A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia

Peter J. Stahl, M.D., a Punet Masson, M.D., a Anna Mielnik, M.S., b Michael B. Marean, M.D., a
Peter N. Schlegel, M.D., a,b and Darius A. Paduch, M.D., Ph.D. a,b

a Department of Urology, Weill Cornell Medical College and b Population Council, The Rockefeller University, New York, New York

Objective: To evaluate the benefit of Y microdeletion testing.

Design: Retrospective analysis.

Setting: University-based male fertility clinic and genetics laboratory.

Patient(s): A total of 1,591 men with sperm concentrations less than 5 million sperm/mL.

Intervention(s): Semen analysis, Y microdeletion testing, microdissection testicular sperm extraction (TESE).

Main Outcome Measure(s): Sperm concentration, incidence and nature of Y microdeletions, microdissection TESE outcome.

Result(s): We identified 149 microdeletions (9.4%). 10.4% of azoospermic men and 10.1% of men with sperm concentrations >0–1 million sperm/mL harbored microdeletions. Two-thirds of microdeletions in azoospermic men were AZFa, AZFb, AZFb+c, or complete Yq deletions. Virtually all microdeletions in oligozoospermic patients were AZFc deletions. Seven hundred eighteen patients underwent microdissection TESE, including 41 with microdeletions. Microdissection TESE failed in all patients with AZFa, AZFb, AZFb+c, and complete Yq deletions. Sperm were retrieved in 15/21 AZFc deleted patients (71.4%). The presence of an AZFc deletion was associated with increased likelihood of sperm retrieval when compared with the 48.8% retrieval rate in 385 idiopathically azoospermic men who consecutively underwent microdissection TESE at our institution during the study period. Clinical pregnancy was achieved in 10/15 azoospermic AZFc deleted patients for whom sperm were successfully retrieved.

Conclusion(s): Of azoospermic and severely oligozoospermic American men, 10% harbor Y microdeletions that alter prognosis for surgical sperm retrieval and are vertically transmissible. Y microdeletion testing is essential for genetic and preoperative counseling in these patients. (Fertil Steril® 2010;94:1753–6. ©2010 by American Society for Reproductive Medicine.)

Key Words: Male infertility, azoospermia, oligozoospermia, Y microdeletions, testicular sperm extraction, sperm retrieval, Y chromosome, genetic testing

Interstitial deletions that occur in the azoospermic factor (AZF) region of the long arm of the Y chromosome (Yq) are referred to as Y chromosome microdeletions. Y microdeletions account for up to 20% of cases of severe idiopathic male infertility (1) and are a leading genetic cause of male factor infertility worldwide. Elegant work performed during the past decade has elucidated the complicated structure of the male-specific portion of the Y chromosome and the de novo genetic events from which Y microdeletions result (2–4).

Similar to all couples considering using advanced reproductive technologies (ART), those affected by Y microdeletions must weigh the significant psychological, financial, and medical costs of undergoing treatment against the chances of achieving pregnancy. They must also consider that sons conceived with sperm from men with Y microdeletions are expected to inherit the abnormal Y chromosome and the subfertile phenotype (5, 6). Most reproductive endocrinologists and male fertility specialists agree that testing for Y microdeletions is an essential part of the evaluation of subfertile severely oligozoospermic or azoospermic men, and testing is recommended by the American Society for Reproductive Medicine (ASRM) practice guidelines (7, 8). Nonetheless Y microdeletion testing is not universally offered to couples affected by severe oligozoospermia or azoospermia (9).

One reason for heterogeneity in Y microdeletion testing practices throughout the world is the well-documented geographic variability in Y microdeletion frequencies that has been observed. In the largest screening studies published to date, the frequencies of Y microdeletions among severely oligozoospermic men from Germany and Italy were 1.8% and 5%, respectively (10, 11). Smaller studies from Australia, Scandinavia, China, India, Japan, and Russia have revealed Y microdeletion frequencies that range from 1%–14% (10). American studies have been limited by small patient populations and have found highly variable microdeletion frequencies that have ranged from 3%–18% (10). In geographic regions where Y microdeletions are less common, the cost effectiveness of testing is diminished and the necessity of testing is less clear.
Other arguments commonly cited against routine Y microdeletion testing of severely oligozoospermic men are that diagnosis of Y microdeletions usually does not alter the prognosis or treatment of affected patients, and that interinstitutional methodological variability in Y microdeletion testing makes it difficult to draw conclusions about the clinical utility of testing. Furthermore, widespread access to intracytoplasmic sperm injection (ICSI) using ejaculated or extracted spermatozoa has enabled some practitioners treating affected couples to proceed with IVF without formal evaluation of the male partner.

