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Germ Cell Quantitation in Human Testicular Biopsy

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Summary. Quantitative analysis of human seminiferous epithelium was carried out using an improved method of glutaraldehyde and osmium fixation with plastic embedding. Part of each biopsy specimen was fixed in Bouin's fixative and embedded in paraffin for comparison. Epon embedded tissue had very little artifactual damage compared with paraffin embedded tissue sections. The germ cell to Sertoli cell ratios were determined by counting the various germ cells per "unit" tubular area. Data obtained by this method reflect a remarkable stability of Sertoli cell number and germ cell-Sertoli cell ratios both between biopsies from different individuals and between biopsies from right and left testes from the same individual. Agreement between the present results and those of earlier studies based on paraffin embedded testicular specimens supports the validity of this method of germ cell quantitation of human testicular biopsy samples.

Key words: Human, Testicular biopsy evaluation, Germ cell-Sertoli cell ratio.

Introduction

Testicular biopsy specimens are prone to artifactual damage during histological preparation because the tissue is soft and loosely packed. Tissue damage may cause difficulty in accurate evaluation of biopsy specimen. Better histological preparation combined with a simple and reliable quantitative procedure may improve the interpretation of human testicular biopsy. In most laboratories, paraffin sections are used for the routine clinical evaluation of human testicular biopsy specimens. Although the techniques of quantitative evaluation of human testicular materials are available [14, 16, 17, 20] they may not be practical for routine use. Previously we reported a simple, definitive method for germ cell quantitation in both human [3, 4] and animal [15] testes. Our technique has three features: i) use of 1 μm semi-thin plastic sections, ii) quantitation of three major germ cells types, i.e. spermatogonia, spermatocytes and spermatids and iii) use of both Sertoli cell and unit tubular area as references for germ cell count. The present report is an extension of earlier studies, describing in detail the technique of evaluation of testicular biopsy samples from seven men.

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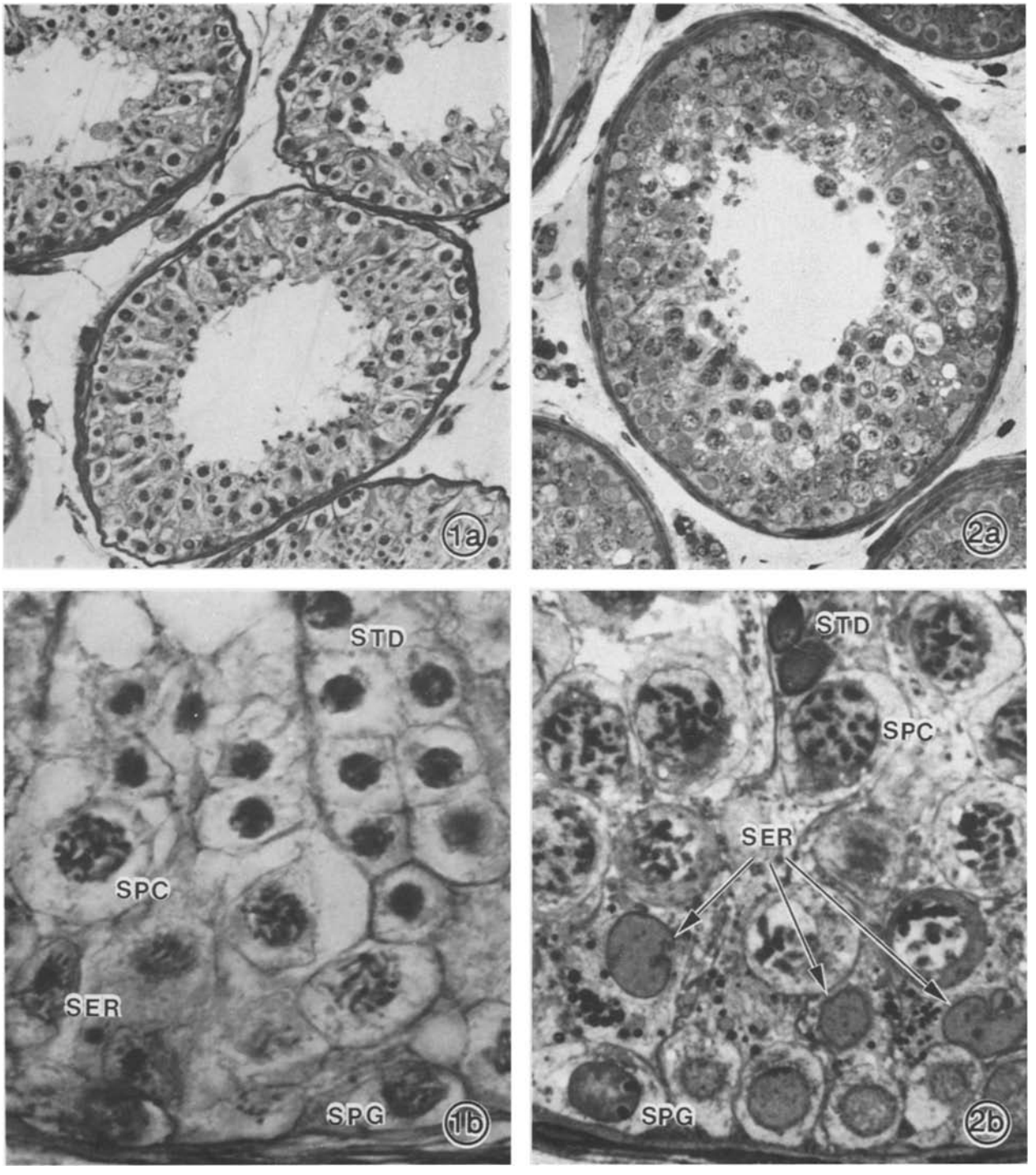
Materials and Methods

