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INTERTUBULAR TOPOGRAPHY OF THE TESTIS OF DONKEY

(With 3 Tables & One Digram and 4 Fig.)

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طبوغرافية المسافات بين أنبوبية في خصية الحمار

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درست طبوغرافية المسافات بين أنبوبية فى خصية الحمار المحقونه والمثبته ، مورفومتريا . وقد تمت الدراسه فى فصل الصيف على خصى أحدى عشر حيوانا تتراوح أعمارها بين ٢ - ٩ سنوات .

تتكون تجمعات الخلايا بين أنبوبية من عدة أنواع : خلايا لايدج وخلايا ليفيه ، كريات كبيره ، وخلايا ملتهمه كبيره . وقد كونت هذه التجمعات الخلويه حوالى ٤ ٪ من المسافات بين أنبوبية فى الحيوانات عمر سنتين ، ثم أصبحت ٨ ٪ عند عمر ٦ سنوات ، ثم قلت النسبه مره أخرى لتصبح ٦ ٪ فى الحيوانات عمر ٩ سنوات .

هذا وتحتوى المسافات بين أنبوبية ، بالاضافه إلى الكتله الخلويه ، على أوعية دمويه وأخرى ليمفاويه . وقد كان للأوعيه الليمفاويه النسبه العظمى فى كل الأعمار موضع الدراسه مما يعضد الأعتقاد بما قد تلعبه هذه الأوعيه الليمفاويه من دور هام فى توزيع الهرمونات الخاصه بالخصيه (الأندروجينز فى الحمار).

SUMMARY

The intertubular topography of the donkey testes was studied morphometrically in perfusion-fixed testes of eleven animals ranging from 2-9 years of age, in the summer season. The intertubular cell population is composed of Leydig cells, fibrocytes, monocytes and macrophages. These cellular elements form about 4% of the intertubular spaces in 2-year-old donkey, and become 8% at age of 6 years, then decline to about 6% at the age of nine years. The intertubular spaces contain, in addition to the cellular mass, blood vessels and lymphatics. The latter have the largest percentage in all ages studied. Lymphatic vessels are believed to play a larger role in the testicular androgen distribution.

INTRODUCTION

Most studies on the testis of Equidae were either volumetric, planimetric or surgical observation on cryptorchid horses *(ARTHUR, 1961; BISHOP; DAVID and MESSERVY, 1964; CORYN, DeMOOR; BOUTERS and VANDEPLASSCHE, 1981; SKINNER and BROWN, 1968; STICKLE and FLESSER, 1978 and WRIGHT, 1963)*. The fine structure of the mule testis in comparison with the horse and donkey was studied by *HERNADEZ-JAUREQUI and MONTER (1977)*. Also, the normal histology of the testis of the zebra was studied by *FAWCETT; NEAVES and FLOVES (1973)*. Otherwise, the rest of works found were dealing with the cytological and chromosomal questions of different Equidae species *(BENIRSCHKE; BROWNHILL and BEATH, 1962; BENIRSCHKE; LOW and BROWNHILL, 1964; CHANDLEY; JONES; DOTT; ALLEN and SHORT, 1974; MOKINO, 1955; MARQUEZ; KOFMAN and HERNANDEZ, 1974 and TRUJILLO, OHNO and JARDINE, 1969)*. Since the intertubular topography of the testis of Equidae in general and that of the donkey in special has not been completely investigated, the present study was undertaken.

MATERIALS and METHODS

The present morphometric study was performed on the testes of eleven donkeys collected from Assiut Province, Egypt. The age ranges between 2-9 years. Immediately after casteration the testes were fixed by perfusion. A canula of suitable size was inserted into straight intratunical portion of the testicular artery and a rinsing fluid was instilled at low pressure *(WROBEL; SINOWATZ and KUGLER, 1978)*.

For light microscopy the right testis of each animal was used, and fixation was accomplished with Bouin's fluid. After fixation, a 0.5-1 cm thick slice was taken from the cranial pole, central part and caudal pole of each testis. After paraffin embedding, 5-7 um thick sections were stained with Haematoxylin and Eosin.

