Proacrosin as a marker of meiotic and post-meiotic germ cell differentiation: quantitative assessment of human spermatogenesis with a monoclonal antibody

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A quantitative immunohistochemical study of human spermatogenesis was performed using the 4D4 anti-proacrosin monoclonal antibody (mAb 4D4) as a marker of meiotic and post-meiotic germ cell differentiation. Cells from 15 testicular biopsies with normal spermatogenesis, 18 with slight and nine with marked hypospermatogenesis and six with maturation arrest were assigned to spermatogenic stages according to both nuclear maturation and proacrosin labelling patterns. The results showed that four spermatogenesis steps (mid- and late-pachytene primary spermatocytes, early and late spermatids) have to be separately considered for the classification of a given biopsy. Conversely, data from primary spermatocytes in the metaphase, anaphase and telophase stages and secondary spermatocytes did not show significant differences between biopsies. We conclude that: (1) slight hypospermatogenesis is due only to fewer cells entering meiosis, whereas in marked hypospermatogenesis there is also germ cell loss during the later meiotic steps and spermiogenesis; (2) the sloughing of germ cells from the epithelium could be of pathological significance; and (3) immunodetection with mAb 4D4 improves the assessment of spermatogenesis because it can label a protein expressed as early as meiotic prophase. In addition, mAb 4D4 labels a protein which is a marker of the Golgi complex allowing the detection of disturbances of cytoplasmic events during meiosis or spermiogenesis. Such an analysis is facilitated by mAb 4D4 labelling of paraffin-embedded sections.

Introduction

A quantitative analysis of the germinal epithelium allows the precise localization of the spermatogenic stage of a disorder and can distinguish maturation arrest from some hypospermatogenesis (Narbaitz et al., 1978; Sigg and Hedinger, 1981). Spermatogenesis has been quantified by means of classical cytological stains to determine stage of nuclear maturation (Rowley and Heller, 1971; Skakkebaek et al., 1973; Weissbach and Ibach, 1976; Zukerman et al., 1978; Johnson et al., 1987; Paniagua et al., 1987; Hirsch and Choi, 1990).

Human spermatogenesis has been analysed by means of probes recognizing germ cell components, such as lectin-binding sites or acrosomal antigens (Flörke-Gerloff et al., 1983; Phi-Van et al., 1983; Jassim and Festenstein, 1987; Jones et al., 1988; Kurpitz et al., 1988; Wollina et al., 1989; Herr et al., 1990; Kurth et al., 1991). The monoclonal antibody mAb 4D4 is specific to the 50–55 kDa primate proacrosin sequestered in the matrix of the anterior region of the acrosome (Gallo et al., 1991) and paraffin embedded sections can be labelled with the 4D4 monoclonal anti-proacrosin antibody (Gallo et al., 1991). Moreover, specific labelling patterns of mAb 4D4 are found at various stages of spermatogenesis from mid-pachytene stage onwards. Identification of germ cell type is therefore possible and cytoplasmic processes of spermatogenesis can be observed because proacrosin labelling during meiosis is a marker of Golgi complex partitioning (Escalier et al., 1991). This new immunohistochemical approach has allowed meiotic arrests due to intra-nuclear disturbances to be distinguished from those resulting from cytoplasmic event impairment (Escalier et al., 1992).
In the present study, quantitative analysis of 55 testicular biopsies including controls and examples with different levels of impaired spermatogenesis was performed using mAb 4D4. Testis specimens were from necropsies and from infertile patients with either excretory obstruction or secretory disorders. Six spermatogenesis steps have been recorded as early as the prophase I step, including germ cells present in the tubular lumen. The aim of this study was to quantify stages in human spermatogenesis more clearly in normal and pathological conditions by means of this precise marker and to compare the classification of spermatogenesis impairment by mAb 4D4 with that normally obtained from nuclear cytology.

