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THE HUMAN TESTIS STUDIED USING STEREOLOGICAL METHODS

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ABSTRACT

The aim of the present pilot study was estimate the total number of Leydig cells and Sertoli cells and the number weighed mean volume of the Leydig cells in men. During the last years, efficient unbiased and relative fast methods to quantificate cells are developed. A principle based on a systematic uniform sampling scheme, the fractionator and a three dimensional counting probe, the optical disector, was used to estimate the total number of Sertoli and Leydig cells in four men. No assumptions about shape, size or orientation of the cells or shrinkage of the organ are needed, when these methods are used. A new method to characterize the volume and the size distribution of cells, the rotator was used to estimate the number weighted mean volume. The mean total number of Sertoli and Leydig cells in normal men was estimated to 1140 $x10^6$ (CE (SEM/mean) = 0.14) and 180 x 10^6 (CV (SD/mean) = 0.20) respectively. The mean volume of Leydig cells was estimated to 3099μm^3 (CV = 0.24).

Key words: optical disector, fractionator, testis, Sertoli cells, Leydig cells.

BACKGROUND

The incidence of testicular cancer has increased worldwide by a factor of three to four during the last four to five decades (Adami et al. 1994) and testicular cancer is now the most common cancer disease among young men in many countries. An apparent decrease in semen quality has been observed during the same period (Carlsen et al. 1992; Auger et al. 1995). The reasons for these changes in male reproductive health are unknown. Experimental and epidemiological studies have shown that excessive exposure to estrogens in fetal life are leading to decreased semen quality and increased incidence of male genital abnormalities in animals and humans (Yasuda et al. 1985; Gill et al. 1977; Whitehead, Leiter, 1981). These observations have led to the hypothesis, that factors acting during the development of gonads in fetal life could be involved in the pathogenesis of both decreasing fertility and increasing incidence of testicular cancer. Increasing exposure to environmental agents with estrogenic activity has been proposed to be of etiological significance in the increasing frequency of abnormalities in the male genital organs (Sharpe, Skakkebæk, 1993). Moreover, in experimental studies the

development of Leydig cells and Sertoli cells is impaired by exposure to estrogens (Yasuda et al. 1985; Yasuda et al. 1986).

Thus, if increasing incidence of testicular cancer is caused by increasing exposure to estrogens in fetal life, disturbances in the development of Sertoli cells and Leydig cells might be expected in men with testicular cancer. These disturbances should be characterized by different volumes and/or numbers of Sertoli cells and Leydig cells compared to healthy individuals.

During the last years efficient, unbiased and relative fast methods to quantificate cells are developed. A principle based on a systematic uniform sampling scheme, the fractionator, and a three dimensional counting probe, the optical disector, is previously reported to be a highly efficient method to estimate the total number of cells in an organ (West et al. 1991). No assumptions about shape, size or orientation of the cells or shrinkage of the organ are needed, when these methods are used. A new and highly efficient method to characterize the volume and the size distribution of cells, the rotator, is recently described (Jensen, Gundersen, 1993). The aim of the present pilot study was estimate the total number of Leydig cells and Sertoli cells and the number weighted mean volume of the Leydig cells in men.

MATERIAL

One testis was sampled from each of four men, aged 25 to 47 years, collected from the Department of Forensic Medicine, Copenhagen, after sudden unexpected death. The four sampled testes were all from the right side. Testicular tissue obtained from orchiectomy of patients with testicular cancer was used to evaluate staining and fixation methods.

METHODS

The structure of the tissue was tested with tissue embedded in 2-hydroxy-methacrylate (Technovit 7100®) and paraffin respectively. Different staining methods including Van Gieson's, Toluidine blue, Giemsa's and Hematoxylin Eosin were evaluated and Hematoxylin Eosin preferred, because of the best differentiation of Leydig cells and Sertoli cells in this staining. The Leydig cells were recognized in the intertubular space as large cells with acidophile cytoplasm. The large round nucleus has one or two eccentrically placed nucleoli. The Sertoli cells were distributed in the tubules and were easy to separate from germ cells because of their typical nucleus with invaginations and a prominent nucleolus.

The testes were weighed before and after fixation in formalin. The first pilot testis was cut into 2.27-mm-thick slices, and the volume was estimated by the Cavalieri principle. Every fourth slice, sampled uniformly, was divided into 2.27-mm-thick bars. Every fourth bar was sampled uniformly and cut into 2.27-mm-wide cubes. Every second cube was sampled systematically at random, rotated uniformly in 3D and embedded in 2-hydroxy-methacrylate (Technovit 7100®) and sliced into 50-µm-thick sections. The sections were cut by a Historange microtome using metal knives. However, slices, bars and cubes of approximately 2 mm thicknesses were difficult to handle, 50-µm-thick sections were difficult to cut with the present equipment and uniform staining was difficult to obtain in these sections. We therefore decided to cut the remaining testes into 4.54-mm-thick slices and every second or third slice was sampled, systematically at random, and divided into 4.54-mm-thick bars. Every second cube was sampled, rotated uniformly in 3D, and embedded in 2-hydroxy-methacrylate and sliced exhaustedly into 40-µm-thick sections.

Every 12-24th section was sampled systematically at random. The optical disector principle was used to count the number of Sertoli- and Leydig cells in a known fraction of the testis. The total number of Sertoli- and Leydig cells was estimated using the fractionator principle (West et al. 1991). The volume of each sampled Leydig cell was estimated by the rotator method (Jensen, Gundersen, 1993).