We do know that men with complete deletions of AZFa, AZFb, and AZFb+c are universally azoospermic without significant hope of testicular sperm retrieval (12, 13). Diagnosis of these Y microdeletions saves patients the potential morbidity of attempted surgical sperm retrieval and enables them to promptly consider using donor sperm or adoption. However, AZFa, AZFb, and AZFb+c microdeletions are relatively rare in azoospermic men with a reported collective prevalence of 4.4% in a large series of azoospermic men screened in Italy (11).

The AZFc microdeletion is by far the most commonly encountered Y microdeletion, comprising 60% of all AZF deletions detected (14). Affected men have variable spermatogenic phenotypes that range from oligozoospermia to complete germ cell absence. Our ability to draw meaningful conclusions about the prognostic relevance of the AZFc deletion has been limited by very small numbers of patients in the existing reports of attempted sperm retrievals. To date no study has demonstrated a statistically significant prognostic benefit to testing for the AZFc deletion in azoospermic men considering surgical sperm retrieval.

We have previously reported our initial experience with surgical sperm retrieval and IVF/ICSI in men with Y microdeletions (12, 13). However, we have yet to report the frequency of Y microdeletion detection among severely oligozoospermic and azoospermic men in our patient population. Furthermore, we have performed considerably more microsurgical sperm retrievals on both deleted and nondeleted azoospermic patients since our prior publications and can now make meaningful statistical assessments of the prognostic relevance of Y microdeletions.

In the present study we report a decade of experience in the diagnosis and surgical management of men with Y microdeletions. Our series of consecutively screened patients is one of the largest reported worldwide, and the single largest series reported from the United States. Furthermore, our series of attempted microdissection testicular sperm extractions (TESE) in men with Y microdeletions is the largest in the literature reported to date.

MATERIALS AND METHODS

The Institutional Review Board (IRB) of the Weill Cornell Medical College approved this study. The study population was an ethnically heterogeneous cohort of 1,591 subfertile men, with sperm concentrations less than 5 million sperm/mL, screened consecutively for Y microdeletions at our laboratory from 1997–2007. Three hundred seventy-six patients were clinically evaluated and treated at our institution during the study period. Idiopathic azoospermia was defined as azoospermia in the absence of cytogenetic or genetic abnormalities, Klinefelter’s syndrome, a history of cryptorchidism, congenital hypogonadism, obstructive azoospermia, history of chemotherapy, or history of pelvic irradiation. For patients in whom sperm were successfully retrieved, pregnancy outcomes of IVF/ICSI cycles were reviewed. ICSI was performed in all cases with fresh spermatozoa. Clinical pregnancy was established by the presence of fetal heartbeats determined by transvaginal ultrasonography (TVUS) approximately 32 days after embryo transfer.

Differences in microdissection TESE and clinical pregnancy rates (PR) between patients with microdeletions and patients with idiopathic azoospermia were evaluated for statistical significance using the Fisher’s exact test.

RESULTS

Of 1,591 severely oligozoospermic and azoospermic men tested, we identified 149 subjects with Y microdeletions (9.4%). The specific microdeletion status, the number of men found to have each deletion, and the results of their semen analyses are depicted in Table 1. One hundred twenty of 1,193 azoospermic men (10.4%) and 26 of 257 severely oligozoospermic men with sperm concentrations less than 1 million/mL (10.1%) were diagnosed with a Y microdeletion, whereas only 3 of 181 men with 1–5 million sperm/mL (1.7%) were positive for microdeletions. All men with complete AZFa, AZFb, and AZFb+c, and AZFa+a+b+c deletions were azoospermic. One patient with a partial AZFb+c microdeletion, in which the centromeric portion of the AZFb region was not deleted, had sperm in his ejaculate. Seventy-eight study patients had AZFc deletions (4.9%). The AZFc deletion was particularly prevalent in severely oligozoospermic men with sperm concentrations between 0 and 1 million sperm/mL, found in 9.7% of these patients.