### Materials and Methods

**Patients and tissues**

The present study was performed on 55 testicular biopsies, 47 were from 31 infertile patients and eight were from five fertile men who died in traffic accidents or from causes not related to testicular or endocrine diseases. Samples were obtained after ethical approval and with the consent of patients. The average age was 34 (range 25–48). Testicular biopsies of patients were obtained by surgical procedures under general anaesthesia, whereas autopsies were carried out between 3 and 5 h after death. The biopsies were bilateral in 19 men and unilateral in 17 men. The semen characteristics of the patients are presented in Table 1. Semen samples were obtained after 3–5 days abstinence. Sperm characteristics were unknown for 11 testis biopsies, eight from five necropsies and three from two infertile patients. Seven testis biopsies exhibiting only Sertoli cell syndrome were excluded from the quantitative evaluation of the results.

**Biopsy processing and immunohistochemistry**

Small fragments (4 mm x 2 mm) of each testicular specimen were fixed in Bouin's solution, embedded in paraffin wax and sectioned at 3 μm. Immunohistochemistry was performed as described using the three-step immunoperoxidase technique with biotin-avidin (Vector Laboratories, Burlingame, CA) (Escalier et al., 1991). The sections were preincubated with PBS-5% BSA (grade V, Sigma, St Louis, MO), and then exposed for 1 h to mAb 4D4 ascite fluid (bio Merieux, Marcy-L'Etoile) diluted 1:1200 in PBS–5% BSA. As specificity of mAb 4D4 for testicular proacrosin has been demonstrated by western blotting (Escalier et al., 1991), the specificity of the labelling of each testicular biopsy was checked by substituting mAb 4D4 with PBS–5% BSA. Unlabelled testis biopsies exhibiting Sertoli cell syndrome were also used as controls. The sections were counterstained with Harris' haematoxylin and mounted in an aqueous medium (Glycergel, Dakopatts, Santa Barbara, CA).

The slices were observed under an Olympus BH-2 photomicroscope. Cell quantification was performed at a magnification of ×400. Photomicrographs were taken at ×630 using Agfa Ultra 50 films and automatic exposure.

**Cell identification criteria**

MAB 4D4 labelling is observed as early as the mid-pachytene step in humans (Escalier et al., 1991). The cell maturation steps were identified taking into account both nuclear features (Clermont, 1963; Holstein and Roosen-Runge, 1981; Schultze and Rehder, 1984) and the cellular location and pattern of mAb 4D4 labelling (Escalier et al., 1991). Briefly, the 4D4 labelling patterns were: (1) in mid-pachytene primary spermatocytes a single 4D4 labelled body about 3 μm in length; (2) in late-pachytene primary spermatocytes two distinct 4D4 labelled bodies, either close to each other or at various distances from each other; (3) in metaphasic, anaphasic and telophasic primary spermatocytes partitioning of the 4D4 labelled bodies into a pattern related to the chromosome movement; and (4) in secondary spermatocytes, two 4D4 labelled bodies. Spermatids were classified in agreement with the criteria of Clermont (1963), Escalier et al. (1991) and Rowley and Heller (1971): Sa spermatids contain a spherical nucleus and a single round 4D4 labelled body; in Sb spermatids, the nucleus is beginning to lose its spherical shape and is becoming slightly elongated; the 4D4 labelled body is more or

### Table 1. Comparison of semen characteristics of patients with rate of spermatogenesis

<table>
<thead>
<tr>
<th>Number of spermatozoa ml⁻¹</th>
<th>Volume (ml)</th>
<th>pH</th>
<th>Vitality (%)</th>
<th>Motility (%)</th>
<th>Normal spermatozoa (%)</th>
<th>Spermatogenesis (number of men)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 20</td>
<td>1, 7–5</td>
<td>7, 4–8, 4</td>
<td>48–87</td>
<td>15–70</td>
<td>11–68</td>
<td>NS (5)</td>
</tr>
<tr>
<td>&lt; 20</td>
<td>1, 4–6, 2</td>
<td>6, 8–7, 2</td>
<td>6–93</td>
<td>0–40</td>
<td>0–46</td>
<td>SH (2), MH (4), MA (1)</td>
</tr>
<tr>
<td>0</td>
<td>2, 5–6, 4</td>
<td>7, 2–8, 1</td>
<td></td>
<td></td>
<td></td>
<td>NS (4)a, SH (4)a, MH (1)a, MA (2)b</td>
</tr>
</tbody>
</table>

