Differentiating between primary and secondary Sertoli-cell-only syndrome by histologic and hormonal parameters

The contribution of histologic differentiation between primary and secondary Sertoli-cell-only (SCO) syndrome in azoospermic men was evaluated. No correlation was found between the presence of sperm cells in the testis and the histologic findings or inhibin B or FSH levels, suggesting a low prognostic value for this differentiation. (Fertil Steril 2005;83:1856-8. ©2005 by American Society for Reproductive Medicine.)

Among men with nonobstructive azoospermia (NOA), it still is a challenge to identify patients who have a better chance of successful testicular sperm extraction, that is, with remnants of normal or slightly disturbed spermatogenesis. Histologic diagnosis of Sertoli-cell-only (SCO) syndrome is confirmed when the examined tissue reveals that all seminiferous tubules contain only Sertoli cells, without any germ cells. However, because of the heterogeneity of the testicular tissue, detection of SCO histologic pattern in one or more biopsies does not preclude focal spermatogenesis in a different location (1, 2).

Sertoli-cell-only syndrome can be divided into two distinctive histologic patterns: primary and secondary SCO (2, 3). The current hypothesis is that primary SCO is caused by a prenatal defect in migration of germ cells into the seminiferous tubules, resulting in sterility. Conversely, secondary SCO is a result of postnatal damage to healthy testicular tissue that may result only in a focal histologic SCO pattern. Primary and secondary SCO differ substantially in tubular wall histology, in morphology and function of Sertoli cells, and in the appearance of the interstitial tissue (2-4). No distinct information has yet been reported regarding the possibility of a different prognosis for the two subgroups of SCO.

In the present study, the prognostic implication (successful sperm retrieval) of discriminating between primary and secondary SCO was tested by using a parametric histologic score. In addition, the hormonal profile (FSH and inhibin B serum levels) was investigated. The study was approved by the institutional review board of our medical center.

Six testicular biopsies (three in each testis) from six different sites were obtained from 41 NOA men (aged 25–53 years; mean ± SEM, 32 ± 1.2 years) for cytological evaluation (Papanicolaou-stained smears) and sperm extraction for intracytoplasmic sperm injection (ICSI) in the IVF unit (1).

Two biopsies (one from each testis) were fixed in Bouin’s solution, embedded in paraplast, and stained with hematoxylin and eosin. From patients showing SCO pattern in both testes, the right testicular biopsy was selected for histologic scoring and basement membrane width (BMW) measurements. From patients with only one biopsy showing SCO pattern (i.e., testis germ cells were observed in the other biopsy), that biopsy was selected for histologic scoring and BMW measurements.

To assemble a histologic score that is capable of distinguishing between the two histologic patterns, five histologic parameters were used in this study: [1] seminiferous basement membrane thickening; [2] Sertoli cell morphology; [3] interstitial hypertrophy; [4] sclerosis and fibrosis of the seminiferous tubules; and [5] homogeneity. For each parameter, a score was allocated on a scale of 1 (minimum) to 7 (maximum). The scores of all parameters were then summarized to provide a histologic score between 5 and 35. Histologic score measurements were expressed in units. Lower histologic scores characterized primary SCO, whereas higher histologic scores characterized secondary SCO.

The BMW measurements were performed at ×400 magnification with an Olympus/Tokyo (Tokyo, Japan) objective micrometer of 0.01 mm and were expressed in units. Mean BMW was calculated as the mean value of 20 BMW measurements, applied in 10 tubules within each biopsy at two sites (the widest and the narrowest) within each tubule.

Serum inhibin B levels were measured by double-antibody, enzyme-linked immunoassay (commercially available from Oxford Bioinnovation, Oxford, United Kingdom). Serum inhibin B values obtained from 10 semen donors, and 11 men with obstructive azoospermia were within normal values (147 ± 21 pg/mL and 122 ± 15 pg/mL, respectively). Serum FSH levels were standardized according to an upper normal limit of 11 IU/L.
Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS), version 11.0 for Windows (SPSS, Chicago, IL). For comparison between two groups of a quantitative variable, a t test for independent samples and Mann-Whitney test were used. Regression analysis was performed by using Pearson’s coefficient for parametric correlation and Spearman’s coefficient for nonparametric correlation. The difference between two qualitative variants was evaluated by χ² test. Descriptive statistics are given as mean ± SEM.