The left testis of each animal was used. Fixation was performed using solutions I and II of *FORSSMAN* et al. *(1977).* Blocks were collected from the three locations as described for light microscopy, postfixed for 3-5 hours in solution II, washed in 0.2 M phosphate buffer, osmicated (1% OsO_4), dehydrated in graded ethanol and embedded in Epon-Araldit. Semithin sections were cut and stained with methylene blue.

Morphometric evaluations of tubular diameter and of tubular / intertubular ratio was performed on Epon-Araldit sections by means of a Morphomat (Zeiss) equipment. A total area of approximately 9 mm^2 of each location and for each age (2-9 years) was measured using 10 randomly chosen sections from each location. The tubular basal lamina was considered as part of the tubule, whereas peritubularly and intertubularly situated cells, intercellular matrix, fibers and vessels constitute the intertubular tissue. Percentage of vessels in the intertubular tissue was assessed in the same Epon-Araldit sections by means of an eyepiece graticle of 900 points. Using a X40 objective lens 4 squares (0.14 mm^2 each) were counted in every section. Lumina and adventitia were considered as parts of the vessels.

The numerical percentage of intertubular cells (fibroblasts, fibrocytes, Leydig cells, peritubular cells, mononuclear free cells) was determined in semithin sections. Cells with visible nuclei were only counted.

RESULTS

The intertubular stroma of the donkey testis is composed of large angular interstices between three or more tubular sections and of narrow strands between adjacent tubules (Fig. 1).

In addition to the Leydig cells, the intertubular spaces contain blood vessels and lymphatics which form peritubular plexuses in the intertubular spaces. The extravascular interstitial tissue is a loose areolar connective tissue which contains fibroblasts, small bundels of collagen fibers and occasional few macrophages. The Leydig cells, are located in the interstices between the convoluted seminiferous tubules. They occur in clusters or cords of varying size, sometimes

closely associated with blood vessels. They do occur also individually as irregularly polyhedral elongated shaped (Fig.2).

The prominant lymph vessels are situated in the central area of almost every large angular interstitial space (Fig. 3). Slender extensions of lymphatic vessels are sometimes seen projecting for some distance into the narrow connective tissue strands between two adjoing tubules. The testicular lymph vessels exhibits thin wall, irregular lumina and contain a flocculent precipitate; whereas the empy blood vessels present round contours (Fig. 4).

The quantitative morphological study on the donkey testis revealed that the intertubular spaces occupy approximately 20% of the testicular tissue in 2-year-old donkey, fluctuate around 22-23% in 3,4,5 year-old donkey, then comprise approximately 27.25% in 9-year-old donkey (Table 1,2,3).

Within the intertubular compartments the histological topography is not uniform and varies during the course of development. In the testes of 2 years intertubular areas are rich in cellular elements (4.44%) which reach its peak (8.44%) at the age of 6 years, then it begins to decrease (6.35%) at the age of 9 years. Meanwhile, the percentage of matrix, cells and vessels (arteries, veins and lymph vessels) increases as the age advances. However, a little drop in the percentage of vessels at the age of four years and of the matrix at the age of five years was observed (Tables 1,2,3 and Digram 1).

The percentage of the vessels is slightly higher in th fifth and sixth years of age (about 6%), while it is about 5% in the second, third and fourth years (Table 3). It could be also concluded that vessels form approximately 5.6% from the intertubular compartments with higher representation of the lymphatic vessels (2.534%), followed by veins (1.768%), then arteries (1.332%).

DISCUSSION

HERNANDEZ-JAUREGUI and MONTER (1977) gave an average of 70 tubules per microscopic field in mules, whereas stallions and donkeys had about 42 and 30, respectively. The latter value for donkey is higher than estimated for the same animal in this study (Table 2). They mentioned also that the average diameters of tubules obtained from the magnified pictures were 0.881 cm in mules, 1.120 cm in stallions, and 1.145 cm in donkeys. The value for the donkeys in this study ranged between 190.11 - 224.73 μm.

