of recovery. With patience, 10 to 20 \( \mu \)L of epididymal fluid is easily recovered in no more than 5 to 10 minutes.

The highest rate of flow is observed immediately following incision of the tubule; however, progressively better quality sperm are found following the
initial washout. Gentle compression of the testis and epididymis enhances flow from the incised tubule. During fluid recovery, a drop is examined under the microscope to confirm the presence of sperm. Since processing techniques, including pentoxifylline incubation, stimulate sperm motility, we aspirate in the presence of both motile and nonmotile sperm. If intact sperm are not encountered, then a more proximal epididymotomy is performed.

The micropipette is connected to a short (3 to 5 cm) segment of medical grade silicone tubing (American Scientific Products, McGaw Park, Ill) (Fig. 2). Alternatively, the tubing attached to a butterfly needle may be used. A 20-gauge needle fitted to a Luer-tip syringe is then placed in line (Fig. 2). The fluid is flushed into a sterile container of buffer solution (0.5 to 1.0 mL) obtained from the sperm-processing laboratory. Once a micropipette has been used, it is discarded. Residual fluid in the pipette will disrupt capillary action. A typical procedure requires 4 to 8 micropipettes.

The sperm bank is instructed to cryopreserve the aspirate in multiple straws ( aliquots), so that several IVF cycles may be used if required. At our institution, epididymal aspirates are diluted in an equal volume of glycerol cryoprotectant. Aliquots are then slowly cooled to 4°C, transferred to a sequential freezer for refrigeration to −90°C prior to immersion in liquid nitrogen (−196°C).

RESULTS

The results of atraumatic epididymal sperm aspirations are presented for the last 25 men undergoing either a VE procedure (n = 15) or the creation of an ASR (n = 10) (Table I). In each case, epididymal fluid was rapidly recovered with no more than 10 minutes of added surgical time. Motile sperm were recovered from the epididymides and cryopreserved in 21 of 25 (84%) men. As the epididymal aspirate was immediately diluted in a buffer solution, aspirate volume and sperm density recorded by the processing labora-

FIGURE 1. The technique of atraumatic sperm recovery by simple capillary action.

FIGURE 2. Materials (micropipettes, silicone tubing) used for atraumatic sperm recovery.

tory do not accurately represent the initial aspirate parameters. Semen parameters reported are limited to percent motility, total sperm per aspirate, and total motile sperm per aspirate.

Two couples, the male partners with congenital absence of the vas deferens and having undergone the microsurgical creation of an ASR, elected to undergo an IVF cycle with aspirated cryopreserved sperm prior to an evaluation of reservoir status. Two ongoing pregnancies have been established for these couples using IVF with intracytoplasmic sperm injection (ICSI).

COMMENT

In reviewing treatment outcomes following 100 consecutive VE procedures, we observed the return of motile sperm in only 52 men.1 Subsequently, 21% (11 of 52), after initial demonstration of patency with motile sperm, have experienced a late anastomotic failure.1 Similarly, motile sperm have been recovered by percutaneous aspiration from only 47% of men undergoing an ASR procedure.2 For the majority who fail epididymal reconstruction, fertility treatment options necessitate additional surgical procedures.

The technique of atraumatic epididymal sperm aspiration presented provides couples with an alternative to surgery following reconstructive failure. The method of sperm recovery presented should not be confused with the technique of epididymal micropuncture with sperm aspiration (MESA).5 Epididymal micropuncture requires highly modified micropipettes used to puncture into the lumen of an individual epididymal tubule. These micropipettes are not commercially available and must be hand-manufactured from commercial stock. The
**TABLE I. Aspirate parameters are summarized for the entire cohort and for men undergoing either a vasoepididymostomy procedure or the creation of an autogenous sperm reservoir**

<table>
<thead>
<tr>
<th></th>
<th>Motility</th>
<th>Total Sperm/Aspirate</th>
<th>Total Motile Sperm/Aspirate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohort (n = 25)</strong></td>
<td>15.2 ± 3.7</td>
<td>25.1 ± 4.8 × 10^6</td>
<td>4.0 ± 1.6 × 10^6</td>
</tr>
<tr>
<td><strong>Range:</strong></td>
<td>0–66%</td>
<td>1.6–106 × 10^6</td>
<td>0–26.5 × 10^6</td>
</tr>
<tr>
<td><strong>ASR (n = 10)</strong></td>
<td>20.2 ± 6.9</td>
<td>32.1 ± 9.6 × 10^6</td>
<td>5.6 ± 2.6 × 10^6</td>
</tr>
<tr>
<td><strong>Range:</strong></td>
<td>0–66%</td>
<td>4.9–106 × 10^6</td>
<td>0–26.5 × 10^6</td>
</tr>
<tr>
<td><strong>VE (n = 15)</strong></td>
<td>11.8 ± 4.0</td>
<td>20.4 ± 4.6 × 10^6</td>
<td>3.0 ± 1.6 × 10^6</td>
</tr>
<tr>
<td><strong>Range:</strong></td>
<td>0–56%</td>
<td>1.6–60 × 10^6</td>
<td>0–25 × 10^6</td>
</tr>
</tbody>
</table>