NS: normal spermatogenesis; SH: slight spermatogenesis; MH: marked hypospermatogenesis; MA: maturation arrest; *genital tract obstruction; †secretory azospermia.
less flattened on the side facing the nucleus; Sc spermatids have a strongly elongated nucleus and a crescent-shaped 4D4 labelled cap; and in Sd spermatids the chromatin has undergone further condensation; the nucleus is reduced in width and anteriorly tapered, as for 4D4 acroosomal labelling. Degenerating germ cells were identified by both dense masses of karyoplasmt and unspecific labelling of the whole cytoplasm.

Measurement of spermatogenesis

The distance between cuts was about 30 μm (every tenth section) to prevent double counting of the cells. Eighteen sections of each testis biopsy were immunolabelled. Determination of the number of germ cells was performed on 25 strictly round cross-sectioned seminiferous tubules for each testis biopsy. Tubules near the biopsy edge were excluded to avoid a pressure artefact. All observations were made by the same individual without any knowledge of the clinical and seminal data of the patients.

The numbers of germ cells at the six following spermatogenic steps were determined in the seminiferous epithelium and in the tubular lumen: (1) mid-pachytene primary spermatocytes; (2) late-pachytene primary spermatocytes; (3) primary spermatocytes at metaphase, anaphase and telophase stages; (4) secondary spermatocytes; (5) early spermatids (including Sa plus Sb spermatids); and (6) late spermatids (including Sc plus Sd spermatids). Moreover, at both the epithelial and luminal levels, the numbers of the following germ cells were also determined: (7) unlabelled late spermatids; (8) multinucleate germ cells; and (9) degenerating germ cells. In addition (10) unlabelled round germ cells and (11) Sertoli cells at the lumen level only were included for quantification. A total of 20 differential cell counts (nine in the seminiferous epithelium and 11 in the lumen) were performed in each of the 25 round cross-sectioned seminiferous tubules considered for each testicular biopsy.

Differential cell counts were expressed calculating the median with 95% confidence limits of each cell type recorded per seminiferous tubule cross-section. After classification of the testicular biopsies by their germ cell distribution (see statistical analysis section), the spermatogenic yield for each testicular biopsy group was expressed calculating the early spermatid:pachytene primary spermatocyte and late spermatid:early spermatid ratios.

Statistical analysis

The distribution of the germ cells at each maturation step and for each testicular biopsy was first controlled using a repeated measure Manova analysis (Winer, 1962).

Scaling unfolding (Young, 1987) and cluster analysis (Hand, 1981) were performed to distribute the testicular biopsies into the new quantitatively defined groups. Comparison of the values was performed by the Kruskal–Wallis test, because the normality or homocedasticity conditions or both conditions were violated.

The germ cell types showing a significant variation between groups were taken into account and a multivariant stepwise discriminant analysis (Hand, 1981) was performed to check whether the groups were effectively defined by the variables and to select the germ cell steps exhibiting a required level of discrimination to predict testicular biopsies attribution to a group with an overall accuracy of 97.92%. Once a set of variables was found that provided satisfactory discrimination between groups, a set of classification functions was derived which permitted the classification of new samples. The changes in the statistical Wilk’s lambda showed as the information in successive discriminant functions was removed.

The number of each cell type present in the seminiferous epithelium and in the lumen were compared by calculating the correlation coefficients within each testicular biopsy group.

Results

Seven of the 55 testicular biopsies studied (four men, 11%) were unlabelled by mAb 4D4 owing to maturation arrest of spermatogenesis before the mid-pachytene primary spermatocyte stage. This result was confirmed by conventional microscopy, ensuring that failure of labelling was not due to processing of testis sections. When labelled by mAb 4D4 (48 testicular biopsies from 32 men), the biopsies showed a normal proacrosin reaction as seen by the step-specific 4D4 labelling patterns (Fig. 1). The immunostaining controls were negative.

