

Effect of Ageing on the Volume, Structure and Total Leydig Cell Content of the Human Testis

Lalit K. Kothari and Anand S. Gupta

Department of Physiology and Surgery, Rabindranath Tagore Medical College, Udaipur, India.

INTRODUCTION

Histopathological examination of the testes was carried out after open biopsy in healthy, fertile males of two different age groups - one young (30-40 years) and the other old (55-65 years). The testicular volume and the total Leydig cell content of the testis was also quantitatively estimated in both. It has been found that ageing leads to a thickening of the tunica propria, inter-tubular fibrosis and progressive hyalinisation and atrophy of some of the tubules. Most of the tubules, however, remain normal with signs of active spermatogenesis. The Leydig cells become more numerous and in 2 cases they had grown into abnormally large clusters. There was no significant difference in the volume of the testes in the young and old subjects, the mean being 13.3 ± 6.0 ml for the whole sample. The total Leydig cell volume showed a significant and interesting increase with age, rising from 2.2 ± 0.5 ml / testis in the young to 2.9 ± 0.9 ml / testis in the old.

During our study of the post-vasectomy changes in laboratory animals as well as human subjects¹⁻⁴, it was observed that the testicular structure shows significant variation with advancing age, involving both the gametogenic and the endocrine components. Screening of the available literature, however, provided little precise information regarding the effect of senility on the testis, particularly in relation to man. Usually it is only mentioned that the changes are inconstant and take form of some inter-tubular fibrosis^{5, 6}. In view of all this, it has been considered pertinent to make a more detailed study of the effects of ageing on the human testis by comparing healthy young adults. Besides histopathological examination by open testicular biopsy and determination of the volume of the testis *in situ*, the total Leydig cell content of the testis has also been precisely estimated, perhaps for the first time in man in this context.

MATERIAL AND METHODS

This study is based on 20 healthy men of two different age groups - ten between 30 and 40 years (young) and the other ten between 55 and 65 years (old). All were clinically normal and married, with at least 2 children to establish their fertility. Care was taken to ensure that none was taking any drugs or suffering from any disease which could possibly affect the testis.

Testicular biopsy: For biopsy, the tunic vaginalis was opened. A nick in the tunica albuginea brought the testicular tissue bulging out and this was carefully taken out by a neat cut. With gentle manipulation, a second and different biopsy piece was then taken through the same cut in the albuginea.

The tissue was fixed in 10% formolsaline and processed for preparing paraffin sections, 5 to 8 μ m thick, in the usual manner.

Testicular volume was determined at the time of the biopsy by measuring the three dimensions of the testis, at their maximum, with the help of a sterilised vernier caliper. The average thickness of the intervening tissues (skin surface to tunica albuginea) was also carefully measured at the site of the biopsy incision and subtracted from the above measurements to get the true testicular dimensions. In some cases the whole testis was delivered out and measured direct but the previous approach was found sufficiently accurate to make this unnecessary.

The volume of the testis was calculated by the formula:

$$V = \frac{4}{3} \cdot \pi \cdot r_1 \cdot r_2 \cdot r_3$$

presuming the testis to be an ellipsoid such that r_1 , r_2 and r_3 are its radii in the three planes⁷.

Total leydig cell volume: The total volume of Leydig cells present in the testis was quantitatively estimated by the histometric point-counting method.

In each of the 20 subjects only the left testis was investigated as a representative of both.

RESULTS

The testicular volume in young and old subjects is given in Table I.

Group	Number of Subjects	Testicular volume, ml.	
		Mean \pm S.D.	Range
Young	10	14.6 \pm 5.4	6.4 - 23.0
Old	10	11.9 \pm 6.5	5.3 - 23.3

It is obvious that the size of the testis can vary considerably even in normal health adults, ranging between 5.3 and 23.3 ml. The mean volume for all the 20 men, young and old included, is 13.3 ± 6.0 ml. Apparently, it tends to be somewhat smaller in the old subjects than in the young (Table I) but the difference is not statistically significant. It can therefore be concluded that the testicular volume remains essentially unaltered, at least from 30 to 65 years of age, in healthy adults.