Bilateral testicular biopsies from seven men between 15 and 40 years of age with irreversible brain death were obtained during kidney retrieval operation. Tissues were immediately fixed by immersion in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 12–14 h, cut into approximately 1 mm^3 pieces, washed in the same buffer, post-fixed in 1% O_sO_4 in 0.1 M s-collidine buffer for 1 h and processed for routine epon embedding [2]. Histological examination and cell counts were performed using a Nikon Optiphot light microscope. Histological examinations of each biopsy specimen revealed normal spermatogenesis and normal extratubular components.

Randomly chosen tubular cross sections and side areas of longitudinally cut tubules with clear lumens were used for counting. Tangentially cut tubules with thicker germinal epithelium were avoided. The identification of various germ cells and Sertoli cells was based on morphology [7]. Differential cell counts were made using an ocular grid (area of 12,000 μm^2) fitted to the eye piece with an 80X objective [4]. Eight to 10 sections from each biopsy were examined systematically by the stepwise movement of the stage.

All spermatogonia (sum of A-long, dark-type A, pale-type A and B spermatogonia), spermatocytes (sum of preleptotene, leptotene, zygotene and pachytene spermatocytes), spermatids (sum of early and late spermatids) and Sertoli cell nuclei having distinct nucleoli within the frame of the grid were counted. Cell counts were finally expressed as number of germ cells per Sertoli cell [14, 16]. The total number of grid fields counted for each biopsy varied



