Histopathologic patterns of testicular biopsies in infertile azoospermic men with varicocele

In azoospermic infertile men with varicocele, testicular biopsy revealed histopathologic patterns that varied from disorganized spermatogenesis with low or moderate sperm scores to early (primary spermatocytes stage) or late (spermatid stage) arrested spermatogenesis or germ cell aplasia and Sertoli cells only. Diagnostic testicular biopsy can be helpful for accurate management of azoospermic infertile men with varicoceles before surgical repair. (Fertil Steril® 2010;94:2482–5. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** Testicular histopathology, varicocele, spermatogenesis arrest, Sertoli cell only, male infertility, azoospermia

Azoospermia is present in approximately 1% of all men and up to 15% of infertile men (1, 2). Defective spermatogenesis and genital tract obstruction are the main causes of azoospermia (3). Azoospermia resulting from testicular disorders is generally irreversible and is classified as nonobstructive azoospermia (4). It has been estimated that 5–10% of infertile men with azoospermia had a clinical diagnosis of varicoceles (5, 6).

Various mechanisms have been proposed to explain testicular damage in infertile men with varicoceles, including testicular hypoxia, venous hypertension, increased temperature, increase in seminal vein catecholamines, and increased oxidative stress (7). The influence of varicoceles on testicular function is variable, leaving it apparently unaltered in some cases, and causing partial or total arrest of spermatogenesis in others (8–10). As a result, infertile men with varicoceles can exhibit abnormal semen quality ranging from oligozoospermia to complete azoospermia (11).

Clinical evidence suggests that spermatogenesis can vary within a damaged testis, resulting in focal areas, or “patches,” of sperm production in an organ largely devoid of germ cells (12). Therefore, it was assumed that an infertile man with low sperm count might have testicular damage reflected by abnormal histologic patterns, such as germ-cell aplasia or maturation arrest, before becoming azoospermic (13).

Diagnostic testicular biopsy (TB) combined with or without surgical exploration of the genital tract is the standard for distinction of defective spermatogenesis from genital tract obstruction as a cause of azoospermia (3). The objective of this study was to determine the histopathologic patterns of TB in a group of infertile men presenting with confirmed laboratory diagnosis of azoospermia and a clinical diagnosis of varicoceles. This study was conducted immediately after obtaining the institutional review board approval. Our study included 37 infertile men with a clinical diagnosis of bilateral varicoceles and a laboratory diagnosis of azoospermia. Diagnosis of varicoceles was made by scrotal examination while the patient in a standing position and during Valsalva’s maneuver. Varicoceles were classified as grade I (palpable only during Valsalva’s maneuver), grade II (palpable without Valsalva’s maneuver), and grade III (visible) (8). Diagnosis of varicoceles was confirmed by scrotal color Doppler ultrasound (14).

Diagnosis of azoospermia was confirmed by the absence of sperms in the centrifuged semen pellet on repeated semen analysis at 2–3 week intervals (15). Testicular volume was assessed by Prader’s orchidometer (16). Serum levels of FSH were measured and normal value ranged 1.4–18.1 mIU/mL (17). After obtaining informed consent from the participants, TBs were taken from all participants using open surgical approach (Window technique). Biopsy specimens were selected from the apparently healthier testes based on size and consistency. All biopsy specimens were preserved in Bouin’s solution, and stained slides were prepared for routine histopathologic examination using a light microscope.

**TESTICULAR HISTOPATHOLOGIC SCORING**

In all three patient groups, testicular specimens were processed further for histopathologic analysis. Tissues were fixed in Bouin’s solution, dehydrated by upgrading from 30 to 100% series of
alcohol and then to xylene each for 1 h, followed by making sections in paraffin blocks to 5 μm thick. The sections were then stained in hematoxylin and eosin following the methods described earlier (18).

Based on the results of histopathologic evaluation of testicular biopsy specimens, patients were classified into three groups:

Group 1: Complete spermatogenesis with disorganization, sloughing and low to moderate sperm scores were found (n = 11/37 [30%]; Fig. 1A and B).

Group 2: Arrested spermatogenesis (n = 14/37 [38%]). In group 2, primary spermatocytes were detected in nine cases and spermatids in five (Fig. 1C and D).

Group 3: Germ cell aplasia and Sertoli cell only (SCO) pattern (n = 12/37 [32%]; Fig. 1E and F).

Statistical analysis was performed using SPSS (Chicago, IL) software package version 13.0 for Windows (Microsoft, Redmond, WA). Data were examined for normal distribution. Comparison between groups was done using the Kruskal-Wallis test. Results were presented as mean ± SD. Statistical significance was established at P < 0.05.

Study Population Description: Infertility Type and Hormonal Study

The mean ± SD of age and infertility duration in our study population (n = 37) were 34.7 ± 7.8 and 7.4 ± 5.8, respectively (Supplemental Table 1, available online). Of 37 infertile azoospermic men enrolled in our study, 30 (81%) patients exhibited primary infertility while 7 (19%) patients exhibited secondary infertility (Supplemental Table 1, available online).
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Supplemental Table 1 shows the mean ± SD of serum FSH in our study population, which was 14.3 ± 5.5 mIU/mL. As shown in Supplemental Table 1, no statistical significant differences were observed between our patient groups regarding their age, duration of infertility, or mean FSH serum levels (P > 0.05). Serum levels of FSH were correlated with neither the histopathologic patterns nor with the grade of varicoceles.