Histological structure: No evidence of any specific disease process was found in any of the 20 subjects. In young adults the testicular biopsies presented the same well known, normal histological picture.⁹ Therefore, only some salient points directly pertaining to the human biopsy material need be described here to provide baseline data for evaluating the senile changes observed in the older subjects.

The seminiferous tubules, in different phases of the spermatogenetic cycle, showed well differentiated spermatogonia (Type A and Type B), primary spermatocytes and spermatids. Fully developed spermatozoa were, however, only seen occasionally in the biopsy material; sperms lying free in the lumen were even rarer. More commonly, there was an sorted mass of cells scattered in the middle of the tubule which is obviously an artifact produced by the disruption at the time of the biopsy. Sertoli cells with their ill-defined cellular outline, could be identified but were not conspicuous.

The lining membrane of the seminiferous tubules – the tunica propria-appeared as a sharply demarcated and compact fibro-elastic connective tissue sheath with numerous flattened fibroblast nuclei interspersed.

A loose areolar connective tissue with fibroblasts, blood vessels and lymphatics filled the spaces between the seminiferous tubules. Present in this interstitial stroma were the characteristic Leydig cells, either singly or more often in small clusters. Their polygonal shape, large pale nucleus with usually a prominent nucleolus, and the vacuolated cytoplasm made them easy to identify. But occasionally there were a typical cells, taking on the characteristics of both fibroblasts and Leydig cells. These were taken to represent the precursors of the mature Leydig cells but were left out while estimating the total Leydig cell volume.

In the 10 elderly males, between 55 and 65 years of age, spermatogenesis was apparently continuing in a normal manner but there were significant differences in the histological picture.

1. The tunica propria forming the limiting membrane of the seminiferous tubules was clearly thickened, compared with that in the younger group. It appeared more lamellated and wavy and its outer border often merged with the surrounding inter-tubular connective tissue. The increase was seen both in the homogenous basement membrane as well as the fibroblast-rich connective tissue covering.
2. There was also an increase in the inter-tubular connective tissue with peritubular fibrosis and, in some cases, considerable fibroblastic reaction. As a result, the seminiferous tubules were less compactly arranged than in the young, and were somewhat reduced in their transverse diameter.
3. A vacuolation of the germ cells, particularly the primary spermatocytes, was present in some of the tubules but it had quite a scattered distribution. Some of the tubules exhibited this vacuolation clearly while the adjacent tubules appeared totally free from it. This vacuolation would represent a cellular degeneration with advancing age.
4. Conspicuous features in the elderly group were the areas of marked hyalinisation and atrophy of the seminiferous tubules. In these atrophic tubules, only the tunica propria was clearly discernible while the germ cells had all practically disappeared. Naturally, these tubules have ceased to function and such areas of atrophy would mean a gradual shut-down of the spermatogenetic activity. This was in sharp contrast to the testes of young males where all seminiferous tubules showed full differentiation of the 4 to 6 layered germinal epithelium and active spermatogenesis.

It must be emphasized, however, that outside these small areas of atrophy the remainder of the tubules still appeared completely normal and active, even in the aged.

5. On careful histopathological examination, an increase in the Leydig cell population was quite evident. Not only were these characteristic cells found with greater frequency in the inter-tubular tissue, but in 2 of the 10 elderly males they had grown into abnormally large clusters. This hyperplasia resulted in areas which under the high-power microscope showed nothing but a mass of Leydig cells. The cells, however, retained their specific appearance and location and there was nothing to suggest an actual tumour formation or malignancy.

Table II			
Group	Number of subjects	Total Leydig cell volume	
		Absolute (ml/testis)	Relative (% of testis volume)
Young	10	2.2 ± 0.5 (1.2 to 3.1) ^a	14.9 (11.8 to 18.1)
Old	10	2.9 ± 0.9 (1.2 to 4.3)	19.2 (17.2 to 21.4)

^a Figures in parentheses give the range.