*No difference in sperm motility was observed for men undergoing either a vasoepididymostomy (VE) or autogenous sperm reservoir (ASR) procedure (P = NS).*

Equipment used for the modification of micropipettes for epididymal micropuncture is not available at many institutions and requires technical skill to fashion a micropipette suitable for epididymal micropuncture. The technique we present has a minimal learning curve and requires no specialized equipment. On the contrary, we have successfully recovered motile sperm using standard hematocrit pipettes attached to butterfly-needle tubing.

Additionally, for men with reconstructive tracts, a simultaneous epididymal micropuncture may compromise surgical results. MESA typically uses epididymal micropunctures at multiple points along the length of the epididymis. VE, performed in conjunction with MESA, thus requires an anastomosis proximal to the highest level of epididymal micropuncture. This results in a sacrifice of potentially viable epididymal length. We have previously reported that no man undergoing a simultaneous ASR with epididymal micropuncture has had sperm recovered from his ASR. The technique of atraumatic aspiration of epididymal sperm with cryopreservation offers the clinician a simple and inexpensive method of sperm collection and the couple the opportunity to maximize their chances for fertility with a single procedure without compromise of the primary reconstructive efforts. For men with congenital absence of the vas deferens in whom a simultaneous IVF/ICSI cycle is to be undertaken with epididymal sperm, epididymal sperm quality should be maximized. This may necessitate multiple epididymal micropunctures.

We do not recommend sperm aspiration and cryopreservation at the time of vasoepididymostomy (VE). As motile sperm are observed in the ejaculate of 98% of men following VE in our hands, we believe that postoperative sperm collection is more economic and practical for men undergoing a VE. We also recommend postoperative sperm banking for all men with motile sperm in the ejaculate following a VE procedure.

The pregnancies established with cryopreserved sperm recovered with this technique from patients undergoing a simultaneous reconstructive procedure reinforces the concept of maximizing a couple’s reproductive options. Pregnancy with cryopreserved sperm requires IVF. Chances for pregnancy will be maximized by IVF with ICSI. At our institution, pregnancy (ongoing) rates of 38.5% per cycle are achieved with IVF/ICSI. Current per cycle costs for IVF/ICSI average $12,000. Couples should be informed of the risks, benefits, and costs for IVF and IVF/ICSI prior to surgery and sperm aspiration. Those unwilling to accept IVF should not be offered sperm cryopreservation.

In our experience, the recovery of epididymal sperm is accomplished quickly with little added operating time. In 21 of 25 cases, motile sperm, in sufficient numbers for multiple cycles of IVF, were cryopreserved by the sperm bank.

**CONCLUSIONS**

It is incumbent on the clinician treating the infertile couple to maximize their options and therefore their chances for a pregnancy with a single surgical procedure. The simultaneous atraumatic recovery of epididymal sperm is advantageous and practical for men in whom epididymal reconstruction is being considered. This technique is rapid, inexpensive, and requires no special or modified instruments.

**REFERENCES**

Use of electively cryopreserved microsurgically aspirated epididymal sperm with IVF and intracytoplasmic sperm injection for obstructive azoospermia

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The Cornell Institute for Reproductive Medicine, New York Weill Cornell Medical Center, New York, New York

Objective: To investigate the efficacy of using intentionally cryopreserved epididymal sperm in selected cases of obstructive azoospermia.

Design: A retrospective, nonrandomized study.

Setting: Academic research environment.

Patients: One hundred forty-one couples undergoing first-time IVF/ICSI using either fresh or cryopreserved epididymal sperm.

Interventions: The epididymides were microscopically aspirated.

Main Outcome Measures: Clinical pregnancy rates.