Figs. 1 and 2. Light micrographs of human testis from paraffin (**1a** and **1b**) and epon sections (**2a** and **2b**) showing less shrinkage and artifactural damage in epon sections (**2a** and **2b**). Seminiferous epithelial cells: Sertoli cells (*SER*); spermatogonia (*SPG*); spermatocytes (*SPC*); and spermatids (*STD*) can be better identified in epon preparations (Fig. **2b**). **1a** and **2a** x 260; **1b** and **2b** x 1,200

from 100 to 130. Results are expressed as the mean ± SE. Statistical comparisons between the left and right testes were made with the student's "t" test. No correction for shrinkage and/or section thickness was necessary as regular semi-thin (1 μm) sections stained with toluidine blue from epon embedded blocks were used [9, 10]. For comparative purposes between epon and paraffin preparations, part of the biopsy was fixed in Bouin's fixative and prepared for conventional histological study following paraffin embedding. 4 μm sections were cut from paraffin embedded blocks and stained with periodic acid-Schiff-hematoxylin [7]. For fine morphological studies, 90–100 nm sections stained with lead citrate were examined under a Philips EM 300 electron microscope.

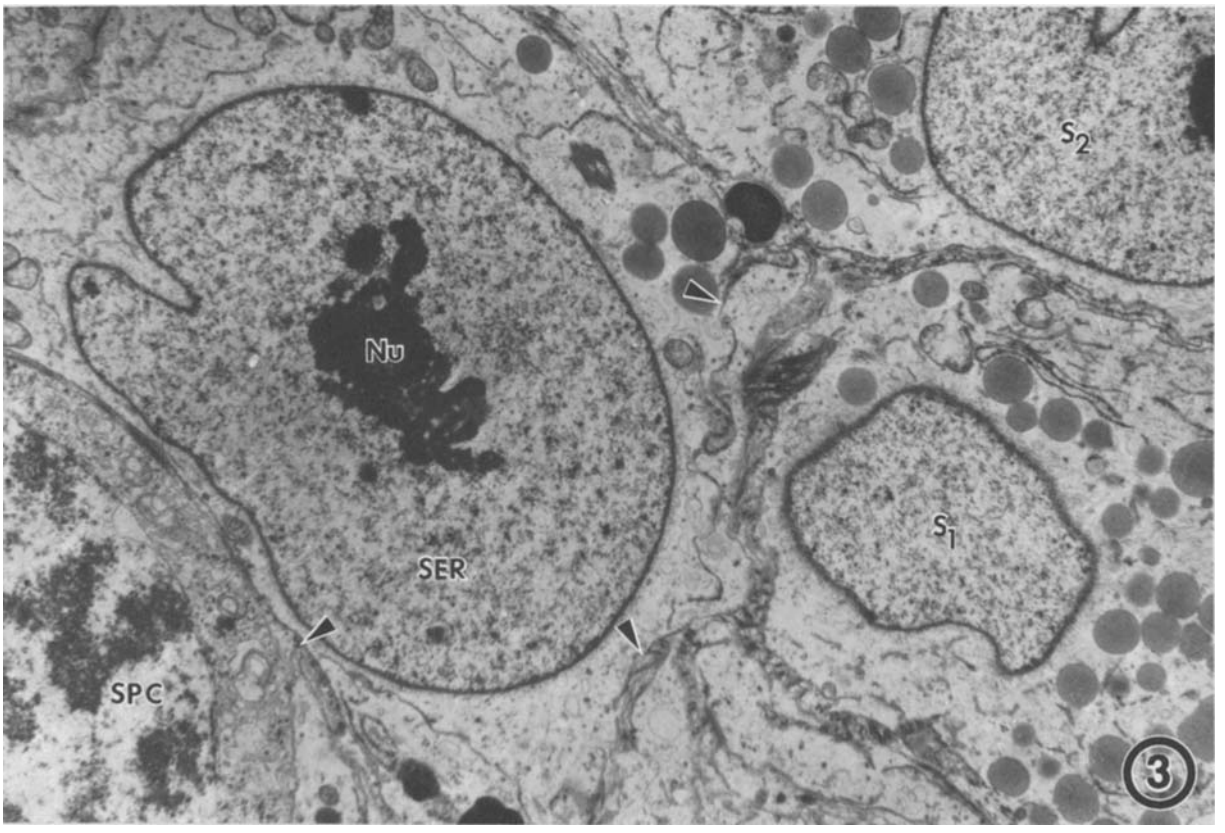


Fig. 3. Electron micrograph of a typical Sertoli cell (*SER*) showing the nucleus containing a nucleolus (*Nu*). Two adjacent Sertoli cells (S_1, S_2) and a primary spermatocyte (*SPC*) are separated by clear boundaries (*arrow heads*). x 8,500

