Image Anal Stereol 2000;19:215-218
Original Research Paper

STEREOLOGICAL QUANTITATION OF LEYDIG AND SERTOLI CELLS IN THE TESTIS FROM YOUNG AND OLD MEN

PETER M PETERSEN[1,2] AND BENTE PAKKENBERG[2,3]

[1]Department of Growth and Reproduction, Copenhagen University Hospital, Rigshospitalet, 2100 Copenhagen Ø, [2]Neurological Research Laboratory, Bartholin Institute, Kommunehospitalet, Øster Farimagsgade 5, 1399 Copenhagen K, [3]Stereological Research Laboratory, Aarhus University, 8000 Århus C, Denmark
(Accepted September 9, 2000)

ABSTRACT

One of the newer stereological methods, the optical fractionator, was applied to the study of the effects of ageing on the human testis. The estimated total number of Sertoli and Leydig cells per testis in men younger than 30 years were 430×10^6 (CV = SD/mean = 0.35) and 117×10^6 (CV = 0.53), respectively, while in men older than 50 years the estimated total Sertoli cell number was 266×10^6 (CV = 0.46) and the mean Leydig cell number 83×10^6 (CV = 0.53). The difference between the number of Sertoli cells in men younger than 30 years compared with men older than 50 years was close to statistical significance ($p = 0.052$) while no differences was found in total Leydig cell number ($p = 0.22$).

Keywords: Leydig cells, Sertoli cells, stereology.

INTRODUCTION

The process of ageing and the changes in different tissues with age have been in focus during the past decade because the number of healthy elderly people has been increasing in many countries. The effects of ageing on testicular function have been widely discussed, and it is generally accepted that androgen insufficiency with increasing gonadotropins evolves in old men (Murray and Meacham 1993; Haidl *et al.*, 1996). One aspect has been the application of androgen substitution therapy in old men. However, only few studies have concentrated on physiological and histologic changes with ageing. A study of men and their grandfathers showed surprisingly small differences in semen quality and reproductive hormones (Nieschlag *et al.*, 1982) while other studies have indicated more obvious differences (Murray and Meacham 1993). Histologic investigations have indicated decreasing Sertoli cell number and Leydig cell number with increasing age (Kaler and Neaves, 1978; Neaves *et al.*, 1985; Paniagua *et al.*, 1987; Johnson *et al.*, 1984). However, the reports are still conflicting, (Murray and Meacham, 1993). Thus, more basic knowledge on the effects of ageing on the testis is needed. We have applied the stereological tool the optical fractionator (Gundersen *et al.*, 1988; West *et al.*, 1991) which has proven to be an efficient stereological method for estimating the total number of Sertoli and Leydig cells (Gundersen *et al.*, 1988; Petersen *et al.*, 1996). The great advantage of this method is that no assumptions are required about shape, size or orientation of the cells or shrinkage of the organ during histological processing.

AIM

The aim of the study was to investigate the effect of ageing on the number of Sertoli and Leydig cells in human testes by use of the optical fractionator principle.

MATERIAL

Right or left testis from each of fourteen males, eight males younger than 30 years (16-30) and six males older than 50 years (53-78), was selected systematically at random (Table 1). All testes from the young men and two of the testes from the older males were sampled from cases who had been taken to the Department of Forensic Medicine in Copenhagen due to sudden, unexpected death, while the remaining testes from old males were obtained at routine autopsy. The cause of death is shown in (Table 1).

METHODS

1) A known fraction of the tissue was sampled systematically at random from each testis in a careful stepwise sampling procedure: a) Each testis was cut into 4-mm-thick slaps, providing 8 - 12 slaps; b) Every 2nd - 3rd slap was sampled systematically randomly and cut into 4-mm-thick bars providing 6 - 10 bars; c) every 2nd - 3rd bar was sampled and cut into cubes; d) every 4th to 6th of these cubes (approximately 8 - 10 cubes) were sampled.

2) The sampled tissue was embedded in 2-hydroxy-methacrylate (Technovit 7100®) and stained throughout with hematoxylin eosin where the Leydig cells and Sertoli cells can be recognised. These cell types was easily recognised also in tissue fixed in formalin when embedded in plastic, in contrast to testicular tissue fixed in formalin and embedded in paraffin.

