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Original research article

Study of Interstitial Leydig Cells In Mammalian Testes

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Abstract

Leydig cells (SLCs), located in the testicular interstitial compartment in the mammalian testes, are capable of differentiating to testosterone-synthesizing Leydig cells (LCs), thus providing a new strategy for treating testosterone deficiency. However, no previous reports have identified and cultured SLCs derived from the pig. The aim of the current study was to isolate, identify, and culture SLCs from pigs.

Keywords: leydig cells in mammalian testes.

Introduction

Testis is male gonad in mammals, two in number and responsible for synthesis of male gametes and male sex hormone “Androgen” for the purpose of reproduction and development of secondary sex character in male. Male gamete is produced by germinal epithelium in seminiferous tubule while androgen is secreted by interstitial cells of the testis.

Testes are the primary reproductive organ or gonads in the male. They are ovoid, reproductive and endocrinal organ responsible for sperm production and testosterone production. They are suspended in the scrotum by scrotal tissue including the dartos muscle and spermatic cord. Average testicular dimentions are 4-5 cm. in length, 2.5 cm in breadth and 3cm in antero posterior diameter, their weight varies from 10.5-14gm. Anterior aspect of testis is convex, whereas the posterior aspect is nearly straight and attached with spermatic cord. Anterior, medial and lateral surfaces and both poles are convex, smooth and covered by the visceral layer of tunica vaginalis, parietal layer and scrotal tissue, in that order from within outwards. Deep to the visceral layer of tunica vaginalis, the whole gland is enclosed by thick fibrous capsule the tunica albuginea, the deep layer of which is very vascular. Posteriorly, tunica albuginea project inwards like a thick fibrous septum called the mediastinum testis. Many thin fibrous septa extend from the mediastinum testis to the tunica albuginea and subdivided the testis in to 200- 300 cone shaped lobule. Each lobule contain 1-3 highly convoluted seminiferous tubules and interstitial tissue of Leydig in between them.

Interstitial cells are distinguishable in all testes examined within 2 days after the gonad differentiated into definite testes. These gonadal cells are probably the source of foetal androgen that controls male differentiation. Interstitial cells of Leydig constitute about 20% of the mass of the testis. Interstitial Leydig cells are almost non-existent in the testes during

childhood but they are numerous in newborn male infant and also in adult male anytime after puberty.

The interstitial cells of different species have common structural feature which have been related to the production of androgen (Christensen, 1965, Christensen and Fawcett, 1966).

Aims and objective

Testis is male gonad that produce male gametes, that fertilize ovum to form Zygote and eventually offspring. This is a way of producing new generation and way of transferring genetic property from father to child via male gametes. The group of interstitial cells of Leydig forms endocrinal part of the testis. **The main aim of the work is to study the features of interstitial cells of Leydig in mammalian testes.**

Materials & methods

Selection of species and gruping:

In Order to carry on the experimentation to study the intertubular tissue of mammalian testis, five groups of animals including human were selected for this project of study. Sexually mature male animals consisted, the Group I- Albion Rat (3 animals of the age of 3 months), Group II- Guinea pig (3 animals of the age of 3 months). These animals are purchase from animal house from market. Group III- Rabbit (3 animal of the age of 9 months). Group IV – Goat (sexually matured of known weight) were inspected at butcher shop and testis of both sides are taken. After the slaughter Group V- Consisted of Adult human male from the fresh human cadavers coming to Anatomy Department IGIMS Patna, for cadaver preparation for dissection.

Collection of organs and fixation of tissue:

The Albino rat, guinea pig were sacrificed at 10 AM. After pithing by pithing needle at cervical region causing instantaneous death. Both testis were removed by holding and cutting the spermatic cord after opening the scrotal sac. The organ weighted and immersed in Bouin's fluid at least ten times more in volume than the volume of the organ. The testis of Rabbit, Goat Human being was also collected in appropriate volume of Bouin's fluid. In case of goat the time lapse between the sacrifice of the animal and collection of testis was approximately 30-40 min where as in case of Human and Rabbit being the time lapse between the death and collection of specimen was rather uncertain. The time lapse varied 24 to 36 hours.

Microscopic observation:

Twenty intact interstitial spaces under the microscope were observed. Leydig cells were identified by the vesicular appearance of the nucleus and eosinophillic granular vacuolated cytoplasm. Such cells were mostly found in clumps of two or three or isolated in the interstitial spaces.

The number of Leydig cells in the selected twenty interstitial space were counted on the basis of their number per field. It was observed that twenty interstitial spaces used to cover up the total span of the section. The value of count per field was recorded for twenty interstitial spaces of each section.