Results: Motile sperm were obtained from all men. For the fresh group, the mean total sperm aspirated was $99 \times 10^6$ with 5.5 vials frozen per patient after ICSI and $82 \times 10^6$ with 4.7 vials frozen per patient in the cryopreserved group. No statistically significant difference in oocyte fertilization rate or number of embryos transferred was noted between groups. Of 108 patients using freshly aspirated sperm, 72 (66.7%) achieved clinical pregnancy. Of 33 patients in the group using cryopreserved sperm, 20 (60.6%) achieved clinical pregnancy ($P=0.47$).

Conclusions: In selected ideal cases of unreconstructable azoospermia, elective open microsurgical epididymal sperm aspiration with cryopreservation yields pregnancy rates similar to that employing fresh sperm. The advantages of this method are: (1) Use of cryopreserved sperm obviates the logistics problems associated with the use of fresh sperm. (2) Abundant high-quality sperm can be cryopreserved in a single procedure for all future attempts at IVF/ICSI. Rarely, viable sperm will not be present after thawing, and fresh retrieval will be necessary. (Fertil Steril 2000;74:696–701. ©2000 by American Society for Reproductive Medicine.)

Key Words: Epididymal sperm, IVF, ICSI, cryopreservation, MESA

The frequency with which obstructive azoospermia is detected among infertile males has been reported to be as high as 7.5%. Approximately 1% of all male outpatient visits are for obstructive azoospermia (1). A standard treatment for unreconstructable obstructive azoospermia has been open microsurgical epididymal sperm aspiration (MESA) timed to coincide with a programmed in vitro fertilization (IVF) cycle undergoing intracytoplasmic sperm injection (ICSI) (2–4). Using this approach, our institution has reported pregnancy rates in excess of 50% (4).

The abundance of spermatozoa in epididymal aspirates after MESA led us to investigate epididymal sperm cryopreservation for use in subsequent IVF cycles. Although several groups have reported on the feasibility of using cryopreserved epididymal sperm in IVF/ICSI, reported pregnancy rates were lower than those achieved at other centers using only fresh sperm (5–10). A possible explanation for the lower pregnancy rates achieved in these studies is their inclusion of couples who had previously failed IVF/ICSI.

The logistics of coordinating IVF with microsurgical sperm retrieval led us to investigate the efficacy of using intentionally cryopre-
served epididymal sperm in couples undergoing IVF/ICSI for the first time. We compared these results to those achieved in couples who underwent first-time IVF/ICSI using freshly aspirated epididymal sperm during a simultaneous programmed ICSI cycle. In an attempt to directly evaluate the use of cryopreserved spermatozoa versus that of freshly retrieved epididymal spermatozoa, we excluded those couples who had undergone IVF/ICSI previously.

MATERIALS AND METHODS

Patient Selection

A retrospective, nonrandomized study of couples undergoing IVF/ICSI was conducted. The IVF/ICSI procedure was performed using either fresh or cryopreserved microsurgically aspirated epididymal sperm. Couples were selected from cycles of IVF/ICSI performed from April 1994 to June 1998 at our institution. Patients selected for cryopreservation of epididymal sperm had levels of serum follicle-stimulating hormone (FSH) that were within the normal range. The patients also had no prior history of MESA. Patients were excluded from our statistical analysis if their cryopreserved epididymal spermatozoa were nonmotile after thawing. The three patients in this group subsequently underwent MESA or testicular sperm extraction (TESE) to salvage their ICSI cycles.

The etiology of obstruction in the group of patients for cryopreservation included the following: congenital bilateral agenesis of the vas deferens in 49% (16/33) of patients; failed reconstruction in 24% (8/33); unilateral absence of vas and contralateral inguinal surgery in 6% (2/33); unreconstructable iatrogenic injury in 6% (2/33); history of epididymitis in 3% (1/33); and obstructive azoospermia of unknown etiology in 12% (4/33).

A total of 33 couples underwent first-time IVF/ICSI using frozen epididymal sperm. Nine men underwent simultaneous sperm retrieval and cryopreservation at the time of vasovasostomy or vasoepididymostomy for obstructive azoospermia. Twenty-four men with obstructive azoospermia underwent MESA without microsurgical reconstruction. A total of 108 couples underwent IVF/ICSI using sperm freshly retrieved by MESA.

Microsurgical Epididymal Sperm Aspiration and Cryopreservation

Epididymal tubule dissection and aspiration were performed using an operating microscope under 15–24X magnification. The techniques used have been described previously (11–13).