Outcomes of microdissection TESE in this patient population are presented in Table 2. Seven hundred eighteen azoospermic study patients underwent microdissection TESE, including 41 patients with Y microdeletions and 385 patients with idiopathic azoospermia. Microdissection TESE universally failed in men with AZFa, AZFb, AZFb+c, and complete Yq deletions. Microdissection TESE was successful in 15 of 21 patients with complete AZFc deletions (71.4%). Microdissection TESE was more likely to succeed in AZFc deleted patients than in nondeleted men with idiopathic azoospermia (P = 0.035), in whom the surgical sperm retrieval rate was 48.8%.
Clinical pregnancy outcomes of IVF/ICSI cycles for couples in whom sperm were successfully retrieved are presented in Table 3. Clinical pregnancies were achieved in 91/188 patients with idiopathic azoospermia (48.4%) and in 10/15 AZFc deleted patients (67.7%) (nonsignificant, P = .19).

**DISCUSSION**

Review of our experience with Y microdeletions re-emphasizes the importance of testing for Y microdeletions in severely oligozoospermic and azoospermic men. For both groups of patients, Y microdeletions are sufficiently prevalent in the United States to justify routine testing in couples considering assisted fertility.

We diagnosed Y microdeletions in 26/257 (10.1%) severely oligozoospermic men with sperm concentrations more than 0 but less than 1 million sperm/mL. This is slightly higher than the 6%–8% reported prevalence of Y microdeletions in severely oligozoospermic men tested and treated at other centers throughout the world (10, 11, 17, 18). The significant prevalence of Y microdeletions in severely oligozoospermic American patients underscores the necessity to perform Y microdeletion screening in these men, even in cases where enough spermatozoa for ART have already been collected. Failure to do so compromises the treating physician’s ability to adequately counsel these patients before ART about the risks of subfertility in their male offspring.

In azoospermic men Y microdeletion testing not only provides essential information for genetic counseling, but it helps patients and their physicians make more informed decisions about surgical sperm retrieval. In this study the prevalence of Y microdeletions in patients with nonobstructive azoospermia was 10.4%. Studies from other geographic areas have reported Y microdeletions in 3%–15% of azoospermic patients (10, 11, 17, 18). Six percent of azoospermic men that we tested were found to harbor microdeletions now established as incompatible with paternity (AZFa, AZFb, AZFc, or AZFa+b+c) (12, 13). Although we report only microdissection TESE results, we have seen no men with complete deletions of the AZFa or AZFb regions that had sperm found on biopsy samples of testis or standard TESE. Clinically, we now recommend primary use of donor sperm rather than TESE for men with deletions that involve complete loss of the AZFa or AZFb regions. For these unfortunate patients, Y microdeletion testing avoids the potential morbidity of TESE and clarifies the need for donor sperm or adoption.

We found AZFc deletions in 4.4% of azoospermic patients tested. Our data are the first to demonstrate that these patients enjoy a statistically better microsurgical sperm retrieval rate (71%) when compared with nondeleted, idiopathically azoospermic men (48%). The idiopathic azoospermia cohort excludes patients with identifiable conditions that have a higher rate of sperm retrieval with microdissection TESE including Klinefelter’s syndrome (retrieval rate 68%) (19) and cryptorchidism (retrieval rate 74%) (20). In our experience this information has been useful when preoperatively counseling couples considering microdissection TESE.

To our knowledge, the tested population reported in the present study is the largest cohort of subfertile men that has been tested

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**TABLE 1**

Prevalence of Y microdeletions among subfertile severely oligozoospermic and azoospermic men stratified by sperm concentration.