Evaluation of observation:

The values for the upper, middle and lower third portions of each testis of each animal of the five groups of species were subjected to statistical analysis, in order to find out the statistical

mean, standard deviation, variance, standard error and statistical significance of differences of Mean for each portion of the testis, each side of the testis, testis of each animal and the value for each group of species.

Method of statistical analysis:

Statistical mean:

The statistical mean was calculated in each case at varying stages by the following formula –

$$X \text{ (Mean)} = \frac{X \times F}{XF}$$

When X is the arithmetical mean widely used in statistical calculation, x is the summation of number of figure.

'x' is the number of cells observed per field and 'F' is the number of times of occurrence of the specific number out of 20 fields.

VARIANCE:

It is the sum of the squares of the deviations of individual observation from the arithmetical mean divided by total frequency and is calculated by the formula –

$$\alpha^2 = \frac{\text{'x' } f (X - \bar{X})^2}{\text{'x' } f}$$

When 'X' is individual number of cells present per field X is the Mean calculated by above mentioned formula and 'f' is the frequency of their occurrence.

Standrad deviation:

The standard deviation is denoted as number root of the variance.

S.D. = $\sqrt{}$ Variance

STANDRAD ERROR:

The standard error of the mean was calculated by the formula.

$$\text{S. E.} = \frac{\text{S. D.}}{\sqrt{n}}$$

When 'n' was the total number of observations.

TEST OF SIGNIFICANCE:

The statistical significance of differences between Mean values was calculated by the formula given below

$$T = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{SE_1^2 + SE_2^2}}$$

Where as $\bar{X}_1$ and $\bar{X}_2$ were the true means and SE_1 and SE_2, their standard errors.

If the value of 'T' was less than 2 the difference was taken to be statistically non-significant, in between 2 and 2.5 doubtfully significant, 2.5 to 3 probably significant and more than 3, statistically significant.

Observation

The observations upon the Leydig cells in the Testis of the species selected for this project comprised of counting the number of interstitial cells of Leydig in the upper, middle and lower third portion of the testis of right and left side in three sexually matured males. The observations of the number of count of Leydig cells in each of the 10 random sections of each portion is given in appendix. The statistical values of the Leydig cells obtained after computation of the result of observations under one thousand two hundred oil immersion

fields at the magnification of 900, in one hundred eight random sections, 10 each from each of the three portions of right and left testis of each subject is taken into account to calculate the mean value of the Leydig cell for each species. The results were based on experimentation in three members of each species consisting of five groups.

GROUP-I

Albino Rat – Three male albino rats of 3 months age weighing 155, 160, 165 gms were selected for the group – I observations. The statistical values of the Leydig cell for the Albino Rat (Group – I) was computed on the basis of observations in three individual members of the group irrespective of the findings regarding statistical significant difference in the mean values for individual portion of the testis, or the side of the testis or individual animal. On the basis of the above mentioned methods of observation the mean value of Leydig cell in Albino rat (Group – I) is 7.46 per oil immersion field with a standard deviation of ±1.59.

GROUP-II

Guinea pig - Three male guinea pig of three months age, weighing 170, 175 and 180 gms were selected for Group –II, observations. The statistical value of Leydig cell the Guinea pig (Group – II) was computed on the basis of observations in three individual members of the group irrespective of the findings regarding statistically significant difference in the mean values for individual portion of the testis, or the side of the testis or individual animal. On the basis of the above mentioned methods of observation the mean value of Leydig cell in Guinea pig (Group – II) is 4.89 per oil immersion field with a standard deviation of ±1.27.

GROUP –III:

Rabbit- Three rabbits of nine months of age weighing 350, 355 and 360 gms were selected for Group–III observation. Statistical value of Leydig cell for rabbit (group – III) was computed on the basis of observation in the three individual members of the group. It was observed that the mean values for individual members of the group were similar without any statistical significant difference. On the basis of above mentioned observation the mean value of Leydig cell per oil-immersion field in rabbit (group – III) is observed to be 9.41±1.30.

GROUP – IV :

Goat- Three male goats of 8to 9 months of age weighing 9 Kg, 10Kg and 10.5Kg were selected from group –IV observation. Statistical values of Leydig cell for the Goat (Group-IV) was computed on the basis of observations in the three individual members of the group. It was observed that the mean values for individual members of the group were similar without any statistical significant differences. It was seen that the mean value of Leydig cell per oil immersion field in goat (Group- IV) is 8.50±1.36

GROUP-V

Human- Three pairs of testis weighing, as shown below were selected from the fresh male dead bodies received from the Anatomy Department of Indira Gandhi Institute of Medical Science Patna, for Dissection. The weight and height recorded for three dead persons are as follows-

Sl. No.	Weight	Height	Weight of right testis	Weight of left testis
1.	50kg	5 ft.4 inches	30 gms.	27 gms.
2.	55kg	5 ft. 6 inches	32 gms.	30 gms.
3.	58kg	5 ft. 8 inches	34 gms.	32 gms.