Oocyte Stimulation, Retrieval, and Preparation

The techniques of ovarian stimulation and retrieval have been described previously (13–15). Briefly, women were desensitized with gonadotropin-releasing hormone agonist (1 mg s.c. daily) (GnRH-a, Lupron; TAP Pharmaceuticals, Deerfield, IL) for an average of 10 days. Ovulation was then induced using a combination of gonadotropins and following a standard step-down protocol (16). Human chorionic gonadotropin (hCG) (4000–10,000 IU) was administered when a minimum of two follicles of at least 16–17 mm in mean diameter were observed on a transvaginal ultrasound.

Oocytes were harvested by transvaginal ultrasound-guided puncture approximately 35 hours after hCG administration. Patients were sedated with i.v. propofol (Diprivan; Stuart Pharmaceuticals, Wilmington, DE). After the cumulus-cell complexes had been evaluated, oocytes were incubated for at least 4 hours at 37°C and cumulus cells were removed by exposure to human tubal fluid (HTF)–HEPES-buffered medium containing hyaluronidase (80 IU/mL) (Type VIII; Sigma Chemical Co., St. Louis, MO). ICSI was performed on all oocytes that reached metaphase II.

ICSI Procedure

Cryopreserved epididymal sperm were thawed in the following manner. The sperm sample was removed from liquid nitrogen and maintained at room temperature (±20°C) for 10 minutes. HTF medium (1 mL) was added to the sperm sample and centrifuged at 300 g for 5 minutes. The formed pellet was resuspended in 50 mL of HTF medium.

The ICSI procedure has been described elsewhere (13–15).

Embryo Formation, Implantation, Therapeutic Implant Support

Oocytes were observed 12–17 hours after ICSI was performed. The appearance of the oocyte cytoplasm and the number and size of pronuclei were noted; 24 hours after ICSI, cleavage was assessed. The morphologically best embryos were transferred into the uterine cavity approximately 72 hours after the microinjection procedure. The number of embryos transferred was dependent on maternal age; up to three embryos were transferred to women less than 35 years old (14).

Starting on the day of oocyte retrieval, methylprednisolone (16 mg/day) and tetracycline (250 mg every 6 hours) were administered to all patients for 4 days. Progesterone administration was started on day 3 after hCG administration (25–50 mg i.m./day) and continued daily until the assessment of pregnancy (17). Clinical pregnancy was defined as the presence of a gestational sac with fetal heartbeat on ultrasound examination.

Statistical Analysis

The two-tailed Student’s t-test, the $X^2$ test, and the Fisher exact test were performed where appropriate. Differences were considered statistically significant when $P<0.05$. 

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TABLE 1
Comparing fresh vs. cryopreserved epididymal sperm for couples undergoing first-time IVF/ICSI.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fresh (n = 108)</th>
<th>Cryopreserved (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (±SD) male age (in years)</td>
<td>38.3 ± 8.8</td>
<td>38.2 ± 11.1</td>
</tr>
<tr>
<td>Mean (±SD) female age (in years)</td>
<td>33.2 ± 5.1</td>
<td>33.1 ± 5.5</td>
</tr>
<tr>
<td>Mean (±SD) total no. of sperm aspirated</td>
<td>99 ± 10^6</td>
<td>82 ± 10^6 ± 110</td>
</tr>
<tr>
<td>Mean (±SD) no. of vials stored</td>
<td>5.5</td>
<td>4.7 ± 2.5</td>
</tr>
<tr>
<td>Mean (±SD) no. of oocytes injected</td>
<td>10.8 ± 5.5</td>
<td>10.1 ± 5.3</td>
</tr>
<tr>
<td>Mean (±SD) no. of oocytes fertilized</td>
<td>8.2 ± 5.1</td>
<td>7.9 ± 4.6</td>
</tr>
<tr>
<td>Mean (±SD) no. of embryos per transfer</td>
<td>3.3 ± 1.0</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>No. of pregnant couples/total no. of</td>
<td>72/108 (66.7)</td>
<td>20/33 (60.6)</td>
</tr>
<tr>
<td>couples (%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Pregnancy is defined as gestational sac detected.


RESULTS

Comparison of Couples Undergoing IVF/ICSI Using Freshly Aspirated or Cryopreserved Epididymal Sperm

Table 1 presents characteristics of the couples undergoing IVF/ICSI with freshly aspirated or cryopreserved epididymal spermatozoa. The mean (±SD) age of men who underwent MESA with cryopreservation for later IVF/ICSI was 38.2 ± 11.1 years. The mean (±SD) age of females who underwent IVF/ICSI with cryopreserved epididymal sperm was 33.1 ± 5.5 years. The mean (±SD) age of women who underwent ICSI with freshly aspirated epididymal spermatozoa was 33.2 ± 5.1 years. The mean (±SD) age of men who underwent ICSI with freshly aspirated spermatozoa was 38.3 ± 8.8 years.

