OBJECTIVES
To evaluate the outcomes of repeated testicular sperm aspirations (TESAs) and to investigate whether the interval between TESA procedures, success in the first attempt, and patient age might influence the results.

METHODS
The patient population in the present study consisted of couples undergoing TESA for intracytoplasmic sperm injection. Of these, 189, 46, 42, and 19 male patients underwent 1, 2, 3, and 4 sperm retrievals, respectively. The effects of the interval between TESA procedures, success of the previous attempts, and patient age on sperm recuperation in a repeated procedure were evaluated.

RESULTS
Of the patients with nonobstructive azoospermia from whom sperm was not retrieved in the first procedure, 16.6% had successful sperm retrieval at the second procedure. In 57.1% of the patients who did not achieve sperm recuperation for the second attempt, sperm could be retrieved in the third procedure. Finally, of the patients without success in the third attempt, 40.0% had successful sperm retrieval at the fourth attempt. Success in the preceding TESA procedure positively influenced the success of sperm retrieval in a repeated attempt, and a trend for a negative effect of paternal age in the success of a repeated TESA attempt was noted. However, the interval between procedures had no influence on TESA success.

CONCLUSIONS
The results of our study have shown that patient age and the success of the first TESA may predict the results of additional attempts. However, TESA may result in sperm recovery even when the first recovery procedure was not successful. Moreover, sperm recovery did not seem to be affected by the interval between procedures.

Azoospermia, the absence of sperm from the ejaculate, is found in about 15% of infertile men and is classified as either obstructive (OA), characterized by normal spermatogenesis, or nonobstructive (NOA), characterized by impaired spermatogenesis. Since its introduction in 1992, intracytoplasmic sperm injection (ICSI) has become the treatment of choice for severe male factor infertility. In addition, ejaculated, testicular, and epididymal sperm can all be used for ICSI, resulting in high fertilization and good pregnancy rates.

Testicular sperm extraction (TESE), testicular sperm aspiration (TESA), and percutaneous epididymal sperm aspiration (PESA), combined with ICSI, offer azoospermic patients the possibility of having their own genetic children. In patients with OA, mature sperm cells can be retrieved in most cases. In patients with NOA, sperm is retrieved in approximately 50% of cases. Some men with NOA have focal areas of spermatogenesis within the testes, even though global spermatogenic function is severely impaired.

However, when sperm cells exist, they might not be present in all testicular samples because the testicular tissue structure might not be homogenous, and spermatogenesis might be present only in minute foci. Even when testicular sperm are found, the development of the embryo and ICSI outcomes are reportedly affected by the sperm origin. Ejaculated and epididymal spermatozoa have been observed to provide better quality embryos than testicular sperm. Furthermore, testicular sperm injection has been reported to result in lower fertilization and pregnancy rates compared with epididymal sperm injection. Moreover, high spontaneous abortion rates were reported with testicular sperm compared with epididymal sperm. Therefore, when spermatozoa are not retrieved in a TESA attempt or if pregnancy is not achieved, repeated testicular biopsies and ICSI cycles may be necessary.

Some studies have been published on the outcome of repeated biopsies for sperm retrieval. Rosenlund et al.
reported that in patients with OA, PESA can be repeated on the same unilateral epididymis ≤3 times, yielding motile spermatozoa in 91%, 89%, and 86% of cases in the first, second, and third attempt, respectively. It has also been reported that motile sperm can be retrieved several times from the same testis while maintaining high fertilization and pregnancy rates in patients with OA or NOA. Ou et al. found no difference in the fertilization, implantation, or pregnancy rates with repeated PESA or TESE cycles compared with those from the first cycles, and Friedler et al. reported that repetition of TESE had clinical value, because pregnancy was achieved in each repetitive trial.

Biopsy of any organ may result in changes consistent with scarring on either ultrasonography or follow-up histologic examination. However, these changes are particularly important in azoospermic patients, who have limited fertility. A potential morbidity of TESE procedures has been previously demonstrated. Scrotal ultrasound evaluation at 3 months after TESE revealed abnormalities, reflecting hematoma formation, inflammation, or diffuse intraparenchymal bleeding.