The time lapse between the death and collation of sample was always greater than six hours. This inevitable delay in collection of samples after death resulted in poor quality of statining of sections in human testis. However, best attempts were made to differentiate the Leydig cells from the surroundings. The vesicular nucleus provided a guide line for recognition of Leydig cells.

Discussion

The size of most endocrine glands is thought to be related to their functional activity and has been related to the level to the level of both, the trophic hormone acting on them and their hormone output. Hudson et al (1967) have observed a reasonably good positive co-relation between the testicular size and plasma testosterone level and a negative co-relation with plasma gonadotrophin levels in boys approaching puberty. For the quantitative evaluation of the testicular biopsies the volume ratio estimation of the Leydig cell was worked out by different workers in late fifties and early sixties, by the application of point counting method.

References

1. A.M. Andersson, N. jorgenesen, E. Rajpet- De Meyts and N.E. skakkeback 2004; impaired Leydig cell function on in fertile men- The journal of clinical Endocrnology & Metablism vol. 89 No. 7316173167.
2. A . S. Gupta, L. K.olhan, A Dhruva, R. Bapna –Surgical sterilization by vasectomy and its effect on the structural and function of the testis in man, British journal of surgery. Vol. 62 issue, page 59-63, Dec. 2005.
3. B.R. Zirkin and L. L. Ewing (1987)- Leydig cell Differentiation during maturation of the Rat Testis :A sterological study of cell Number and Ultrastructure. The Anatomical Recors, Vol. -219, p. 157-163.
4. Barry R. Zirkin and Haolin Chen 2000 Regulation of leydig cell steroidogenic function during aging; Biology of Reproduction 63 (4); 977-981.
5. Belanger, A, B. Candas, L. Gomer and F. Labria Change in serum concentration of conjugated and unconjugated steroid in 40 to 80 yr. old men j. Clinical Endocrinology Metab 1994, 79: 1086-1090.
6. Chandak Sengoopta – 'Dr. steinach 'coming to make old young' sex gland vasectomy and the quest for rejuvenation in roaring twenties, Endeavour vol. 27 No -3 september 2003.
7. Chen H, L. Luo and B.R. zirkin, Leydig cell structure and function during aging. In payne. AH, Hardy Mp, Russell LD (eds), The leydig cell, IIIinois Coche River press 1996: 222-230
8. F. p. prince, (2001), The triphasic nature of Leydig cell development in humans, and comments on nomenclature. The journal of Endocrinology, 01/03/2001, 168 (2); 213-6
9. .J .R. W Dykes (1969) –Histometric Assessment of human Testicular Biopsies. J .path, vol. -97,p. 429-439.
10. Keeney D.S.,.S.M. L. C., Mendis –Handagama, B.R. Zirkin and L.L. Ewing, Effect of long term deprivation of Luteinizing hormone on Leydig cell volume, LC number and steroidogenic capacity of rat testis Endocirinology 1988, 123: 2905- 2915.
11. L.K. Kothari & Pushpa Mishra (1972)- Vasectomy and The Endocrine Function of the Testis. The Lancet, vol. –I, p. 438-439.
12. L.K. Kothari and Pushpa Mishra (1973) – Histocchemical changes in testis and epididymis after vasectomy. Int. J. Fertil., Vol. -18, p. 119- 125.
13. L. K. Kothari, A.S. Gupta, A. K. Dhruva, M.L. Jain –A second look at Steinach second procedure: Testiculoepididymal occlusion in man and Dog, System Biology in Reproductive Medicine Vol. 2, page 77- 84 issued 1979.

14. Cunningham's Manual of Practical Anatopmy Volume-2, 15th edition, P 108-109, G.J. ROMANES, OXFORD UNIVERSITY PRESS.
15. M. A. H. Matabagani, Morphological and Morphometric stuby on the effect of simulated microgravity on rat testis. The Chines Journal of physiology 01/09/2007. 50 (4), 199-209
16. P. L. R. DIAS, the effect of vasectomy on Testicular volume Brithis Journal of urology, Vol. 55, lssue!, page 83-84 Nov. 2008.
17. Peter J. O' shalghnessy, Healthes Johnston, Louise Willerton and paul J. Baker 2002, failure of normal adult leydig cell development in androgen- receptor- deficient mice Journal of cell Science 115, 3491- 3496 (20002).

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