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ESTIMATION OF SERTOLI CELL NUCLEAR VOLUME ON VERTICAL SECTIONS

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ABSTRACT

Sertoli cell nuclear volume is a potential indicator of cellular activity, however, it's direct estimation on transverse sections of tubules using the point sampled intercept family of methods may be complicated by anisotropy. In the present study the volume weighted mean volume ($\bar{v}_V$) and the number weighted mean volume ($\bar{v}_N$) of Sertoli cell nuclei were estimated on vertical sections using the point sampled intercept method, the "Selector" and "Nucleator" methods with both sine weighted and arbitrarily directed lines. The results were then compared to estimates of Sertoli cell nuclear volume obtained assuming isotropy from transverse sections. Volume weighted mean volume was $578 \pm 21\mu m^3$ measured using sine weighted directions and $610 \pm 29\mu m^3$ measured using arbitrary directions, the nucleator estimates were $543 \pm 24\mu m^3$ and $575 \pm 40\mu m^3$ respectively whilst the selector estimates were $570 \pm 22\mu m^3$ and $586 \pm 13\mu m^3$ respectively. Sertoli cell nuclear volume estimated on transversely sectioned tissue was $570 \pm 37\mu m^3$. This study confirms previous estimates of Sertoli cell nuclear volume performed on tubule cross-sections.

Key words: Sertoli cell nuclei, stereology, point sampled intercept, selector, nucleator.

INTRODUCTION

The Sertoli cell is the sustentacular cell of the seminiferous epithelium, it is thought to both nurture and control the differentiation of the germ cells during the process of spermatogenesis. Sertoli cell nuclei are distributed around the periphery of the seminiferous tubule and their individual nuclear volume is a potentially valuable estimator of Sertoli cell function. In previous studies nuclear volume, estimated using the point sampled intercept, has been shown to increase with age (Wang et al, 1989; Zhengwei et al, 1990). The seminiferous tubules have an orientation which lies predominantly parallel to the long axis of the testis (Clermont and Huckins, 1961); because of the unique cellular associations identifiable on cross section of the tubules this is the preferred section orientation. Estimation of Sertoli cell nuclear volume (in the rat) has been performed on transverse sections using the point sampled intercept family of methods (Wang et al, 1989; Zhengwei et al, 1990) and using serial reconstruction and a Cavalieri type estimator (e.g. Ghosh et al, 1992). The point sampled intercept family of methods are preferable to the Cavalieri type estimators because they are fast and applicable to single sections. The disadvantage of these methods is that they are biased by any anisotropy present in the measured object. Ideally estimation of Sertoli cell nuclear volume should be performed on "vertical sections" (Baddeley et al, 1986), however it is simpler to measure it on transverse sections contemporaneously with other counting and measuring operations which are routinely performed on such sections. The present study was undertaken

to determine the extent of any bias resulting from using transverse rather than vertical sections.

MATERIALS AND METHODS

Animals. Five adult (90 days) male Sprague -Dawley rats maintained on a 14 hour light and 10 hour dark cycle and fed ad libitum were obtained from the Central Animal House at Monash University. The study was approved by the Monash University Animal Ethics Committee.

Fixation and processing of tissues. Animals were injected with heparin (porcine mucous, 15 IU / g body weight) and anaesthetised with ether. The descending thoracic aorta was cannulated and the vascular system was flushed with saline before fixation with a solution containing 5% glutaraldehyde, 3% formaldehyde, 0.75% calcium chloride and 0.15% picric acid buffered to pH 7.4 with 0.15M cacodylate (Kerr and de Kretser, 1975). The testis was removed after approximately 15 minutes perfusion and placed in the fixative solution to await sampling. Prior to sampling the testis was weighed and 3 uniformly spaced 2mm slices were cut (orthogonal to the long axis) in a systematic uniform random manner from the upper middle and lower parts of the testis. These slices were then further sampled using the method shown in Gundersen et al (1988, fig 7). Nine blocks were sampled from each testis. The resultant blocks each with an identifiable axis parallel to the long axis of the testis were processed into Epon-Araldite and sets of 10-15 serial 2 µm sections were prepared and stained with toluidine blue. A separate set of blocks was prepared using the same methods but sectioned in the usual manner (orthogonal to the long axis) to give the commonly used transverse sections of tubules.

Stereology:
Selection of fields. The upper section from each stack was chosen and the upper left hand corner was photographed, additional fields were sampled by moving diagonally across the section photographing alternate fields. This procedure was repeated on the serial sections with identical fields being photographed using Kodak Ektachrome 64 ASA (tungsten) film. Measurements were performed on the projected image at a final magnification of 1136x using a linear ruler.