3) The blocks of methacrylate, each containing 8-10 cubes of testicular tissue, were cut exhaustely into 40-μm-thick sections.

4) Approximately 10 sections were sampled from each testis and the optical fractionator principle used to estimate the total number of Sertoli and Leydig cells in a known fraction of the tissue. To avoid bias from cutting artefacts a disector height of 15 μm was chosen. By this sampling procedure the coefficient of error (CE = SEM/mean) at each sampling level is estimated and kept below 10%.

5) Approximately 150 of each cell type were counted per testis.

6) The total numbers of Sertoli and Leydig cells were estimated by multiplication of the number of sampled cells by the inverse of the sampling fraction. For example in one testis every 2nd slap, every 3rd bar, every 6th cube, and every 12th methacrylate section were sampled and counting of Leydig cells was performed in 1/4973 of the sampled tissue section. Thus the global sampling fraction was 1: (2×3×6×12×4973). The estimated number of Leydig cells was equal to the inverse of the global sampling fraction times the number of sampled Leydig and Sertoli cells. For further details, see Petersen *et al.* 2000.

Table 1. *Cause of death, age, fixation data and number of Sertoli and Leydig cells in young and old males.*

Side	Cause of death	Time from death to processing (days)	Fixative	Age (years)	Weight (gr.)	Number of Sertoli cells counted	Total Sertoli cell number (10^6)	Number of Leydig cells counted	Total Leydig cell number (10^6)
Left	Sud	1	F	16	13	292	665	103	47
Right	Sud	-	F	18	22	123	281	94	43
Left	Sud	-	F	18	19	344	388	233	53
Left	Sud	3	F+A	22	19	124	400	237	168
Right	Sud	2	F	24	19	113	253	143	65
Left	Sud	3	F +A	28	13	158	318	251	111
Left	Sud	1	F + A	29	19	371	571	323	75
Left	Sud	2	S	30	23	303	564	256	72
Mean (range)				23.1 (16-30)	18.4 (13-23)	228 (123-371)	430 (253-665)	205 (94-323)	117 (43-168)
Right	Sud	2	S	52	21	148	394	95	56
Right	Sud	1	S	57	16	282	344	506	93
Right	-	2	F	75	19	390	156	164	66
Right	C.esophagei/COPD	2	S	75	9	346	86	606	151
Left	AMI/NIDDM	3	S	63	16	254	361	271	127
Left	AMI	3	F	80	19	197	255	122	52
Mean (range)				67.0 (52-80)	16.7 (9-21)	269 (148-390)	266 (86-394)	294 (95-606)	83 (52-151)

Sud: Sudden unexpected death, F: Formalin, A: Acetic acid, S: Stieves Fixative (formalin, mercuric chloride, acetic acid), COPD: Chronic obstructive pulmonary disease, AMI: acute myocardial infarct, NIDDM: non-insulin dependent diabetes mellitus.

STATISTICS

The mean values and coefficient of variation (CV = SD/mean given in parentheses) between testes were calculated for each cell type and for testicular weight. The hypothesis that no differences were present in testicular weight, and number of Sertoli and Leydig cells between men younger than 30 and men older than 50 years was tested using an unpaired two-tailed t-test. The data on Leydig cell number was log transformed in order to obtain normal distribution. The remaining data were normally distributed.

RESULTS

The mean number of Sertoli and Leydig cells counted per testes was 254 (113 - 394) and 236 (94 - 606) (see Table 1), respectively.

The estimated CE due to sampling was below 10% at all sampling levels and the overall estimated CE due to sampling was 0.15 for both Sertoli and Leydig cells. The estimated number of Sertoli cells per testis was 430×10^6 (0.35) and Leydig cell number 117×10^6 (0.53) in males less than 30 years. The corresponding numbers in men older than 50 years were 266×10^6 (0.46) for Sertoli cells and 83×10^6 (0.53) for Leydig cells (Table 1). The difference between number of Sertoli cells in males younger than 30 years of age and older than 50 years was borderline statistically significant ($p = 0.052$) while no difference was seen between the total number of Leydig cells in the two groups ($p = 0.22$). No difference was found with respect to testicular weight ($p = 0.43$) (Table 1). As seen in figure 1 the biological variation of total Leydig and Sertoli cell number seems to be very high in both groups.