ARIGHI et al. (1987) estimated the tubular diameter in the horse abdominal, inguinal and scrotal testis as 66.2, 83.6 and 146.6 μm, respectively. They commented that the mean tubular diameters were significantly greater ($P<0.05$) in the scrotal than in the retained testes, and is not significant ($P>0.05$) between the abdominal and inguinal testes. They added that interstitial spaces and the number of interstitial cells (of Leydig) seemed to be increased while spermatogenesis appeared to be arrested in the retained testes. The tubular diameter of donkey in the present study was 193.33 μm at the age of 2 years, 224.73 μm at 6 years, then reduced to 191.90 μm at 9 years of age.

FAWCETT et al. (1973) mentioned in the common zebra that the seminiferous tubules are separated by very large areas of endocrine tissue irrigated by a rich network of small blood vessels. These course through a sparse connective tissue stroma that form delicate septa traversing the masses of closely compacted Leydig cells. They mentioned also that the interstitial lymphatics are poorly developed, and that the lymph vessels may be either centrally placed or close to the tubules at the periphary of the intertubular areas - in agreement with the findings of this study in donkey. The same authors came to the conclusion that lymphatics are least well developed in those species that have the greatest volume of Leydig cells (e.g. boar, wart hog, and zebra). However, the oppossum seems to be intermediate, having conspicuous lymphatics deposite the unusual cellularity of its interstitial tissue. The results of this study in donkey revealed larger percentage of the lymphatic than the blood vessels in relation to the cells in all studied ages. This finding could indicate that in donkey the testicular lymphatic vessels may have a larger role for androgen transport to general blood circulation or for the local testicular androgen supply.

The interstitial tissue of the donkey testis exhibits rather remarkable species differences in the relative volume of the Leydig cells. In the human they are said to make up 12% of the volume of the testis, whereas in the boar they may account for 35-40% *(BLOOM and FAWCETT, 1975)*. In this study the Leydig cells as well as the other cells found in the intertubular spaces were counted as one cell mass.

Lymph vessels are a common feature of the testicular intertubular spaces in mammals and thus also in ruminants *(HUNDEIKER, 1969; FAWCETT et al., 1973; ODUOR- OKELO, 1974) . FAWCETT et al., 1973; ODUOR-OKELO, 1974; CLARK, 1976; HOLSTEIN et al., 1979,* studied the distribution of testicular intertubular lymph vessels using different techniques in a

number of mammals, including man. Special situation in the mouce, rat, chinchilla, guina pig and armadillo were described where a system of extensive peritubular lymphatic sinusoids surrounds almost the entire tubular circumference *(FAWCETT et al., 1973; CLARK, 1976 and WEAKER, 1977)*, and in man where lymphatic vessels are restricted to the septula *(HOLSTEIN et al., 1979)*.

Specific differences in the intertubular arrangement are reported by *FAWCETT et al. (1973)*, who described three patterns of organization:

1) Guina pig, chinchilla, rat and mouce posseses a small volume of Leydig cells, a minimum of interstitial connective tissue and extensive peritubular lymphatic sinusoids.
2) Ram, bull, hyrax, elephant, monkey and man have clusters of Leydig cells in a very loose connective tissue stroma and lymphatic vessels placed centrally in the intertubular spaces.
3) Boar, wart hog, zebra, opossum and naked mole rat exhibit a large number of closely packed epitheloid Leydig cells, very little interstitial connective tissue and few lymph vessels of small caliber.

According to the results of this study the donkey could be catigorized between the second and third groups of *FAWCETT et al.* patterns because its lymphatic vessels are large and not only centrally placed in the intertubular spaces but also in the angular interstices and peritubularly situated. Also, the Leydig cells are found in both the cluster and individual forms.

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LEGEND

Fig. 1: Donkey intertubular testicular tissue organized in large interstices between three or more tubules and in narrow strands between two tubules. Donkey 3 years old, methylene blue, X 350.