The coefficient of error (CE=SEM/mean) and the coefficient of variation between the individuals (CV=SD/mean) were calculated for the different steps of sampling and these values were used to estimate the required number of sampled objects at the different steps of sampling as described previously (West et al. 1991).

RESULTS

The estimated total number of Sertoli- and Leydig cells are shown in Fig. 1, and the estimated number weighted mean volume of Leydig cells and total Leydig cell volume are shown in the Table 1.

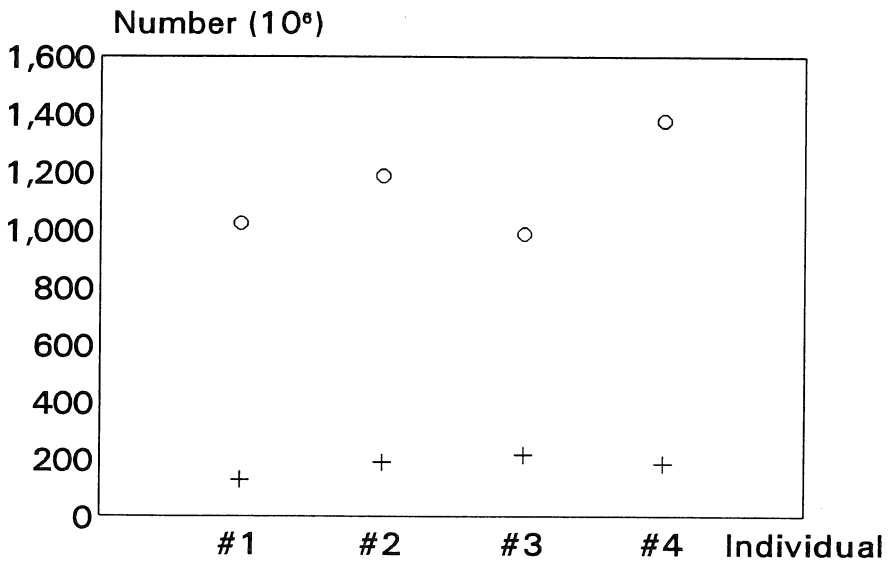

Fig. 1: Total number of Sertoli cells (○) and Leydig cells (+) per individual.

The mean total number of Sertoli cells in the four men was 1340 x 10^6, with CV = 0.14.

The CE originating from sampling of slices was 0.12 - 0.16 when about five slices were sampled from each testis and 0.03, when 11 slices were sampled.

The structure of the tissue was well-preserved, when the tissue was embedded in 2-hydroxy-methacrylate, but poorly preserved in paraffin.

DISCUSSION AND CONCLUSION

To our knowledge this is first time, the fractionator principle combined with optical disectors is used to estimate the total Leydig cell and Sertoli cell number in humans. In previous studies the estimated total Sertoli cell numbers were in the range from 390 to 3700 x 10^6 and total Leydig cell number from 400 to 800 x 10^6 calculated from two-dimensional profile counting (Paniagua et al. 1987; Kaler, Neaves, 1978; Cortes et al. 1987). However, it is previously shown in experimental studies, that conventional counting methods may bias the estimated number of Leydig cells in unpredictable ways (Mendis-Handagama, Ewing, 1990; Mendis-Handagama, 1992). The total number of Leydig cells was estimated to 23 x 10^6 in rats and 3 x 10^6 in mice, estimated by the disector principle (Mendis-Handagama, 1992; Mendis-Handagama et al. 1990).

Table 1: Estimation of number weighted mean volume, total cell number and total Leydig cell volume per individual.

	Number weighted mean volume of Leydig cells (μm^3) (CE)	Leydig cell number (10^6) per individual (CE)	Total Leydig cell volume per individual (ml)
Testis 1	2757 (0.08)	128 (0.17)	0.353
Testis 2	2807 (0.08)	190 (0.27)	0.533
Testis 3	2979 (0.06)	216 (0.19)	0.643
Testis 4	3852 (0.05)	184 (0.16)	0.709
Mean	3099 (0.07)	180 (0.20)	0.560
CV	0.14	0.20	0.24

CE: coefficient of error, CV: coefficient of variation.

Although formalin is known to be inferior to other fixatives as Stieve's and Boin's fixative to preserve testicular tissue when the tissue are embedded in paraffin, the structure of the tissue was well preserved embedded in 2-hydroxy-methacrylate.

The CE originating from sampling of slices is 0.12 - 0.16, when only five sections were sampled but only to 0.03 when 11 slices were sampled.

As previously mentioned the cells were easy to distinguish, when the tissue was stained by Hematoxylin Eosin and embedded in historesin. We can therefore conclude:

- The mean total number of Sertoli cells in normal men was estimated to 1140 x10^6 (CV = 0.15, CE = 0.07).
- The mean total number of Leydig cells was estimated to 180 x 10^6 (CV = 0.20, CE = 0.10).
- The mean volume of Leydig cells was estimated to 3099μm^3 (CV = 0.24).
- With a sampling design of seven cubes and 11 slices the total number of Sertoli and Leydig cells can be estimated with a CE of approximately 15% in testes fixed in formalin and embedded in 2-hydroxy-methacrylate.
- Estimation of the number of Sertoli and Leydig cells and complete size distribution of Leydig cells in normal human testis can be done in one to two days.

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