Table 1. Sertoli cell number and germ cell – Sertoli cell ratios in man

Subject No.	Side	Sertoli[a] cell no.	Germ cell-Sertoli cell ratios		
			spermatogonia	spermatocytes	spermatids
1.	Left	4.09	1.02	2.16	2.91
	Right	4.20	0.90	1.73	2.58
2.	Left	3.90	0.79	2.41	2.75
	Right	3.78	0.80	2.53	3.03
3.	Left	3.46	0.83	2.45	3.31
	Right	3.32	0.78	2.90	3.35
4.	Left	2.97	1.10	2.81	3.44
	Right	3.34	1.17	2.45	3.32
5.	Left	3.44	0.87	2.32	2.71
	Right	3.03	1.05	2.73	2.73
6.	Left	3.03	1.08	2.49	3.20
	Right	2.78	1.03	2.60	3.42
7.	Left	4.23	0.87	1.67	2.40
	Right	4.33	0.79	1.66	2.40
	Mean	3.56	0.93	2.35	2.96
	(Range)	(2.78–4.33)	(0.78–1.17)	(1.66–2.90)	(2.40–3.44)

[a] cell count is expressed as number per unit tubular area

Table 2. Comparison of Sertoli cell number and germ cell-Sertoli cell ratios between left and right testes of the subjects studied

Cell types	Left testes (7)	Right testes (7)	*P*
Sertoli cells	3.58 ± 0.19[a]	3.54 ± 0.22	N.S.
Sertoli-germ cell ratios			
Spermatogonia	0.94 ± 0.05	0.93 ± 0.06	N.S.
Spermatocytes	2.33 ± 0.13	2.37 ± 0.18	N.S.
Spermatids	2.96 ± 0.15	2.97 ± 0.15	N.S.

Number in parenthesis represents total number of biopsies

[a] cell count is expressed as number per unit tubular area. Values are given as mean ± SE. *P* values for each cell type do not vary significantly between right and left testes (significant level: $P < 0.05$)

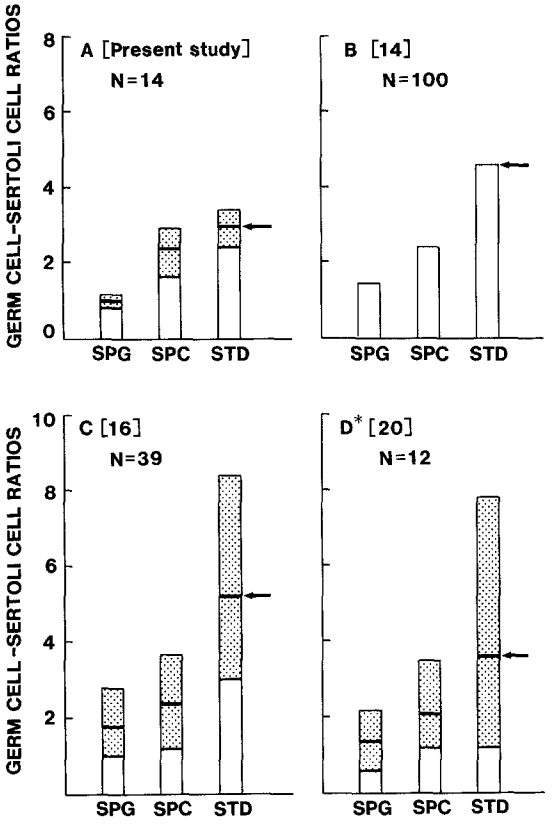


Fig. 4A–D. Comparison of germ cell-Sertoli cell ratios between epon (A) and paraffin (B, C, D) sections. Values are given as mean (*arrows*) and range (*Stippled bar*). * From subjects with sperm count ranged from 16.0 to 89.0 x 10^6/ml. Labels in the figure are spermatogonia (*SPG*); spermatocytes (*SPC*); and spermatids (*STD*)