Fig. 2: Donkey intertubular testicular tissue with individual Leydig cells and blood (Bv) and lymph (Ly) vessels, semithin section, methylene blue, X 560.

Fig. 3: Donkey intertubular testicular tissue with blood vessels (Bv), thin-waled, lymphatic vessels (Ly), and clumps of Leydig ceils (Lc). Donkey 4 years old, semithin section, methylene blue, X 560.

Fig. 4: Donkey quadrangular stroma region between four tubules, semithin section, methylene blue, X 560. Lymph vessel (Ly) displays a central position, blood vessel (Bv).

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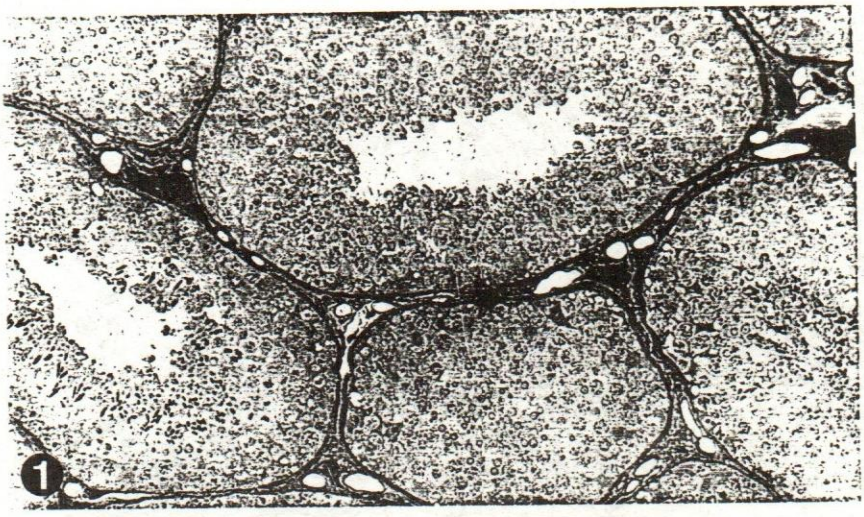
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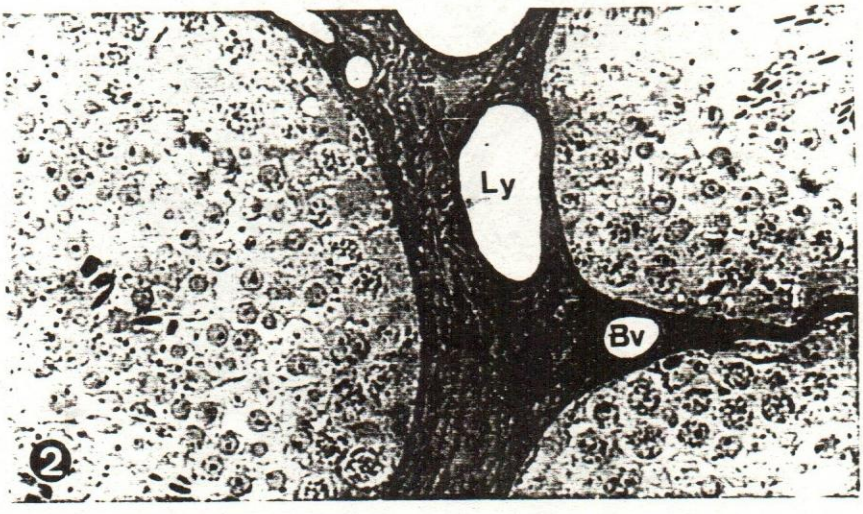
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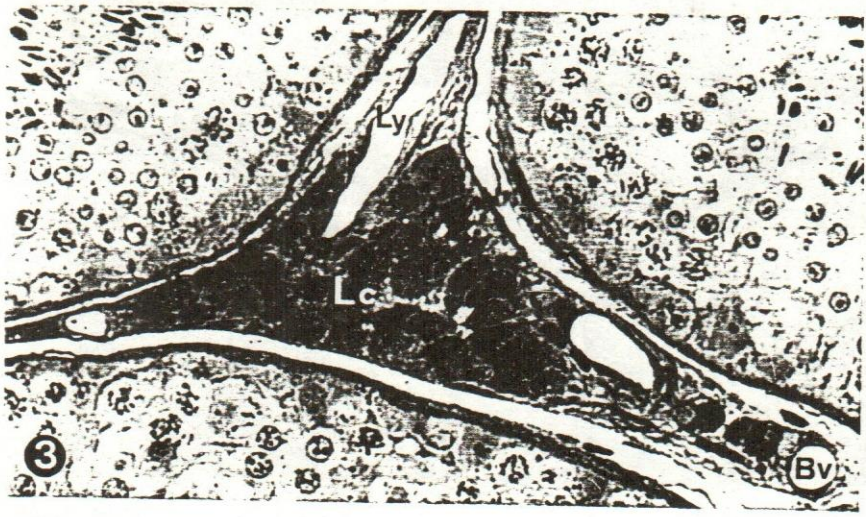
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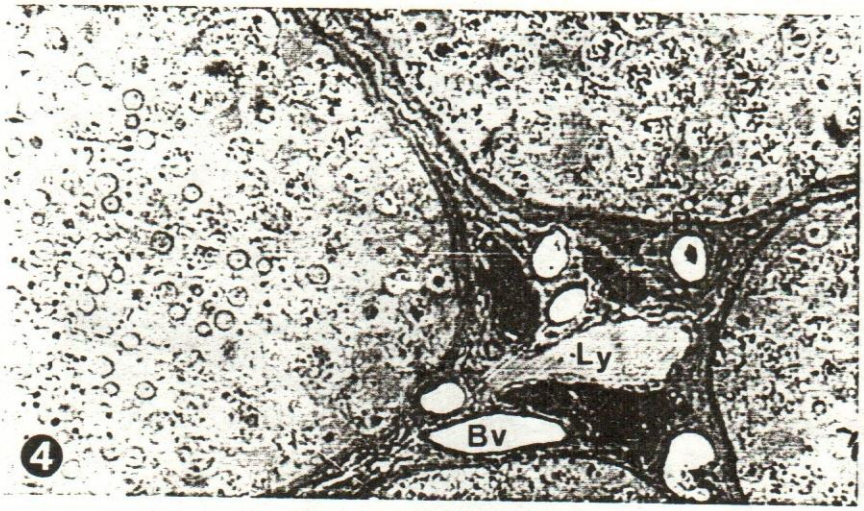
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Table (1) : The percentage of tubules, matrix, vessels and cells in the testis of donkeys, 2-9 years old.