Motile sperm were obtained from all 141 men for IVF/ICSI. The mean (±SD) total sperm aspirated in the group using cryopreserved sperm was 82 × 10^6. The mean (±SD) number of vials stored was 4.7. In the group using freshly aspirated sperm, the mean (±SD) total aspirated was 99 × 10^6 and the mean (±SD) number of vials stored was 5.5.

Between the two groups, no statistically significant difference was noted in either the mean number of oocytes injected and fertilized or in the mean number of embryos transferred. Of the 108 patients who underwent IVF/ICSI with freshly aspirated sperm, 67% (72) became pregnant. Of the 33 patients in the group using cryopreserved sperm, 61% (20) achieved clinical pregnancy. The difference in pregnancy rates between the two groups did not achieve statistical significance.

Thus far in the group using cryopreserved sperm, 17 couples have delivered 27 children, and three women have miscarried. This group has a delivery rate of 51% (17/33). In the group using freshly aspirated sperm, 62 couples have delivered 93 children, nine women have miscarried, and one couple has terminated the pregnancy because of a neural tube defect. This group has a delivery rate of 57% (62/108); P = 0.45.

Comparison of Couples Undergoing IVF/ICSI Using Cryopreserved Epididymal Sperm: Pregnancy vs. No Pregnancy

To further evaluate the use of cryopreserved epididymal sperm in patients with obstructive azoospermia, we compared the characteristics of couples who achieved pregnancy and those who did not. Table 2 demonstrates the outcome of
this comparison.

A statistically significant difference in male age was found between the two groups. In group 1, the mean (±SD) male age was 34.2 ± 7.4 years as compared to 44.3 ± 13.2 years in group 2 (P=0.008). No statistically significant difference was demonstrated in total sperm aspirated or sperm quality as measured by percent motility and progression. Also, no statistically significant differences were demonstrated in the mean number of vials stored, mean testicular volume, or mean testosterone.

Effect of Microsurgical Reconstruction on Retrieved Semen Parameters and ICSI Outcomes

Of 33 males who underwent first-time IVF/ICSI with cryopreserved sperm, nine had experienced failure of a previous vasovasostomy or vasoe epididymostomy. In each case, cryopreserved epididymal sperm obtained during the time of repair were available for ICSI. Table 3 demonstrates the difference in sperm quality and pregnancy rates between this group and those men who underwent MESA for cryopreservation without repair.

The mean total sperm aspirated in the aspiration-only group was higher than in the repair-plus-aspiration group (100 ± 126 × 10⁶ and 34 ± 31 ± 10⁶, respectively); however, this difference was not found to be statistically significant. No statistically significant difference in mean motility or mean progression was found between the two groups. The clinical pregnancy rate between the two groups was similar: 62.5% in the aspiration-only group and 55.6% in the repair-plus-aspiration group (P=0.69).

DISCUSSION

The use of cryopreserved surgically retrieved epididymal sperm as compared to freshly aspirated epididymal sperm in IVF/ICSI yields similar fertilization rates (78.2% and 76.9%, respectively) and clinical pregnancy rates (60.6% and 66.7%, respectively). This was true in our study of select couples who underwent this form of assisted reproduction for the first time. In previous studies, Nagy et al., Oates et al., Holden et al., Friedler et al., Hutchon et al., and Madgar et al. have similarly demonstrated no difference in fertilization and pregnancy rates between cryopreserved and freshly aspirated sperm when ICSI is used (5–10).

Our study included only men who had motile sperm available after cryopreservation. For three couples, previously cryopreserved epididymal spermatozoa were not used because they were not motile. In these cases, an additional MESA or TESE procedure was necessary to salvage these ICSI attempts (18). As a result of this experience, we now recommend that an aliquot of all epididymal sperm aspirates be reserved for a test thaw. If no motility is found, these patients should be prepared to undergo a backup MESA procedure or percutaneous testicular sperm aspiration under local anesthesia.

Fertilization failure is associated with the use of cryopreserved epididymal sperm that have poor post-thaw motility (19). This observation supports the use of MESA or TESE as a backup during ICSI cycles with cryopreserved sperm of marginal motility.