Patients Study: Histopathologic Pattern

Supplemental Table 1 shows a comparison of the patients’ right and left testicular volumes among the three study groups. Low testicular volumes were observed in right and left testes in group 3 vs. group 1 (P = 0.001 and P = 0.001) and group 2 (P > 0.05 and P = 0.04) respectively. Testicular volume was significantly larger in group 1 compared with group 2 (P = 0.04 for right and P = 0.04 for left) and with group 3 (P = 0.001 for right and P < 0.001 for left). Supplemental Table 2 (available online) shows distribution of patients in the three histologic groups in relation to the grade of varicocele. The testis volume in azoospermic men was not significantly correlated with the histopathologic patterns of TB (P > 0.05).

Varicocele can result in progressive deterioration of Sertoli cells, leading to the release of spermatogenic cells before their full maturation (19, 20). Testicular histology was reported as the infertile men with varicoceles (5, 21). In our study, histopathologic evaluation of TB from infertile azoospermic men with varicoceles has shown complete spermatogenesis with disorganization, sloughing and low to moderate sperm counts in 30%, arrested spermatogenesis in 38%, and SCO pattern in the remaining 32% of cases. A recent study has shown complete spermatogenesis and hypospermatogenesis in 49% of azoospermic men with varicoceles (22). Another study has shown complete spermatogenesis in 27.8%, arrested spermatogenesis in 33.2%, and SCO pattern in 33% (23).

Our results indicate that the grade of varicoceles was not significantly correlated with the histopathologic pattern of TB. This finding is in agreement with previous reports that the degree of histopathologic impairment seems to be independent of the clinical stage of varicoceles (9, 19, 20). This observation is interesting and may have important clinical implications. Our findings indicated that azoospermic infertile men with grade 3 varicoceles may have similar incidence of having complete or hypospermatogenesis to those with grade 1 varicoceles. Extensive spermatogenesis disturbances up to germ cell aplasia could be observed in azoospermic infertile men with varicoceles regardless of the grade of varicocele, because similar testicular damage was equally associated with either grade 1 or grade 3 varicoceles.

A recent study has shown a significant increase in testicular sizes in varicocele patients after varicocelectomy (13). In light of these observations, it can be speculated that the finding of small testicular volume in infertile azoospermic men with varicoceles is of negative prognostic value and may indicate severe testicular damage and poor outcome. In our study, serum levels of FSH were correlated with neither the histopathologic patterns nor the grade of varicoceles. However, serum levels of FSH were significantly higher in infertile men with varicoceles as compared with fertile men with or without varicoceles (27). TB has important diagnostic and prognostic value in the management of azoosperma with varicoceles. Based on the results of TB, azoospermic patients with complete spermatogenesis can benefit from varicocelectomy repair after excluding obstruction. Future research is warranted to show whether azoospermic men with different testicular pathologies will benefit from varicocelectomy.

REFERENCES


### SUPPLEMENTAL TABLE 1

Comparison of patients’ age, infertility duration, serum FSH levels, and right and left testis volume between the patient groups (n = 37).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 11)</th>
<th>Group 2 (n = 14)</th>
<th>Group 3 (n = 12)</th>
<th>P value (1 vs. 2)</th>
<th>P value (1 vs. 3)</th>
<th>P value (2 vs. 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>38 ± 8</td>
<td>33 ± 5</td>
<td>32 ± 6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Infertility duration (y)</td>
<td>9 ± 5</td>
<td>7 ± 7</td>
<td>5 ± 4</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>15 ± 3</td>
<td>16 ± 2</td>
<td>18 ± 5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Right testis volume (mL)</td>
<td>19 ± 3</td>
<td>16 ± 2</td>
<td>13 ± 3</td>
<td>0.04</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Left testis volume (mL)</td>
<td>20 ± 2</td>
<td>16 ± 3</td>
<td>13 ± 2</td>
<td>0.04</td>
<td>&lt;0.0001</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**Note:** Group 1: patients with complete spermatogenesis with disorganization, sloughing and low to moderate sperm scores (n = 11/37 [30%]). Group 2: patients with arrested spermatogenesis (n = 14/37 [38%]). Group 3: patients with germ cell aplasia and SCO pattern (n = 12/37 [32%]). Values are presented as mean ± SD. P < 0.05 was significant. NS = not significant.

<table>
<thead>
<tr>
<th>Varicocele grade</th>
<th>Group 1, n = 11 (%)</th>
<th>Group 2, n = 14 (%)</th>
<th>Group 3, n = 12 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 (36.4)</td>
<td>4 (28.6)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>2</td>
<td>4 (36.4)</td>
<td>5 (35.7)</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>3</td>
<td>3 (27.2)</td>
<td>5 (35.7)</td>
<td>3 (25.0)</td>
</tr>
</tbody>
</table>

Note: Group 1, patients with complete spermatogenesis with disorganization, sloughing, and low to moderate sperm scores (n = 11/37 [30%]); group 2, patients with arrested spermatogenesis (n = 14/37 [38%]); group 3, patients with germ cell aplasia and SCO pattern (n = 12/37 [32%]).