These lines of evidence raise questions about the optimal interval between biopsies. Therefore, the purposes of the present study were to evaluate the outcomes of repeated TESA and to investigate whether the interval between the procedures, the success of the first attempt, and patient age influenced results.

**MATERIAL AND METHODS**

**Experimental Design**

The patient population in the present study consisted of couples undergoing TESA for ICSI, of whom 189, 46, 42, and 23 male patients underwent 1, 2, 3, or 4 sperm retrievals, respectively. The local institutional review board approved the study, and all patients provided written informed consent, in which they agreed to share the outcomes of their ICSI cycles for research purposes.

To classify the patients as having either OA or NOA, testicular histologic examination was performed. Patients with NOA were those presenting with Sertoli cell-only syndrome, hypospermatogenesis, maturation arrest, or tubular hyalinization.

For surgical sperm retrieval in patients with NOA, TESA was performed. Patients with OA, underwent PESA as the first attempt, with TESA performed if the former method failed. Positive sperm retrieval was defined as the presence of motile sperm in the biopsied material.

We evaluated the influence of the following variables on motile sperm recuperation: (a) the interval between TESA approaches, (b) a positive response in the previous attempt, and (c) patient age. In addition, the results of repeated sperm retrieval before and after 3 and 6 months were compared.

**Testicular Sperm Aspiration**

After administration of cord block anesthesia, TESA was performed by longitudinally inserting a 21-gauge butterfly needle into the superior testicular pole, avoiding the epididymis. Forward and backward movements were made, and the needle direction was slightly changed to achieve 8 different spots in the testis, increasing the chance of contacting a spermatogenic focus. In addition, the negative pressure, applied using a 10-mL syringe connected to the end of the butterfly setting catheter during needle aspiration, allowed for the extraction of part of the seminiferous tubes for additional dissection to search for germ cells.

The aspirated material was collected in a Falcon tube and washed with a minimal volume of culture medium at 37°C. The recovered material was checked for the presence of spermatozoa and centrifuged at 300g for 8 minutes. When necessary, the fraction was diluted or concentrated. The material was placed in a 5 × 40-mm glass culture dish (WillCo-dish; distributor, Bellco Glass, Vineland, NJ), and motile sperm were immobilized, aspirated into the microinjection needle, and injected into the oocytes. The testicular sperm aspiration results were considered positive when motile sperm were retrieved. Surgical sperm recuperation was performed on the oocyte retrieval day, and repeated TESA procedures were performed if spermatozoa had not been retrieved in a previous attempt or if pregnancy was not achieved.

**Assessment of Fertilization, Embryo Quality, and Embryo Transfer**

Fertilization was assessed 18 hours after ICSI, and normal fertilization was declared if 2 clearly distinct pronuclei were present. Embryo quality was evaluated under an inverted microscope (Eclipse TE300; Nikon, Tokyo, Japan), and embryo transfer was performed on the third day of development. For each couple, 1-4 embryos were transferred, depending on the embryo quality and the woman’s age.

**Statistical Analysis**

Categorical variables were compared using the χ² test, and continuous variables were compared using Fisher’s exact test. To study the influence of the interval between TESA procedures, the success of the previous attempt, and patient age on motile sperm recuperation, binary logistic regression models were constructed. The results are presented as odds ratios, with the corresponding 95% confidence interval calculated from the logistic regression model. Results were considered significant at P < .05. Data analysis was performed using Minitab, version 14, statistical software (State College, PA).

**RESULTS**

Of the patients undergoing their first TESA procedure, 80 (42.3%) men had OA and 109 (57.7%) had NOA. The mean follicle-stimulating hormone value and mean testicular size was 5.2 ± 2.43 and 13.3 ± 1.78 IU/L and 12.7 ± 2.13 and 9.2 ± 0.86 mL for the patients with OA and NOA, respectively.

The testicular histologic patterns for the patients with NOA were Sertoli cell-only syndrome (39.4%), hypospermatogenesis (21.1%), maturation arrest (25.7%), and tubular hyalinization (13.7%).

Of the patients undergoing repeat TESA, 5 (11.6%) had OA and 41 (89.1%) had NOA. The causes of azoospermia among the patients undergoing TESA are summarized in Table 1.