<table>
<thead>
<tr>
<th>Sperm concentration (million/mL)</th>
<th>Total screened</th>
<th>AZFa (%)</th>
<th>AZFb (%)</th>
<th>AZFb+c (%)</th>
<th>AZFc (%)</th>
<th>Complete Yq (%)</th>
<th>Any Y microdeletion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZoospermic</td>
<td>1,153</td>
<td>4 (0.3%)</td>
<td>17 (1.4%)</td>
<td>31 (2.7%)</td>
<td>50 (4.3%)</td>
<td>18 (1.6%)</td>
<td>120 (10.4%)</td>
</tr>
<tr>
<td>&gt;0–&lt;1</td>
<td>257</td>
<td>0</td>
<td>0</td>
<td>1 (0.4%)a</td>
<td>25 (9.7%)</td>
<td>0</td>
<td>26 (10.1%)</td>
</tr>
<tr>
<td>1–&lt;5</td>
<td>181</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (1.7%)</td>
<td>0</td>
<td>3 (1.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>1,591</td>
<td>4 (0.3%)</td>
<td>17 (1.1%)</td>
<td>32 (2.8%)</td>
<td>78 (4.9%)</td>
<td>18 (1.1%)</td>
<td>149 (9.4%)</td>
</tr>
</tbody>
</table>

* Partial AZFb+c deletion that spared the centromeric portion of the AZFa region.


| TABLE 2 |

Outcomes of microdissection TESE in azoospermic men stratified by Y microdeletion status.

<table>
<thead>
<tr>
<th>Etiology of azoospermia</th>
<th>Sperm retrieved</th>
<th>Sperm not retrieved</th>
<th>Total</th>
<th>Retrieval rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZFa</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>AZFb</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>0%</td>
</tr>
<tr>
<td>AZFb+c</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>0%</td>
</tr>
<tr>
<td>AZFa+b+c</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0%</td>
</tr>
<tr>
<td>AZFc</td>
<td>15</td>
<td>6</td>
<td>21</td>
<td>71.4%a</td>
</tr>
<tr>
<td>Nondeleted, idiopathic</td>
<td>188</td>
<td>197</td>
<td>385</td>
<td>48.8%a</td>
</tr>
</tbody>
</table>

* Note: TESE = testicular sperm extraction; AZF = azoospermic factor.
* Comparison of retrieval rates in AZFc deleted men and idiopathically azoospermic nondeleted men, P < .05 (Fisher’s exact test).


| TABLE 3 |

Clinical pregnancy outcomes in idiopathically azoospermic men and in azoospermic AZFc deleted men in whom sperm were surgically retrieved.

<table>
<thead>
<tr>
<th>Etiology of azoospermia</th>
<th>Sperm retrieved</th>
<th>Clinical pregnancies</th>
<th>Clinical pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>188</td>
<td>91</td>
<td>48.4%a</td>
</tr>
<tr>
<td>AZFc deletion</td>
<td>15</td>
<td>10</td>
<td>66.7%a</td>
</tr>
</tbody>
</table>

* Not significantly different (P = .19, Fisher’s exact test).

for Y microdeletions in the United States, and our experience with microdissection TESE in men with Y microdeletions is the largest such series published to date. Nonetheless, the ability of our findings to be generalized may be limited. Our institution specializes in the genetic evaluation and treatment of male factor infertility. The high incidence of Y microdeletions we observed might reflect selection bias due to our referral pattern. In addition, as a tertiary care center for men with severe infertility, our screened population of infertile men may be more phenotypically severe than men treated at other centers. This is reflected by the high percentage of azoospermia (70%) in our screened population. Finally, our sperm retrieval rate of 71% in azoospermic men with AZFc deletions is higher than the retrieval rates reported by other centers, which range from 28%–55% (12, 13). This variability in retrieval rates likely reflects differences in retrieval techniques used at different centers (i.e., use of the operating microscope and extent of testicular dissection).

Despite these limitations we believe that our study emphasizes the need for Y microdeletion testing in severely oligozoospermic and azoospermic American men. Ten percent of these patients harbor Y microdeletions. Diagnosis of Y microdeletions is critical for preconception genetic counseling and provides valuable prognostic information to couples considering surgical sperm retrieval.

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REFERENCES