Classification of testicular biopsies

From the scaling unfolding and cluster analysis data, the series of 48 testicular biopsies were separated into four groups (Table 2 and Fig. 2). The testicular biopsy groups were defined by the mean number of spermatids per seminiferous tubule cross-section (Table 2) according to Nistal and Paniagua (1984) and Nistal et al. (1987).

Normal spermatogenesis. In this group (group 1; n = 15 biopsies) there were 32.36 (30.60–33.84) early spermatids and 26.12 (24.68–29.68) late spermatids per seminiferous tubule cross-section. By contrast, the number of mid- and late-pachytene primary spermatocytes varied (5.96 (3.24–7.40) and 2.27 (1.32–2.98), respectively) (Fig. 2a). The early spermatid:pachytene primary spermatocyte ratio was about 3.93 and the late:early spermatid ratio about 0.83.

Slight hypospermatogenesis. In comparison with group 1, the medians of mid-pachytene primary spermatocytes in this group (group 2; n = 18 biopsies) and the number of early and late spermatids were 20%, 25%, and 33% lower, respectively (Fig. 2b). Compared with group 1, the early spermatid:pachytene primary spermatocyte ratio was unchanged (3.97) and the late:early spermatid ratio was lower (0.70).

Marked hypospermatogenesis. This group (group 3; n = 9 biopsies) was characterized by the presence of 6.92 (3.76–8.96) late spermatids per seminiferous tubule cross-section (Fig. 2c). The ratios of early spermatid:pachytene primary spermatocytes (2.50) and late:early spermatids (0.49) were much lower than those of Group 1.
Fig. 1. Diagram of the different stages of spermatogenesis according to both nuclear features and the cellular location and patterns of mAb 4D4 labelling.

Maturation arrest. In this group (group 4; n = 6 biopsies), there were few early spermatids (2.40 (0.00–3.04)), late spermatids (0.16 (0.00–0.36)) and late-pachytene primary spermatocytes (0.70 (0.00–1.04)). In contrast, the medians of mid-pachytene primary spermatocytes (6.48 (4.68–8.60)) were greater than those of groups 2 and 3 (4.82 (3.40–5.48) and 3.92 (1.84–4.04), respectively) (Fig. 2d).

Discriminative spermatogenesis steps for classification of biopsies

Two classes of germ cell could be distinguished by the median value per seminiferous tubule cross-section (as estimated by the Kruskal-Wallis test) (Table 2). The median for mid- and late-pachytene primary spermatocytes, and for early and late spermatids varied significantly from one testicular biopsy group to another, indicating that the germ cell steps concerned could be used for classification of biopsies. The median values for primary spermatocytes at metaphase, anaphase and telophase stages, and for secondary spermatocytes showed no significant variation from one group of biopsy to another, indicating that this factor could not be used to characterize impairment of spermatogenesis.

The median for unlabelled late spermatids varied significantly from one biopsy group to another. Compared with total late spermatids there were 14.41%, 14.79%, 25.75% and 33.33% in groups 1, 2, 3 and 4, respectively. Failure of mAb 4D4 labelling was very occasional for the other cell types, in all of the testicular biopsy groups. In addition, few degenerating germ cells were found in any group, although their median varied significantly from one biopsy group to another.

Stepwise discriminant analysis allowed the germ cell steps suitable for quantitative discrimination to be distinguished between groups with an overall accuracy of 97.92%. The germ cell type arrangement according to decreased discriminating significance as expressed by Wilk’s lambda values was (1) early spermatids (0.09), (2) late spermatids (0.05), (3) mid-pachytene primary spermatocytes (0.04), (4) late-pachytene primary spermatocytes (0.03) and (5) unlabelled late spermatids (0.02).