With the use of ICSI, fertilization and pregnancy rates are independent of sperm quality as long as viable sperm are available for injection. A variety of sperm-retrieval techniques, including MESA, percutaneous epididymal sperm aspiration (PESA), and TESE are currently in use. Testicular sperm extraction yields a lower concentration of sperm with less motility. The extracted sperm are not as suitable for cryopreservation as are epididymal sperm (20). In addition, Schlegel and Su found that among the physiological consequences of open testicular biopsy is the potential for temporary or permanent ischemic testicular injury due to devascularization (21). Therefore, use of TESE should be limited to cases in which the epididymis is absent or non-obstructive azoospermia is present, or to the very rare cases (4%) of obstructive azoospermia in which motile spermatozoa cannot be retrieved from the epididymis (22).

PESA, introduced in 1994 by Craft and Shrivastav (23,24), is a less invasive technique. This simple procedure need not be performed by an experienced microsurgeon. However, higher pregnancy rates have been reported to date with the use of MESA as compared to published results for patients using fresh or cryopreserved percutaneously retrieved epididymal sperm (5,25). MESA allows for atraumatic sampling of intraluminal fluid; thus, sperm samples contain fewer contaminating blood products and require less processing before IVF. Although this subject has not been formally studied, the presence of high-quality epididymal sperm before cryopreservation may be related to post-thaw quality. With the use of PESA, amounts of sperm sufficient for cryopreservation were obtained in only 33% of cases (26).

In our study, motile sperm for cryopreservation were
obtained from all 141 men who underwent MESA. The mean (+SD) total number of sperm aspirated in all MESA procedures was 95 × 10⁶; the mean number of vials cryopreserved was nearly 5. Abundant high-quality sperm can be obtained for cryopreservation in a single MESA procedure, possibly for all future IVF/ICSI attempts. A recent cost-effectiveness analysis found that the per-delivery cost of PESA/ICSI is higher than for MESA/ICSI (27). Holden et al. attempted to identify pre-freeze predictors of successful ICSI. They concluded that a poor IVF/ICSI outcome can be expected when pre-freeze sperm vitality is <20%, presumably because lower-quality sperm are less capable of surviving the cryopreservation process (8).

In the event that a thawed sample is inadequate, an unplanned fresh MESA or TESE operation must be performed to salvage the IVF cycle. Hutchon et al. reported a few cases in which sperm failed to thaw successfully. Improved laboratory technique, however, prevented failure (6). In our study, only couples with motile sperm recovered after cryopreservation were analyzed.

If the three cases previously cited (i.e., those with nonviable sperm after cryopreservation) were included in this analysis, then the pregnancy rate using cryopreserved samples would likely have been significantly lower than that achieved for fresh MESA. IVF/ICSI cycles involving nonviable frozen–thawed sperm were managed with the use of fresh simultaneous MESA retrieval. In some women, especially those of advanced age, cryopreserved sperm cannot be used for ICSI because ovarian hyperstimulation may not be successful. This situation could result in an unnecessary sperm-retrieval procedure unless the couple opted for the use of donor oocytes and a suitable donor were available. Therefore, it is important to select couples carefully before performing sperm retrieval with elective cryopreservation.

Abundant high-quality sperm for cryopreservation can be obtained at the time of microsurgical repair if care is taken to isolate these spermatozoa from the proximal epididymis. This option is important to consider because most couples likely would prefer a naturally conceived pregnancy following microsurgical repair. The results of vasectomy reversal are good, with patency rates as high as 99% (28). When sperm are present in vasal fluid, an estimated overall pregnancy rate of 52% and a live delivery rate of 47% have been cited (27, 28). In addition, repair has been shown to be more cost-effective than IVF/ICSI using epididymal sperm (27). Vasopididymostomy has a lower patency rate of 50%–80%. Therefore, cryopreservation of epididymal sperm at the time of microsurgical repair can serve as back-up insurance in the case of failure to have sperm return to the ejaculate, or in the event of late anastomotic stricture (29). Physicians should discuss costs and benefits of the aspiration procedure as well as IVF/ICSI with couples before surgery.

If only immotile sperm are found in the vas deferens or epididymis at the time of repair, testicular biopsy will almost always yield viable cryopreservable sperm. In our study, sperm cryopreserved during microsurgical repair of obstructive azoospermia had similar quality and yielded similar pregnancy rates when these spermatozoa survived cryopreservation. Therefore, routine cryopreservation of sperm at the time of microsurgical repair of obstructive azoospermia is strongly recommended.