Estimation of Sertoli cell nuclear volume. Volume weighted Sertoli cell nuclear volume was estimated using the "Point sampled intercept" (Gundersen and Jensen, 1985). Number weighted nuclear volume was estimated using the "Selector" Cruz-Orive (1987a) and the "Nucleator" (Gundersen et al ,1988). All methods were applied using vertical sections with both sine weighted and arbitrary directions (in practice parallel to the bottom of the micrograph). Sine weighted directions were selected using the protractor described by Cruz-Orive (1987b) with an increment of 37 from a random start.

(1) Volume weighted mean volume was estimated on the first and last section from each stack (this ensured that the measurements were performed on different sets of nuclei); approximately 800 intercepts were measured per testis.
(2) Selector estimates were performed on nuclei sampled using a "disector" (Sterio,1984) applied to the middle sections of each stack. Nuclei were then followed through the stack in either direction; approximately 100 nuclei were sampled per testis.

(3) Nucleator estimates were performed using the nucleolus as a sampling point. All profiles from two sections which contained a well defined nucleolus were sampled and measured from the centre of the nucleolus to the nuclear boundary. Two measurements were recorded for each nuclear profile; approximately 300 nuclei were sampled per testis. Nucleator estimates were also performed on transversely sectioned tubules; approximately 50 nuclei were sampled per testis.

All measurements were classified using an l_0 ruler in mm. Data was then manipulated using a spreadsheet program.

Statistical analysis. Data reported in the text is mean ± sd. Comparison of volume estimates derived using sine weighted directions and an arbitrary direction were made using a paired Student t-test. Comparison of volume estimates made using the different methods was made using the Peritz F test (Harper 1984) for multiple comparisons.

RESULTS

Vertical sections invariably contained elliptical profiles of the seminiferous tubules reflecting the predominant orientation of the seminiferous tubule. Glancing sections of the tubules were relatively common where the lumen was not apparent, these tubules were difficult to stage with respect to spermatogenesis and hence not suitable for estimating germ cell number in the testis. In contrast, transversely sectioned material invariably presented circular profiles of the tubules which could be readily staged facilitating the recognition of germ cell categories. In all sections nucleolated Sertoli cell nuclear profiles were readily identified but sections not containing the nucleolus presented some difficulty necessitating reference to the adjacent sections.

Using the vertical sections there was no difference in the estimate of number weighted mean volume made using the "selector" with sine weighted (570 ± 22 μm^3) or arbitrary directions (586 ± 13 μm^3). Similarly there was no difference in the estimates of number weighted mean volume made using the "nucleator" with sine weighted (543 ± 24 μm^3) or arbitrary directions (575 ± 34 μm^3). Volume weighted mean volume was also similar when measured using sine weighted directions (578 ± 21 μm^3) or arbitrary directions (610 ± 29 μm^3). Nucleator estimates performed on transverse sections with arbitrary direction of measurement (570 ± 37 μm^3) were consistent with the estimates obtained on vertical sections.

Comparison of the mean estimates obtained by the different methods showed only one significant difference, volume weighted mean volume estimated with arbitrary directions was significantly greater ($p<.05$) than number weighted mean volume estimated using sine weighted directions.

DISCUSSION

Sertoli cell nuclear volume has potential as an indicator of Sertoli cell functional activity. However the unequivocal recognition of germ cell and Sertoli cell nuclear profiles requires transverse sections of the seminiferous tubules. The geometry of the seminiferous tubule within the testis makes this impossible to achieve with vertical sections. In this study there is good consistency between Sertoli cell nuclear volume estimates obtained on vertical sections measured in sine weighted directions and the estimates obtained either on the same sections measured in arbitrary directions or transverse sections measured in arbitrary directions.

Estimates of $\bar{v}_V$ and $\bar{v}_N$ obtained in this study were consistent with estimates of $\bar{v}_V$ and $\bar{v}_N$

obtained in previous studies (Zhengwei et al, 1990; Wang et al 1989) on transversely sectioned material using identical methods. The estimates of $\bar{v}_V$ in the present study were consistently higher than the corresponding estimates of $\bar{v}_N$ which is consistent with their known relationship, ie $\bar{v}_V = \{1+CV^2(v_N)\}\bar{v}_N$ (Cruz-Orive, 1987b). The data also further validates the estimates of Sertoli cell number given in our earlier work (Zhengwei et al, 1990; Wang, et al 1989) which depended on the assumption that $\bar{v}_V \approx \bar{v}_N$ for Sertoli cell nuclei. In practice the nucleator estimate is the easiest to apply using our current instrumentation, giving an acceptable estimate of Sertoli cell nuclear volume from about 50 nucleolated profiles.

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