Age	Tubul. (x)	Matr. (x)	Vessels (x)				Cells (x)
			Aa.	Vv.	Ll.	sum	
2 years	80.03	9.03	1.28	1.36	2.75	5.39	4.44
3 years	77.17	10.00	1.22	1.81	2.31	5.34	5.42
4 years	78.03	11.17	0.72	1.89	2.33	4.94	5.00
5 years	77.86	9.50	2.00	1.50	2.58	6.08	5.67
6 years	73.03	11.47	1.44	2.28	2.61	6.33	8.44
9 years	72.75	15.50	0.25	1.8	2.8	4.85	6.35

Table (2) : Volumetric percentage of tubules and intertubular compartments (pooled) in the testis of donkey.

Age	% of tubules (pooled) (x)	% of inter-tubular compartment (pooled) (x)	Tubular diameter (µm) (x)	No.of tubules/ microscopic field
2 years	80.03	19.97	193.33	11-20
3 years	77.17	22.83	190.11	4-16
4 years	78.03	21.97	194.69	3-9
5 years	77.86	22.14	201.47	4-6
6 years	73.03	26.97	224.73	7-9
9 years	72.75	27.25	191.90	

Table (3) : Tubular percentage and diameter in the testis of donkey.

Age	Tubules % (x)	SE	Tubules dia. µm (x)	SE
2 years	80.03	± 4.078	193.33	± 18.611
3 years	77.17	± 6.525	190.11	± 23.607
4 years	78.03	± 3.040	194.69	± 22.428
5 years	77.68	± 4.112	201.47	± 25.474
6 years	73.03	± 5.942	224.73	± 20.107
9 years	72.75	± 3.359	191.90	± 23.454

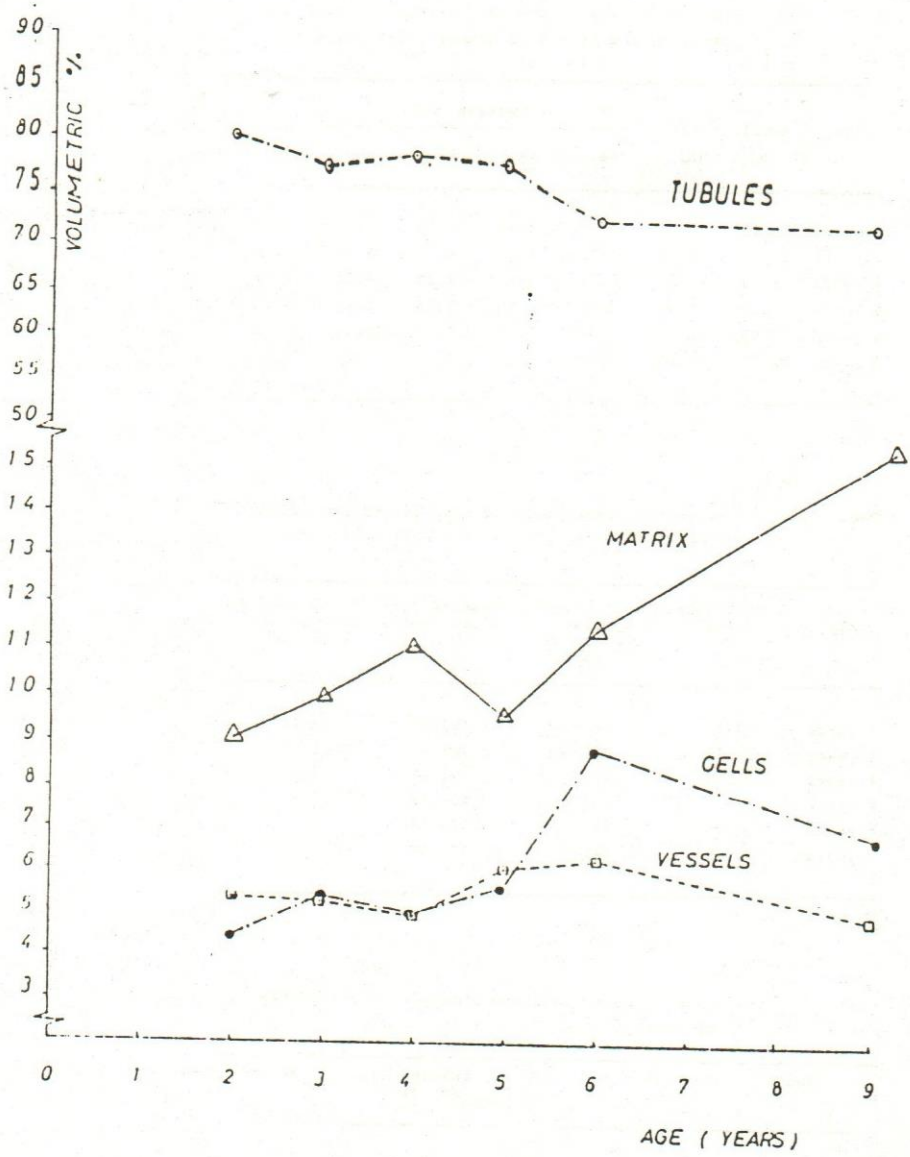

Diagram (1)

Volumetric percentage of tubules, matrix, cells, and vessels in the testis of donkey.