A single TESA attempt was performed in 143 patients. Of the patients in whom sperm could not be retrieved in
the first attempt or pregnancy was not achieved, the procedure was repeated 2 (n = 46), 3 (n = 42), and 4 times (n = 19). The sperm retrieval rate for the first, second, third, and fourth attempt was 75.6%, 69.5%, 71.8%, and 63.1%, respectively.

Stratified by the type of azoospermia, sperm could be retrieved from patients with OA and NOA at a rate of 93.7% and 62.3% (P < .001) and 100.0% and 65.8% (P = .023) in the first and second TESA procedure, respectively. All the patients undergoing a third or fourth attempt had NOA.

Of the patients with NOA from whom sperm was not retrieved in the first attempt, 16.6% had successful sperm retrieval at the second procedure. Of the patients who did not achieve sperm recovery for the second attempt, sperm could be retrieved in the third procedure in 57.1%. Finally, when sperm had still not been retrieved in the third attempt, 40.0% had successful sperm retrieval with the fourth attempt (Table 2).

The cumulative sperm retrieval rate was 89.1%, and the retrieval rate after previous failure was 52.6%. The cumulative pregnancy rate was 31.7%. Of the patients with NOA, the cumulative sperm retrieval rate was 67.2%, and the retrieval rate after previous failure was 35.1%. For NOA patients, our findings showed that when the first attempt was successful, the chance of sperm retrieval in a repeated TESA increased nearly threefold. Moreover, a trend for a negative effect of paternal age in the success of repeated TESA was noted. However, these findings most likely did not reach statistical significance because of the small number of cycles evaluated in the present trial.

No influence from the interval between procedures in the success of a repeated TESA attempt was observed (Table 3). The interval between biopsies in patients in whom sperm could be retrieved was 8.3 months. When sperm was not retrieved, it was 13.7 months (P = .220). Furthermore, no significant difference in sperm retrieval was observed when the procedure was repeated before vs after 3 (76.4% vs 58.6%, P = .3376) or 6 (67.7% vs 60.0%, P = .6053) months.

**COMMENT**

In azoospermic patients, surgical retrieval of spermatozoa provides the opportunity for fertility despite quantitatively limited sperm production. However, questions still exist whether repetitive testicular biopsies result in motile sperm recuperation and whether the interval between biopsies influences the result.

Studies to date have focused on the potential to surgically retrieve viable spermatozoa from the testis. However, little is known about the consequences of testicular manipulation on testicular function. Spermatogenesis is a complex 74-day process involving mitotic cell division, meiosis, and spermatogenesis. Each of these steps represents a key element in the spermatogenic process, and defects in any of them can result in failure of the entire process.

The present study examined the influence of the interval between repetitive TESA procedures on motile sperm retrieval using a binary logistic model. In addition, we compared the results of procedures that were repeated before vs after 3 months because of the nearly 3-month duration of spermatogenesis and those repeated before and after 6 months because the active inflammation caused by testicular manipulation has frequently resolved by 6 months. Our findings did not demonstrate any effect from the interval between repeat procedures on motile sperm recuperation; furthermore, no significant difference in sperm retrieval success was detected when the procedure was repeated before vs 3 or 6 months after the first procedure.

Conversely, a previous study of the histologic effects of TESE on the testes demonstrated that TESE causes a decrease in seminiferous tubular volume within the testicular parenchyma and might have at least a transient adverse effect on spermatogenesis. Although sperm retrieval can be successful in ≤80% of patients, second TESA procedures have been found to be much more difficult and to require more biopsy sites. After using

**Table 1. Causes of azoospermia in patients undergoing testicular sperm aspiration**

<table>
<thead>
<tr>
<th>Etiology</th>
<th>OA</th>
<th>NOA</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptorchidism</td>
<td>—</td>
<td>18</td>
<td>9.52</td>
</tr>
<tr>
<td>Genetic</td>
<td>—</td>
<td>11</td>
<td>5.82</td>
</tr>
<tr>
<td>Congenital absence of the</td>
<td>9</td>
<td>—</td>
<td>4.76</td>
</tr>
<tr>
<td>vas deferens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropathy</td>
<td>10</td>
<td>—</td>
<td>5.29</td>
</tr>
<tr>
<td>Orchitis</td>
<td>17</td>
<td>8.99</td>
<td></td>
</tr>
<tr>
<td>Sexual dysfunction</td>
<td>10</td>
<td>41</td>
<td>21.7</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>—</td>
<td>4</td>
<td>2.11</td>
</tr>
<tr>
<td>Testicular tumor</td>
<td>—</td>
<td>13</td>
<td>6.88</td>
</tr>
<tr>
<td>Toxic</td>
<td>3</td>
<td>1.60</td>
<td></td>
</tr>
<tr>
<td>Epididymal cysts</td>
<td>5</td>
<td>2.64</td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>48</td>
<td>—</td>
<td>25.4</td>
</tr>
</tbody>
</table>