DISCUSSION

The mean number of Sertoli cells in testes from adult males is approximately 400×10^6 and the number of Leydig cells approximately 90×10^6. The total Leydig and Sertoli cell numbers, estimated by use of stereological methods, differed from the results obtained from most previous studies. The estimated total Sertoli cell numbers have been reported to range from 390 to 3700×10^6 and the total Leydig cell number from 400 to 800×10^6, calculated from two-dimensional profile counting (Kaler and Neaves, 1978; Neaves *et al.*, 1985; Cortes *et al.*, 1987; Paniagua *et al.*, 1987). These large ranges may be ascribed to the difficulties in interpretation of results obtained from assumption based designs as previously described (Mendis-Handagama and Ewing, 1990; Mendis-Handagama, 1992). The finding of decreasing number of Sertoli cells with increasing age fits with previous observations while the data on Leydig cells are more conflicting (Kaler and Neaves 1978; Johnson *et al.*, 1984; Neaves *et al.*, 1985; Paniagua *et al.*, 1987; Murray and Meacham 1993). Some investigators claim that Leydig cell hyperthrophy evolves with increasing age while other studies have shown decreasing number of Leydig cells with increasing age (Murray and Meacham 1993).

In the present material we did not find any correlation between the total number of Sertoli or Leydig cells and testis size, probably because of the large biological variation of the total number of these cell types, combined with the rather limited sample size. Furthermore, it should be kept in mind that conclusions based on cross sectional data always may be confounded by secular changes.

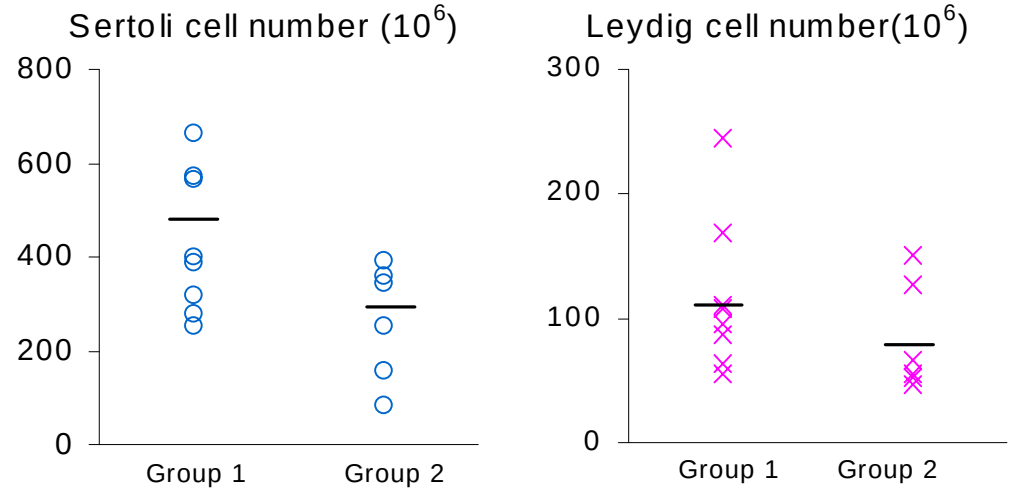

Fig. *Estimation of the total number of Sertoli and Leydig cells per human testes in the age-groups: 1: 16-30 years, 2: 50-80 years.*

The imprecision of the estimates of Sertoli and Leydig cells (CE approximately 15%) should be seen in view of the observation of apparently very large biological variations (CV of Sertoli cells > 0.34 and Leydig cells CV > 0.50). The very large biological variation implies that even the relatively large imprecision of estimates will be acceptable in most clinical situations and that the most important factor in clinical studies comparing groups of patients will be the number of individuals included. In the present study more testes from the older age group are needed before firm conclusions can be made.

In summary, new information about the mechanism of the age-related changes in human testis can be obtained by stereology. In this pilot study decreasing total Sertoli cell number was seen with ageing, while no correlation was detected between total number of Leydig cells and age.

This paper was presented at the Xth International Congress for Stereology, Melbourne, Australia, 1-4 November 1999.

AKNOWLEDGEMENTS

The economic support given by The Hartmann Foundation, Direktør Emil Hertz and Wife Inger Hertz Foundation, The NOVO Nordic Foundation, and Fabrikant Einar Willumsens Mindelegat is highly appreciated.

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