Total Leydig cell volume: Table II gives the values obtained in the twenty young and old males. Obviously the Leydig cells have increased in the elderly males above 55 years of age. The mean figure has risen from 2.2 ± 0.5 ml/testis to 2.9 ± 0.9 ml/testis. The increase is statistically significant ($p < 0.05$) and is of the order of 32% above the young control males. Correspondingly, the relative volume of Leydig cells has also increased from 14.9 to 19.2% of the testicular volume, which again denotes an increase of about 30%. The fact that both the relative and the absolute values have

increased in the same proportion corroborates the earlier finding that the testicular size has remained essentially unchanged in the two age groups.

Thus, both by histopathological examination as well as by a quantitative estimate of the total Leydig cell volume, a distinct increase in the endocrine component of the testis has been established in the elderly males above 55 years of age.

DISCUSSION

It is well known that the senile involution of the reproductive system in the human female is associated with abrupt and well defined changes which are clinically obvious at menopause. Attempts have been made to establish a similar condition in the male also, which has been aptly called the 'male climacteric'. In contrast to the female, however, fertility can be maintained in the male well past this climacteric. All the same, changes do occur in the testis with ageing and they need closer attention than has been given to it in the past.

The volume of the testis has been estimated, *in situ*, for all the 20 subjects by a method which involves exposing the testis and measuring its 3 dimensions. The essential accuracy of this computation has already been fully established by comparison with direct estimate of volume after orchidectomy⁷. Although much more accurate than many previous methods,¹¹ it cannot be put to general use since it requires surgical intervention.

Two main facts emerged from our data (Table I). Firstly, the size of the testis varies considerably even amongst normal fertile males, ranging from 5.3 to 23.3 ml - a wide variation indeed. Secondly, there is no consistent and statistically significant difference between the testicular volume of young and old subjects. It can, therefore, be concluded that the testicular volume remains essentially unchanged within the span of at least 30 to 65 years of age.

On histopathological examination a definite pattern of change has been observed in the testis on account of ageing. The tunica propria, forming a connective tissue sheath for the seminiferous tubules, becomes gradually thickened. This has also been noted by McKeown⁵. It may be pertinent to mention here that several unrelated conditions, all associated with depressed spermatogenesis, also exhibit a similar thickening of the tunica propria. This includes Klinefelter's syndrome, exposure to ionising radiations,¹² and bilateral vasectomy^{2,3}. The common mechanism involved in all these diverse conditions remains uncertain. The thickening of the tunica propria, with age, could certainly affect its two main functions, i.e. regulation of fluid movement into the tubule and maintaining an adequate pressure for the onward transport of the sperms. But, as pointed out by Johnson *et al.*,⁶ little is known so far about the effects of old age on the fluid secretion by the tubules and all this would require further elucidation. It is interesting to note that a similar thickening of the tunica albuginea investing the testis as a whole has been demonstrated by Yoshimura and Fukunishi¹³ which had an almost linear progression with age.

Spermatogenesis is well maintained even in advanced old age. There is, however, a progressive degeneration of some of the tubules leading to complete hyalinisation and atrophy. One can, therefore, visualise a gradual shut-down of the spermatogenetic function, slow and irregularly distributed, although enough tubules may still be left normal looking even in extreme old age. On the other hand, fertility may actually decline much earlier in most cases as the quantity and quality of the sperms formed drops below the minimum requirements because of this partial atrophy of the tubules.

The Leydig cells have shown an interesting change with ageing. Far from regressing and diminishing in numbers as suggested by several authors,^{6,14} they have actually shown a hyperplasia. In 2 out of the 10 elderly males examined, they had grown into prominent and large clusters

exclusively made up of these characteristic cells. Interestingly enough, in senile rats spontaneous Leydig cell tumours appear to be common¹⁵.

