Introduction: We aimed to determine pregnancy and miscarriage rates following intracytoplasmic sperm injection (ICSI) cycles using retrieved epididymal and testicular sperm in azoospermic men and ejaculated sperm in oligospermic and normospermic men.

Materials and Methods: This retrospective study was carried out on 517 couples who underwent ICSI. They included 96 couples with azoospermia and 421 with oligospermia or normal sperm count in the male partner. Of the men with azoospermia, 69 underwent percutaneous epididymal aspiration (PESA) and 47 underwent testicular sperm extraction (TESE). In the 421 men with oligospermia or normal sperm count, ejaculated sperm was used for ICSI. The differences in the outcomes of ICSI using PESA or TESE and ejaculated sperm were evaluated. The main outcome measures were pregnancy and miscarriage rates.

Results: No significant differences were seen in pregnancy and miscarriage rates with surgically retrieved and ejaculated sperm. The pregnancy rates (including frozen embryo transfer) were 43.5%, 36.2%, and 41.4% in couples with PESA, TESE, and ejaculated sperm, respectively ($P = .93$). The miscarriage rates were 16.7%, 23.5%, and 12.1%, respectively ($P = .37$).

Conclusion: Intracytoplasmic sperm injection in combination with PESA and TESE is an effective method and can successfully be performed to treat men with azoospermia. The outcomes with these procedures are comparable to ICSI using ejaculated sperm.
aspiration, percutaneous epididymal aspiration (PESA), testicular sperm extraction (TESE), and testicular sperm aspiration. Percutaneous epididymal sperm aspiration, a less invasive procedure for retrieval of epididymal spermatozoa, can be performed under local anesthesia. If PESA fails, then TESE can also be attempted, as it has been shown to be an efficient minimally invasive method. It has been shown that hormone level and testicular histology are unable to predict which men with azoospermia will have sperm retrieved by PESA or TESE. There are few published studies comparing the results of ICSI between epididymal or testicular retrieved sperm and ejaculated sperm. The aim of this study was to compare the outcomes of ICSI cycles using PESA or TESE in azoospermic men with those using ejaculated sperm in oligospermic and normospermic men.

MATERIALS AND METHODS

Patients
This retrospective study was carried out in a private center for assisted reproduction from January 2004 to December 2006. The cohort included 517 infertile couples treated by ICSI. These included 96 couples with azoospermia and 421 with oligospermia or normal sperm count in the male partner, according to the World Health Organization criteria. A total of 116 successful retrieval procedures were performed in the 96 azoospermic men, 69 of which were PESA and 47 were TESE procedures. In the 421 men with oligospermia or normal sperm count, 437 ICSI cycles were performed using ejaculated sperm.

Procedures
All men presenting with infertility who had azoospermia on at least 2 semen analyses were further evaluated. The patients had a comprehensive history taken followed by physical examination including inguinoscrotal examination. Testicular volume was determined by a Prader orchidometer and the status of the epididymis and the presence or absence of vas deferens was noted. Hormone levels including follicle-stimulating hormone were evaluated in all azoospermic men. Other tests for hormones such as serum levels of testosterone, luteinizing hormone, and prolactin were done more selectively as dictated clinically. If there was suspicion of obstructive azoospermia, further evaluation, including transrectal ultrasonography, was performed to rule out ejaculatory duct obstruction. All men with azoospermia were counseled for need of further workup including genetic testing. Data, however, is not included in the present analysis due to its lack of direct bearing on the objectives of the study.

Patients with a testicular volume of 15 mL or greater underwent a diagnostic PESA. If no spermatozoon was found, TESE under the same local or general anesthesia was performed. On confirmation of the spermatozoon on PESA or TESE, the couple was counseled to undergo therapeutic PESA or TESE on the day of ovum pickup. Cycle treatment with controlled ovarian hyperstimulation was started 6 to 8 weeks after the diagnostic procedure.

Percutaneous epididymal aspiration was performed on an outpatient basis under local or general anesthesia. Negative pressure was utilized with a 27.5-gauge insulin syringe prefilled with 0.1 mL of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-buffer human tubal fluid. In order to aspirate the maximum quantity of material possible, the needle was directed into the most prominent part of the epididymal head. The sample was further divided using 2 needles of 1 mL (29 gauge) and examined under a magnification of × 400 in the microscope for the presence of sperm cells. Discontinuous density gradients (isolation) were used in all cases to separate spermatozoa and spermatids. The gradient scales and compositions applied were 3 minidensity gradients containing volumes of only 0.3 mL to 0.5 mL of different density gradient materials (50%, 70%, and 85% isotonic solutions). After centrifugation (300 rpm), another medium bath was made up (0.3 mL) into a pellet-containing spermatozoa. All metaphase II oocytes were injected for ICSI according to the standard protocols.