Elevated FSH levels (higher than the upper limit of normal range) were detected in 35 patients (86%). In one of the patients with normal FSH values, sperm was present in one testis, whereas five other patients showed SCO in both testes and absence of sperm in all locations. A significant negative correlation \( (r = -0.44; P < .05) \) was found between FSH and inhibin B levels. Inhibin B levels were below the lower limit of the standard range in all patients. A negative correlation \( (r = -0.32; P < .05) \) was found between inhibin B levels and histologic score. Nevertheless, the histologic score did not correlate with FSH, or with the presence of sperm in cytology of at least one biopsy. The histologic score cutoff between primary and secondary SCO was set at 28 units, according to the significant difference in inhibin B levels found in this cutoff \( (P = .041) \). In accordance with this, mean inhibin B levels were 19 ± 3.1 pg/mL in primary SCO (26 men), and 9 ± 3.2 pg/mL in secondary SCO (15 men; Table 1). When separately analyzing different parameters included in the histologic score, only the parameter of ghost tubules abundance correlated significantly with serum inhibin B levels \( (r = -0.37; P = .018) \). All other parameters did not correlate with FSH or inhibin B values. Follicle-stimulating hormone did not differ significantly between primary and secondary SCO (Table 1).

A statistically significant negative correlation was found between BMW and serum inhibin B values \( (r = -0.43; P = .006) \), as well as between BMW and FSH levels \( (r = 0.5; P = .01) \).

In 16 patients with histology of SCO (39%), sperm cells were present in cytology of at least one of the six biopsies (focal SCO pattern), and in 25 patients (61%), no sperm was found in any of the biopsies (complete SCO pattern). Not inhibin B, FSH levels, nor BMW measurements correlated with the presence of sperm in cytology. Moreover, there was no significant difference in serum inhibin B or FSH levels between the group of patients with focal SCO pattern and the group of patients who showed a complete SCO pattern in any of the biopsies performed. Furthermore, sperm cells were found in 44% of patients with a very low histologic score \( (<10 \text{ units}) \) and in 23% of patients with a very low BMW (BMW of \(<4.5 \text{ units}) \). No significant correlation was found between the presence of sperm in cytology and the histologic diagnosis (primary or secondary SCO). Sperm was found in 11 patients (42%) with primary SCO and in 5 patients (33%) with secondary SCO (Table 1).

The literature contains limited data on primary and secondary SCO, the two histologic subgroups of SCO syndrome. Anniballo et al. summarized this data and suggested the prognostic implication of discriminating between primary and secondary SCO. In our study, histologic criteria were used to create a parametric histologic score that is capable of distinguishing between two SCO histologic subgroups: primary and secondary.

The decrease of serum inhibin B levels observed in our study in the secondary SCO group could be explained by the decrease in the number of functioning Sertoli cells, as reflected by the large percentage of ghost tubules in the secondary SCO group. The relation between serum inhibin B and testicular histologic appearance in men with NOA has been previously examined by numerous studies. However, the role of inhibin B in predicting the presence of sperm within the testis in patients with NOA is still controversial.

The correlation of inhibin B and FSH with BMW measurements suggests that the thickened tubular wall of the seminiferous tubules probably impairs the relationship be-

### TABLE 1

**Histologic and hormonal parameters of primary and secondary Sertoli-cell-only (SCO) groups.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Primary SCO</th>
<th>Secondary SCO</th>
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<tbody>
<tr>
<td>Histologic score (unit)</td>
<td>16 ± 1.36</td>
<td>32 ± 0.39</td>
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<tr>
<td>Basement membrane width (unit)²</td>
<td>3.8 ± 0.24</td>
<td>4.8 ± 0.47</td>
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<tr>
<td>Inhibin B (pg/mL)²</td>
<td>19 ± 3.1</td>
<td>9 ± 3.2</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>17 ± 1.5</td>
<td>18 ± 1.9</td>
</tr>
<tr>
<td>Men with spermatozoa in other location, n (%)</td>
<td>11 (42)</td>
<td>5 (33)</td>
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</table>

² Significant difference \( (P < .05) \) between the two groups by Mann-Whitney and t tests.

tween the inner tubular Sertoli cells population and the interstitium. This impairment may affect hormone permeability, influencing inhibin B and FSH levels in the peripheral blood. In the present study, no differential diagnosis was performed for the detailed composition of the lamina propria, consisting of a basal lamina, myofibroblasts, fibroblasts, collagen and elastic fibers, and extracellular matrix (8).

Follicle-stimulating hormone levels did not differ significantly in the primary and secondary SCO groups, nor was there a significant difference in the probability of detecting sperm in biopsies from other sites in six different locations within the testis. Thus, histologic score (as defined in this article) and BMW measurements cannot be used as prognostic indicators for success of sperm retrieval, and the differentiation between primary and secondary SCO probably has low, if any, prognostic value. Moreover, our data raise doubts concerning the pathogenesis of primary and secondary SCO.

In conclusion, the present study contributes to the understanding of the complex relationship between the function and histology of the seminiferous tubule. The role of BMW in determining blood hormonal levels in pathologic and physiologic states needs further evaluation.

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