The histopathological impression of Leydig cell hyperplasia has been fully substantiated by a quantitative estimate of the total Leydig cell volume. We have already established experimentally the reliability of the method employed⁸. It has been found that the Leydig cells constitute about 15% of the testicular volume in young adults below 40 years of age. In absolute terms it comes to 2.2 ± 0.5 ml of Leydig cell tissue per testis. This is a little higher than the two other estimates for human testis available for comparison. Dykes¹⁶ has reported a figure of 1.67 ml while Ahmad *et al.*,⁷ have found it to be 1.41 ml per testis. Some difference could possibly arise because both have included only a small number of normal individuals in their largely pathological sample and their ages were also not taken into consideration.

In the case of elderly males between 55 and 65 years of age, the Leydig cell population has grown so as to make up 19.2% of the testicular volume, on an average. In absolute terms, the total Leydig cell volume has increased from 2.2 ± 0.5 ml/testis in the young to 2.9 ± 0.9 ml/testis in the old which is a statistically significant increase of about 32% ($p < 0.05$). Incidentally, this appears to be the first quantitative estimate of the total Leydig cell volume in man in relation of ageing

A very plausible explanation can be offered for this senile hyperplasia of the Leydig cells. With advancing age the secretory activity of the Leydig cells declines, leading to a fall in the blood testosterone level and the associated symptoms of low virility, etc. Through the normal feed-back mechanism the low testosterone stimulates the secretion of pituitary gonadotrophin. Indeed, such a rise in the blood gonadotrophin level has been found in men complaining of the 'male climacteric', in young males after castration and in cases of primary testicular atrophy¹⁰. It is only to be expected that the Leydig cell content of the testis increases under this increased gonadotrophin stimulus, mostly perhaps by transformation of undifferentiated precursor cells. But obviously, this Leydig cell hyperplasia cannot make up for the declining testosterone secretion in old age, much to everyone's disappointment.

REFERENCES

1. Kothari, L.K. and Mishra, P., Vasectomy and the endocrine function of the testis. *Lancet* (1972): I, 438.
2. Kothari, L.K., Mishra, P., and Mishra, R.K., Effect of bilateral vasectomy on the structure and function of the testis. *Am. J. Surg.* (1973): 126, 84.
3. Kothari, L.K. and Mishra, P., Histochemical changes in the testis and epididymis after vasectomy. *Int. J. Fertil.* (1973): 18, 119.
4. Kothari, L.K., Vasectomy for contraception - clinical problems and physiological perspectives. *Ind. J. Physiol. Pharmacol.* (1973): 17, 209.
5. McKeown, F., *Pathology of the Aged*. Butterworths, London, (1965): p. 241.
6. Johnson, A.D., Gomes, W.R. and Vandemark, N.L., *The Testis*, vol. 1, pp. 36, 192. Academic Press, New York, 1970.
7. Ahmad, K.N., Lennox B. and Mack, W.S., Estimation of the volume of Leydig cells in man. *Lancet* (199): II, 461.

8. Kothari, L.K., Srivastava, D.K., Mishra, P. and Patni, M.K., Total Leydig cell volume and its estimation in dogs and in models of testis. *Anat. Rec.* (1972): 174, 259.
9. Ham, A.W., *Histology*, 6th edition (Asian), p. 937. J.B. Lippincott Co., Philadelphia, 1969.
10. Heller, C.G. and Myers, G.B., The male climacteric, its symptomatology, diagnosis and treatment. *J. Am. med. Assoc.* (1944): 126, 472.
11. Synder, C.H., A simple orchidometer for measurement of testes of boys. *J. Pediat.* (1966): 69, 461.
12. Velardo. J.T., *Essentials of Human Reproduction*. p. 234. Oxford University Press, New York, 1958.
13. Yoshimura, M. and Fukunishi, M., Studies on the change with ageing of human testicle. Estimation of age from thickness of tunica albuginea. *Osaka city Med. J.* (1985): 11, 1.
14. Davies, D.V. and Coupland, R.E., *Gray's Anatomy*, p. 1554. Longmans Green & Co., 34th Edition, 1967.
15. Reddy, J. and Svoboda, D., Microbodies in Leydig cell tumours of rat testis *J. Histochem. Cytochem.* (1972): 20, 793.
16. Dykes, J.R.W., Histometric assessment of human testicular biopsy. *J. Path.* (1969): 97, 429