Ovarian stimulation was performed using long protocol of standard technique of gonadotropin-releasing hormone agonist downregulation and
controlled stimulation with recombinant follicle-stimulating hormone. Cycle was monitored by ultrasonography and estradiol levels from the 7th day of stimulation. Once the follicular sizes exceeded 1.7 × 1.7 cm, ovum pickup under general anesthesia and ultrasonographic control was conducted 36 hours after injection of human chorionic gonadotropin. The total number of oocytes retrieved in a cycle was 10 or more. On the same day, sperm retrieval was performed by PESA or TESE. After retrieval and preparation of an adequate number of sperm, ICSI was performed on mature eggs. Fertilization was confirmed 24 hours later and embryo transfer was performed on day 3 of ovum pickup. The number of embryos transferred was 2 to 3 per cycle. Only high-quality embryos (grades 1 and 2) were transferred. If more than 3 good embryos were left after embryo transfer, they were cryopreserved, thawed, and transferred in frozen embryo transfer cycle.

Statistical Analyses

Data were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 14.0, SPSS Inc, Chicago, Ill, USA). Results were expressed as mean ± standard deviation for continuous variables. The independent sample t test was used to compare the mean differences in testicular volume and ages of each partner between PESA and TESE groups. The chi-square test was used to compare presence of vas deferens, pregnancy rate, and miscarriage rate between the groups of couples. A P value less than .05 was considered significant.

RESULTS

The mean ages of men in the PESA and TESE groups were 36 ± 8 years and 37 ± 7 years, respectively. While the mean ages of the male and female partners in the PESA and TESE groups were not significantly different (Table 1), those of the couples with normal sperm count or oligospermia in the male partner were slightly higher (31 ± 5 years and 41 ± 6 years, respectively). The mean testicular volume was not significantly different between the two groups of PESA and TESE as shown in Table 1.

The average fertilization rate in our private center is 80% and the average implantation rates for ICSIs with PESA, TESE, and ejaculated sperm are 20%, 18.8%, and 18.2%, respectively. The pregnancy rates after PESA-ICSI and TESE-ICSI were 43.5% and 36.2%, respectively. These were comparable with the pregnancy rate achieved with ejaculated sperm (41.4%; P = .93). There was no significant difference in miscarriage rates between the three groups of couples (Table 2). Overall, 38 normal deliveries yielded birth of 54 babies in the PESA/TESE group and 159 normal deliveries led to birth of 226 babies in the ejaculated sperm group. The rates of multiple pregnancies were not significantly different between the two groups with PESA/TESE and

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PESA</th>
<th>TESE</th>
<th>Ejaculated Sperm</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment cycles†</td>
<td>69</td>
<td>47</td>
<td>437</td>
<td>...</td>
</tr>
<tr>
<td>Number of couples</td>
<td>53</td>
<td>43</td>
<td>421</td>
<td>...</td>
</tr>
<tr>
<td>Frozen embryo transfer cycles</td>
<td>18</td>
<td>3</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>30 (43.5)</td>
<td>17 (36.2)</td>
<td>181 (41.4)</td>
<td>0.93</td>
</tr>
<tr>
<td>Miscarriage‡</td>
<td>5 (16.7)</td>
<td>4 (23.5)</td>
<td>22 (12.1)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*Ellipses indicate not applicable. Values in parentheses are percents.
†Treatment cycles included transvaginal ovum pickup plus embryo transfer.
‡Percentages are calculated with the number of pregnancies as denominator.
ejaculated sperm (Table 3).

The total number of women who got pregnant after PESA/TESE cycles in all age groups was 47/116 (40.5%). A higher pregnancy rate was achieved among female partners with an age between 20 and 29 years, and those older than 35 years had a significantly lower conception rate. The same results were found in the group of the couples with ejaculated sperm (Table 4).