The accuracy of evaluation of spermatogenesis by this method led to about 50% discrepancy (Table 3) compared with evaluation by a pathologist using a conventional non-quantitative method according to diagnosis criteria of Colgan.
Table 2. Germ cells per seminiferous tubule cross-section in men

<table>
<thead>
<tr>
<th>Classification of spermatogenesis</th>
<th>Normal</th>
<th>Hypospermatogenesis</th>
<th>Maturation arrest</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of biopsies</td>
<td>15</td>
<td>18</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Mid-pachytene</td>
<td>5.96±</td>
<td>4.82±</td>
<td>3.92±</td>
<td>6.48±</td>
</tr>
<tr>
<td></td>
<td>(3.24, 7.40)</td>
<td>(3.40, 5.48)</td>
<td>(1.84, 4.04)</td>
<td>(4.68, 8.60)</td>
</tr>
<tr>
<td>Late-pachytene</td>
<td>2.27±</td>
<td>1.48±</td>
<td>1.64±</td>
<td>0.70±</td>
</tr>
<tr>
<td></td>
<td>(1.32, 2.98)</td>
<td>(1.04, 1.76)</td>
<td>(0.52, 2.20)</td>
<td>(0.00, 1.04)</td>
</tr>
<tr>
<td>Metaphase, anaphase and telophase</td>
<td>0.2±</td>
<td>0.12±</td>
<td>0.12±</td>
<td>0.08±</td>
</tr>
<tr>
<td></td>
<td>(0.00, 0.28)</td>
<td>(0.00, 0.28)</td>
<td>(0.00, 0.24)</td>
<td>(0.00, 0.24)</td>
</tr>
<tr>
<td>Secondary spermatocytes</td>
<td>0.2±</td>
<td>0.36±</td>
<td>0.52±</td>
<td>0.12±</td>
</tr>
<tr>
<td></td>
<td>(0.20, 0.36)</td>
<td>(0.24, 0.44)</td>
<td>(0.12, 1.00)</td>
<td>(0.00, 0.40)</td>
</tr>
<tr>
<td>Early spermatids</td>
<td>32.36±</td>
<td>25.01±</td>
<td>13.92±</td>
<td>2.40±</td>
</tr>
<tr>
<td></td>
<td>(30.64, 33.84)</td>
<td>(22.48, 27.04)</td>
<td>(9.60, 14.52)</td>
<td>(0.00, 3.04)</td>
</tr>
<tr>
<td>Late spermatids</td>
<td>26.12±</td>
<td>17.62±</td>
<td>6.92±</td>
<td>0.16±</td>
</tr>
<tr>
<td></td>
<td>(24.68, 29.68)</td>
<td>(16.60, 19.36)</td>
<td>(3.76, 8.96)</td>
<td>(0.00, 0.36)</td>
</tr>
<tr>
<td>Unlabelled late spermatids</td>
<td>4.40±</td>
<td>3.06±</td>
<td>2.40±</td>
<td>0.08±</td>
</tr>
<tr>
<td></td>
<td>(3.84, 4.76)</td>
<td>(2.48, 3.76)</td>
<td>(1.04, 3.64)</td>
<td>(0.00, 0.16)</td>
</tr>
<tr>
<td>Multinucleate germ cells</td>
<td>0.44±</td>
<td>0.42±</td>
<td>0.28±</td>
<td>0.06±</td>
</tr>
<tr>
<td></td>
<td>(0.20, 0.72)</td>
<td>(0.24, 0.56)</td>
<td>(0.12, 0.36)</td>
<td>(0.00, 0.12)</td>
</tr>
<tr>
<td>Degenerating germ cells</td>
<td>1.24±</td>
<td>0.96±</td>
<td>0.60±</td>
<td>0.26±</td>
</tr>
<tr>
<td></td>
<td>(0.80, 2.20)</td>
<td>(0.72, 1.04)</td>
<td>(0.20, 1.00)</td>
<td>(0.04, 0.36)</td>
</tr>
</tbody>
</table>

Values are medians with 95% confidence limits. *ab* Values with different superscripts are significantly different (P < 0.05). NS: not significant.