OA = obstructive azoospermia; NOA = nonobstructive azoospermia.

**Table 2. Sperm retrieval rate for patients with nonobstructive azoospermic undergoing repeat TESA**

<table>
<thead>
<tr>
<th>TESA Attempt</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>23/28 (82.1)</td>
<td>3/18 (16.6)</td>
</tr>
<tr>
<td>Third</td>
<td>23/28 (82.1)</td>
<td>8/14 (57.1)</td>
</tr>
<tr>
<td>Fourth</td>
<td>10/14 (71.4)</td>
<td>2/5 (40.0)</td>
</tr>
</tbody>
</table>

TESA = testicular sperm aspiration.

* Because pregnancy not achieved (positive sperm retrieval) or spermatozoa not retrieved in previous attempt (negative sperm retrieval).
serial ultrasonography to evaluate the physiologic consequences of TESE on the testes, Schlegel and Su\textsuperscript{15} recommended that \( \geq 6 \) months elapse before repeating TESE.

However, those reports studied the effect of TESE, and not TESA, on testicular function. TESE is performed by opening a slit in the scrotal skin and tunica albuginea, followed by excision of a small peripheral testicular tissue fragment, which is immediately processed for sperm recovery. In the present study, fine needle aspiration, a much less-invasive alternative, was the technique of choice to recover sperm from the testes. The probable less traumatic nature of TESA has been supported by the low rates of postoperative clinical complications.\textsuperscript{16}

According to Donoso et al.,\textsuperscript{19} an ideal surgical technique would enable, with minimal trauma to the testis, the retrieval of a sufficient number of motile spermatozoa to inject all available oocytes. Nevertheless, none of the currently available techniques fulfills these criteria. TESE with multiple biopsies was proposed as a method to enhance the sperm retrieval rate of TESA, given that in many cases of NOA only isolated regions of spermatogenic tissue are present. However, although it has been reported that TESE is more effective than TESA,\textsuperscript{1} our results have demonstrated the effectiveness of TESA among patients with OA and NOA, because after a fourth attempt, sperm was retrieved from 89.1\% of the patients. In the present study, during the TESA procedure, the needle direction was slightly altered, which might have increased the chances of encountering a spermatogenesis focus.

Evidence that the success of a previous sperm retrieval attempt influences the result of the repeat procedures corroborates reports from a previous study showing that repeated testicular sperm retrieval was successful in 69.7\% of patients with positive results after the first TESA but in only 11.1\% of patients with negative results. The investigators of that study suggested that after a negative result, patients should be strongly advised to use donor backup if a second attempt is planned.\textsuperscript{20} However, in view of the highly successful sperm recovery with repeat procedures after a negative result in the first attempt, such as was found in our study, we suggest that repeat TESA should be offered to all patients, even if a previous TESA procedure has failed.

The effects of advancing paternal age on assisted reproductive technology outcomes are controversial. The male reproductive functions do not cease as they do in women, but some factors become fundamentally changed with age.\textsuperscript{21} We evaluated whether male patient age would influence the outcome of repeat TESA. A decline in semen parameters, such as volume, concentration, motility, and morphology, in men of increasing age has been previously reported.\textsuperscript{22-25} Although it did not reach statistical significance, we also observed a trend for a negative effect of paternal age in the success of repeated TESA.

### CONCLUSIONS

Our evidence suggests that the success of the first TESA procedure and patient age might predict the results of additional attempts; however, TESA may result in sperm recovery even when the first recovery procedure has failed. Moreover, sperm recovery did not seem to be affected by the interval between procedures.

### References