**DISCUSSION**

The approach to azoospermic patients has changed significantly with the introduction of sperm retrieval techniques and assisted reproduction, especially the ICSI. In addition to improving pregnancy rates using sperm from ejaculated semen, ICSI has opened new possibilities for achieving pregnancy with sperm retrieved from the epididymes or testes which have been performed for more than 10 years. Satisfactory results have been achieved in various studies using these techniques.\(^{2,3,11}\)

The results of this study showed that there was no significant difference in the treatment outcome following ICSI using surgically retrieved sperm (PESA/TESE) and ejaculated sperm. Pregnancy rates of 43.5% and 36.2% from PESA-ICSI and TESE-ICSI were similar to that of 41.4% found in the cases of ICSI with ejaculated sperm. The miscarriage rate was 16.7% and 23.5% in PESA and TESE, whereas in ICSI cycles with ejaculated sperm the miscarriage rate was 12.0% \((P = .37)\). There was also no significant difference in miscarriage rates between PESA and TESE groups \((P = .85)\). Other researchers have reported that the use of testicular sperm leads to a higher spontaneous miscarriage rate.\(^{12,13}\) On the other hand, Aboulghar and colleagues and other authors did not find any significant difference between the treatment results following ICSI with ejaculated sperm and with epididymal sperm.\(^{8,9}\) A recent meta-analysis of surgical sperm retrieved in azoospermic patients, however, concluded that sperm origin does not affect cycle outcome.\(^{14}\)

Age of the female partner, however, has been shown to exert substantial influence on the success of ICSI treatment. In line with our findings, Devroey and associates found that the delivery rate in women younger than 40 years was 25.4%, while in those older than 40 years, it was 8.5%.\(^{15}\) Silber and colleagues also reported low delivery rate in women aged more than 37 years.\(^{16}\) Of the other factors considered for prediction of successful ICSI are hormone profile, testicular volume, and histopathology findings; few studies have reported that these parameters are unable to predict which procedure would be successful in azoospermic men.\(^{7,17}\) In our study, the main emphasis was on genital examination to measure the testicular volume with orchidometer, the status of epididymis, and presence or absence of the vas deferens. We used PESA procedure as a diagnostic technique in all azoospermic men. It is

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**Table 3.** Multiple Pregnancies Following Intracytoplasmic Sperm Injection Using Sperm Supplied by Percutaneous Epididymal Aspiration (PESA), Testicular Sperm Extraction (TESE), and Ejaculation*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PESA</th>
<th>TESE</th>
<th>Ejaculated Sperm</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successful Delivery</td>
<td>25</td>
<td>13</td>
<td>159</td>
<td></td>
</tr>
<tr>
<td>Twins</td>
<td>7 (28.0)</td>
<td>5 (38.5)</td>
<td>43 (27.0)</td>
<td>0.68</td>
</tr>
<tr>
<td>Triplets</td>
<td>1 (4.0)</td>
<td>1 (7.7)</td>
<td>12 (7.5)</td>
<td>0.81</td>
</tr>
<tr>
<td>Multiple pregnancy, %</td>
<td>32.0</td>
<td>46.2</td>
<td>34.6</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*Ellipses indicate not applicable. Values in parentheses are percents.

**Table 4.** Pregnancy Rates According to Age Groups of Female Partners*

<table>
<thead>
<tr>
<th>Age, y</th>
<th>PESA/TESE</th>
<th>Ejaculated sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Pregnancy (%)</td>
</tr>
<tr>
<td>20 to 29</td>
<td>52</td>
<td>29 (55.8)</td>
</tr>
<tr>
<td>30 to 35</td>
<td>28</td>
<td>15 (53.6)</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>16</td>
<td>3 (18.7)</td>
</tr>
</tbody>
</table>

*Frozen embryo transfer cycles are included in the rates. PESA indicates percutaneous epididymal aspiration and TESE, testicular sperm extraction.
desirable to perform a diagnostic PESA procedure before planning an ICSI and committing resources to ovulation induction treatment and to select the patients with greater chance of successful retrieval. This facilitates counseling of couples and has financial and medicolegal benefits, as well. As pregnancy rates are acceptable in men with azoospermia, ICSI associated with sperm retrieval techniques should be employed in assisted reproduction centers. It allows good pregnancy rates in relation to patients with azoospermia. On the basis of our results, PESA should be the first option offered to patients. If spermatozoa cannot be recovered, TESE can be offered.

CONCLUSION
In conclusion, our study confirms that minimally invasive techniques of PESA and TESE can be successfully performed to retrieve sperm for ICSI in the treatment of azoospermic men. It should be emphasized that ICSI for the treatment of severe male-factor infertility is especially important in our culture, because the use of donor spermatozoa is forbidden. The result of this study indicates that treatment outcomes of PESA/TESE-ICSI including frozen embryo transfer cycles compare favorably with that of ICSI using ejaculated sperm.

CONFLICT OF INTEREST
None declared.

REFERENCES