Table 3. Comparison of the human testicular biopsies distribution obtained by the 4D4 method and the routine analysis*

<table>
<thead>
<tr>
<th>Quantitative analysis (4D4 method)</th>
<th>Routine analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Number of men</td>
</tr>
<tr>
<td>Normal spermatogenesis</td>
<td>15</td>
</tr>
<tr>
<td>Slight hypospermatogenesis</td>
<td>18</td>
</tr>
<tr>
<td>Marked hypospermatogenesis</td>
<td>9</td>
</tr>
<tr>
<td>Maturation arrest</td>
<td>6</td>
</tr>
<tr>
<td></td>
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<td></td>
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</tbody>
</table>

Carried out according to the diagnosis criteria of Colgan et al., 1980, based on the histological appearance of the seminiferous epithelium.

et al. (1980). Testicular biopsies affected by either slight or marked hypospermatogenesis were frequently overestimated by routine analysis.

Classification of testicular biopsies and semen characteristics

Comparison between classification of testicular biopsies and semen characteristics could be made for only 26 men because the right and left testicular biopsies were classified in different groups in the six other patients. For three men, discrepancies between the two testicular biopsies were that there was normal spermatogenesis on one side and slight hypospermatogenesis on the other side. In the other three patients, the paired biopsies showed slight hypospermatogenesis and marked hypospermatogenesis. In five of these patients, the best quantitative parameters were on the left testis.

The production of more than \(20 \times 10^6\) spermatozoa ml\(^{-1}\) corresponded to normal spermatogenesis (group 1; five men) (Table 1). Production of fewer than \(20 \times 10^6\) spermatozoa ml\(^{-1}\) corresponded to (i) slight hypospermatogenesis (group 2; two men); (ii) marked hypospermatogenesis (group 3; four men); and (iii) maturation arrest (group 4; one man). For azoospermia, testis data corresponded to the various groups...
Fig. 2. Monoclonal antibody 4D4 immunolabelling of human seminiferous epithelium (Bouin’s fixed, paraffin-embedded testicular biopsies labelled by the avidin–biotin–immunoperoxidase procedure). Bar represents 20 µm. (a) Seminiferous tubule from a biopsy of Group 1 (normal spermatogenesis) showing germ cells at various stages of spermatogenesis (LS: late spermatid). (b) Seminiferous tubule from a biopsy of Group 2 (slight hypospermatogenesis) showing fewer pachytene primary spermatocytes and spermatids (ES: early spermatid). (c) Seminiferous tubule from a biopsy of Group 3 (marked hypospermatogenesis) characterized by much fewer germ cells at all stages. Note the high degree of germ cell sloughing (LP: late-pachytene primary spermatocyte). (d) Seminiferous tubule from a biopsy of Group 4 (maturation arrest) showing germ cell differentiation arrest at the mid-pachytene primary spermatocyte step (MP: mid-pachytene primary spermatocyte).

defined in this study including normal spermatogenesis and all were associated with genital tract obstruction, except for the cases exhibiting maturation arrest.

Distribution of sloughed germ cells

The cell types exhibiting significant variation between groups (as estimated by Kruskal–Wallis test) were almost identical to those identified in the seminiferous epithelium, except for sloughed late-pachytene primary spermatocytes (Table 4). In contrast to the results obtained at the epithelium level, the medians of primary spermatocytes in the metaphase, anaphase and telophase stages and of secondary spermatocytes were greater in groups 3 and 4. For the four testicular biopsy groups, the number of each type of germ cell in the lumen was poorly correlated with that of cells present in the epithelium.
except for late spermatids of group 4 (r = 0.95). The rate of cell sloughing was: group 1 (10.01%); group 2 (12.75%); group 3 (18.50%); and group 4 (20.79%). In all testicular biopsy groups, the medians of degenerating germ cells (< 0.6), unlabelled round germ cells (< 0.8) and Sertoli cells (< 0.1) were very low and showed no significant differences among groups. There was no difference between testicular biopsies from autopsies and from infertile patients with normal spermatogenesis.