Results

The quality of structural preservation of the human testicular biopsies following plastic embedding makes it favorable for quantitative analysis. The cellular integrity is well maintained and there is very little artifactual damage in comparison with paraffin preparations (Figs. 1a and 1b). Paraffin embedded tissue showed a variable degree of shrinkage, with loss of compactness and artifactual damage which caused difficulties in identification and accurate estimation of cell types. Germ cells at various stages of differentiation and the Sertoli cells could be better identified in epon preparations (Figs. 2a and 2b). Light microscopic observations on semi-thin epon sections and ultrastructural studies on Sertoli cells by serial sectioning confirmed that each Sertoli cell contained only one nucleus (Figs. 2b and 3). Thus, the number of Sertoli cell nuclei appears to be indicative of the number of Sertoli cells in the human testis.

The Sertoli cell count and the germ cell-Sertoli cell ratios obtained from 14 biopsies (seven bilateral) are presented in Table 1. Quantitation of Sertoli cells per unit tubular area using epon sections reflects a constancy of cell counts between biopsies of different individuals (Table 1). The interindividual variation of germ cell-Sertoli cell ratios is almost negligible (Table 1). No significant deviation of Sertoli cell number or the germ cell-Sertoli cell ratios has been detected between the right and the left testes of the same individual (Table 2).

Discussion

Epon-embedded, 1 μm-thick toluidine blue stained sections, used for the present investigation provide an integrity of tissues which allows improved identification of intratubular elements. However, the major problem of using plastic sections for evaluation of testicular biopsy is that the small size of the specimens required for epon sectioning, may not provide reliable information for critical assessment of spermatogenesis. The results of the present study and our previous studies [3] compare favourably with other studies [14, 16, 20] in (Fig. 4). Methods used by earlier investigators to obtain quantitative information on human testicular biopsies such as differential cell counts are mainly based on paraffin preparations [14, 16, 17, 20]. Germ cell counts are expressed either per unit length of seminiferous tubule circumference [17] or per Sertoli cell [14, 16]. Agreement in overall estimation of germ cell-Sertoli cell ratios between our own and the previous studies support the validity of using epon embedded small biopsy materials for quantifying human seminiferous epithelium.

It is known that in adult human testes, the Sertoli cells do not divide [1, 8, 13, 18]. Also these cells are highly resistant to external challenge such as ischemia [12], increased temperature [6, 19], and experimental torsion of the spermatic cord [5]. Therefore, it would appear logical to use the Sertoli cell as reference for germ cell quantitation. However, some investigators reported a high interindividual variation of Sertoli cell number in normal testes and concluded that Sertoli cell number should not be presumed stable in human testes [11, 17, 20]. Therefore, the Sertoli cell would not be an ideal reference cell type for germ cell quantitation. Whereas, the present results clearly demonstrate a remarkable stability of Sertoli cell number

and germ cell ratios both between individuals and between right and left testes of the same subject (Tables 1 and 2). This investigation justifies the validity of use of the Sertoli cell as a reference cell type for quantitation of human germ cell population, because it was demonstrated that the relative number of Sertoli cells per "unit" tubular area of all biopsy samples remained constant. This method also minimised the variation of cell count due to shrinkage during tissue preparation. The present report thus lends further support to the earlier notion of using the Sertoli cell as a reference for quantitative analysis of human seminiferous epithelium [14, 16]. The present method does not require the knowledge of intricate details of the "staging" of the seminiferous epithelium and the cell counts are expressed as number per unit area of the seminiferous tubule. The method is easy to use, once the morphologic details of the cell types are learned.

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