CONCLUSION

In selected cases, elective open microsurgical epididymal sperm aspiration with cryopreservation yields pregnancy rates similar to that employing fresh sperm. Abundant high-quality sperm can be cryopreserved in a single procedure sufficient for all future attempts at IVF/ICSI. In rare instances thawed epididymal sperm are not motile, necessitating a fresh retrieval. Therefore, we recommend that an aliquot of aspirated epididymal sperm be subjected to a test thaw prior to IVF/ICSI.

Use of cryopreserved epididymal sperm aspirated at the time of simultaneous microsurgical reconstruction yielded pregnancy rates similar to the aspiration-only group. Elective microsurgical epididymal sperm aspiration with cryopreservation for future IVS/ICSI is a highly successful treatment for obstructive azoospermiain select couples.

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References


FERILITY & STERILITY®
Sperm cryopreservation and in vitro fertilization/intracytoplasmic sperm injection in men with congenital bilateral absence of the vas deferens: a success story

In this study we evaluate the use of cryopreservation of sperm obtained at the time of surgical exploration in men with congenital bilateral absence of the vas deferens. We assess the impact of cryopreservation on pregnancy rates after IVF/intracytoplasmic sperm injection. Intraoperative cryopreservation of sperm at the time of microsurgical epididymal sperm aspiration in men with congenital bilateral absence of the vas deferens resulted in a 100% live delivery rate per couple, providing the highest pregnancy rates of any infertility treatment. (Fertil Steril® 2004;82:1452-4. ©2004 by American Society for Reproductive Medicine.)

Congenital bilateral absence of the vas deferens (CBAVD) is a relatively rare cause of male factor infertility that occurs in 1 in 1,000 men in the population (1). It is found in 1% of patients attending male infertility clinics (2) and affects approximately 2% of men with azoospermia (2, 3). Men with CBAVD have a form of obstructive azoospermia not amenable to surgical correction (4) and must therefore rely totally on assisted reproduction to achieve pregnancy.

Fertilization with intracytoplasmic sperm injection (ICSI) has greatly expanded the indications for cryopreservation of sperm in these men (5). In this study, we evaluated the outcomes associated with the use of cryopreserved epididymal sperm obtained at the time of microsurgical epididymal sperm aspiration (MESA) in men with obstructive azoospermia (OA) due to CBAVD. We assessed the impact of cryopreservation on pregnancy rates.

The records of 30 consecutive men with OA due to CBAVD who underwent open MESA were retrospectively reviewed in accordance with the Helsinki Declaration of 1975 on human experimentation. Preoperatively, each had undergone a thorough history and physical examination followed by testing for the presence of a cystic fibrosis (CF) gene mutation.

MESA was performed as described elsewhere (6, 7). Sperm obtained intraoperatively were cryopreserved for subsequent IVF and ICSI. The intraoperative parameters that were analyzed included fluid volume, sperm concentration, motility, and number of vials stored. All cryopreserved specimens were tested for post-thaw motility. Patients were followed over time to evaluate the number of IVF/ICSI cycles performed in addition to pregnancy and delivery rates.

The average patient age was 33.0 years, and the average partner age was 30.4 years. All patients had a diagnosis of CBAVD based on physical examination that was confirmed on surgical exploration. In the 24 men who had CF genetic data available, 11 were carriers of a CF mutation (46%), one had symptomatic CF, and 12 had no CF mutation. Of the 12 men with no mutation, one had congenital absence of the right kidney as demonstrated on ultrasound.

At the time of MESA, an average of 6.1 vials were cryopreserved per patient. Each vial had an average volume of 1.1 mL including a media (range 0.5-2.5 mL), concentration of 29.0 million sperm/mL and motility of 37.1%.

Of the 30 men, 13 were either followed up outside the United States, were lost to follow-up, or had not yet undergone IVF/ICSI cycles. Seventeen couples underwent 27 IVF/ICSI cycles at our institution (see Table 1). From these 27 cycles, 20 pregnancies occurred (74.1% pregnancy rate/cycle) and one couple miscarried, for a live delivery rate per cycle of 70.4% (19/27). The overall delivery rate per couple was 100%, with two couples undergoing two successful IVF cycles with deliveries. Deliveries included 12 singleton births, five sets of twins, and two sets of triplets. The total multiple birth rate was 36.8%.