**Discussion**

The specific 4D4 labelling pattern at various stages of spermatogenesis allows the identification of germ cell stages. Identification is particularly clear for mid- and late-pachytene primary spermatocytes, and for secondary spermatocytes and early spermatids, which are difficult to distinguish from conventional techniques (Paniagua et al., 1987; Skakkebaek et al., 1989). Moreover, in disturbed spermatogenesis, germ cells in the first meiotic prophase can show swollen or disorganized chromat (Holstein et al., 1988). The 4D4 labelling pattern allows their identification at both epithelial and luminal levels, unless cell changes have induced proacrosin alteration.

The study of the testicular biopsies on the basis of discriminative variables obtained from differential germ cell counts and statistical methods have allowed the analysis of spermatogenesis on the basis of individual biopsies rather than on individual patients and, thus studies of unilateral biopsies. There were quantitative differences between the right and the left testes in six men. Nevertheless, bilateral biopsies showed moderate differences such as normal spermatogenesis and slight hypospermatogenesis, or slight hypospermatogenesis and marked hypospermatogenesis. This result is probably related to regional differences (i.e. vascularization) between the two testes and confirms the importance of performing bilateral testis biopsies (Nistal and Paniagua, 1984; Holstein et al., 1988). In another study, there was no difference in the total number of germ cells between right and left testicular biopsies (Guarch et al., 1992). Such differences between studies confirm the importance of quantitative evaluation.

Slight hypospermatogenesis with about 20 late spermatids per seminiferous tubule section results in the production of more than 10 × 10⁶ spermatozoa ml⁻¹ (Silber et al., 1981; Nistal et al., 1987). Since men with 5 × 10⁶ or more spermatozoa ml⁻¹ can be of normal fertility (Jouannet et al., 1988), slight hypospermatogenesis could be considered as being without functional consequences. In five of these cases, genital tract obstruction was present and this is known to affect spermatogenesis (Sigg and Hedinger, 1981; Holstein et al., 1988).

Until now it was unclear whether the number of either spermatids or of spermatids and spermatocytes was lower in hypospermatogenesis (Narbaitz et al., 1978; Nistal et al., 1987). The method used in the study reported here shows that the number of both pachytene primary spermatocytes, and early
and late spermatids have to be considered for the classification of a given biopsy. The data suggest that slight hypospermatogenesis may be due to fewer cells entering meiosis, which may be related to the decreased spermatogonia which is attributed to the spermatogonial stem cells (Nistal et al., 1984). In contrast, marked hypospermatogenesis appears to have, in addition, loss of germ cells during later meiotic steps and spermiogenesis.

The most common form of maturation arrest in humans affects primary spermatocytes (Nistal and Paniagua, 1984; Francavilla et al., 1989). In the testicular biopsy series studied, the 4D4 labelling revealed that maturation arrest could occur at the level of either mid-pachytene primary spermatocytes or earlier as seen by the absence of 4D4 labelling in the excluded cases. In addition, as some cells escape the blockade, maturation arrest has been considered as a type of hypospermatogenesis (Honoré, 1979), and its existence as a separate entity has been questioned (Guarch et al., 1992). However, in the study reported here, significant differences were found between these two disturbances of spermatogenesis, supporting the notion that these disorders must be of different origin (Nistal and Paniagua, 1984; Skakkebaek et al., 1989).

It is not known whether the frequent presence of immature germ cells in the lumen of seminiferous tubules is due to specimen processing (Honoré, 1979; Pesce, 1987) or is of pathological significance (Cameron et al., 1980; Bairati et al., 1985). Data from the study reported here appear to agree with the second hypothesis. Indeed, the number of sloughed germ cells (of any type) was not proportional to that of germ cells in the epithelium. Moreover, there were more sloughed germ cells in marked hypospermatogenesis and in maturation arrest than in the other testicular biopsy groups.

The 4D4 immunolabelling of testicular biopsies allows the establishment of new characteristics of normal and disturbed human spermatogenesis and the study of germ cell sloughing. The differential 4D4 labelling patterns at each stage of spermatogenesis should allow an automated analysis of human testicular biopsies and allow a reliable and fast method for evaluating spermatogenesis.

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