Whereas almost all men with CF have CBAVD, only a small proportion of men with CBAVD have a medical history of pancreatic insufficiency, respiratory disease, or chronic sinusitis; therefore it is considered a mild phenotype of CF (1, 8).
TABLE 1

Results of microsurgical epididymal sperm aspiration with IVF/ICSI in couples with congenital bilateral absence of the vas deferens.

<table>
<thead>
<tr>
<th>No. of cycles per couple</th>
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Note: There were 27 total cycles, 20 total pregnancies, and 19 total deliveries, with 12 singletons, five sets of twins, and two sets of triplets (36.8% multiple births).


Concern exists surrounding the risk of transmission of CF gene mutations when IVF is used in these men. However, Shin et al. found that CBAVD was present in only 5% of brothers of men with CBAVD, consistent with incomplete penetrance for the CBAVD phenotype after inheritance of CF gene mutations (12). For couples in which the man has CBAVD and the female partner tests negative for standard CFTR gene mutations, the maximum risk of having a child with CBAVD is less than 1% (12). Incomplete penetrance may account for a low prevalence of CBAVD in the population and may lower the risk of having a child with CBAVD for couples undergoing sperm retrieval and assisted reproductive techniques (12).

In a study byPhillipson et al., 60% of men with CBAVD carried a single CF gene mutation, 20% were compound heterozygotes, and 20% of men had no CF mutations (1). DeltaPSO8 is the most common mutation found (13). The presence of detectable CFTR gene mutations does not affect fertility or pregnancy rates for men with CBAVD when IVF and micromanipulation are applied (1, 14).

Intraoperative cryopreservation of sperm at the time of MESA in men with CBAVD resulted in a 100% live delivery rate per couple after IVF/ICSI. Freezing multiple vials at one time can provide sufficient sperm for multiple future IVF/ICSI cycles, eliminating the need for repeated invasive procedures. These men have high-quality sperm and fertile wives, resulting in the highest pregnancy rates of any infertility treatment. It is important for physicians involved in the care of men with CF to convey the message that prospects for fatherhood are excellent with current technology (4).

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References

ASPIRATION OF EPIDIDYMAL AND VASAL SPERM: POST-OPERATIVE CARE

1. IT IS LIKELY THAT YOU WILL HAVE SOME DISCOMFORT FOR THE FIRST 2-3 WEEKS AFTER SURGERY. At the time of discharge from the hospital you will have been given a prescription for pain medication. When taking pain medication, be careful as you walk or climb stairs. Dizziness is not unusual.

2. SWELLING AND BRUISING OF THE PENIS AND SCROTUM ARE NORMAL AND WILL TAKE ABOUT 3 WEEKS TO COMPLETELY RESOLVE.

3. APPLYING ICE TO THE INCISION FOR 48 HOURS POST-OPERATIVELY WILL HELP DECREASE PAIN AND SWELLING.

4. A small amount of bright red blood showing through the gauze dressing is to be expected. DO NOT BE ALARMED. If you feel the amount is excessive, call the office. You may replace bloody bandages with clean ones. If there isn’t any bleeding the wound need not be covered with gauze.

5. Do not make any important judgement decisions or sign any legal documents for 24 hours after anesthesia.

6. A low-grade fever [to 101° F] is common 2-3 days post-operatively. This fever can be lessened by coughing, deep breathing and walking. There is no danger that these activities will disrupt your incisions. Taking pain medication one hour before activities and placing a pillow over your lower abdomen when coughing will help decrease discomfort.

7. There are no stitches that need to be removed. The stitching is beneath the skin and dissolves.

8. YOU SHOULD SHOWER 48 HOURS AFTER THE SURGERY. BEFORE SHOWERING, REMOVE THE SCROTAL SUPPORTER AND
GAUZE DRESSINGS. Dry yourself well after showering and wear a clean scrotal supporter daily.

9. Do not take tub bathes for at least one week after surgery.

10. Do not drive for one week after surgery, but you can ride in a car if someone else is driving.

11. No heavy work, strenuous exercise or sports are allowed for 3 weeks post-operatively.

12. If your job involves only desk work and light activity, you may return to work 2 to 3 days after surgery.

13. No sexual intercourse is allowed for one week post-operatively.

14. Thereafter, you may resume normal activities as you feel up to it.

15. Remember that your pain medication can cause constipation. To avoid straining increase your fiber intake [fruits, vegetables, whole-grains, etc.]. Drinking lots of water can also help.

16. **Follow-up Visit:** As soon as possible you should call the office for an appointment to see Dr. Goldstein one month after surgery.

**If you have any questions, please feel free to call our office.**