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Morphological aspects of the spermatic cord of mice (*Mus musculus*)
Aspectos morfológicos dos componentes do funículo espermático do camundongo (*Mus musculus*)

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Abstract

A histological study of the morphological aspects of 33 spermatic cord pairs of adult mice (Mus musculus) showed that its their components were ensheated by a thin mesothelium capsule in 3 pairs. Under this capsule, completely surrounding the cord, there was a dense layer of adipose tissue. Its components were surrounded by loose connective tissue with a predominance of collagen fibers among reticular and elastic fibers. The testicular artery varied in diameter and was involved by the testicular veins, consisting of an inner layer formed by endothelium and an inner limiting elastic membrane, a middle layer formed by a thick smooth muscle layer supported by a network of reticular fibers, and an outer layer consisting of dense connective tissue close to the intervascular connective tissue and the adventitia layers of the veins. The testicular veins form the pampiniform plexus which partially surrounds the artery, presenting wide and irregular lumens, thin walls consisting almost exclusively of endothelium, with no valves and intimately related to the testicular artery. The vas deferens is located at the periphery of the subcapsular adipose tissue which partially surrounds it and is accompanied by arterioles, venules, lymphatic vessels and nerves. The cast of the segment of testicular artery obtained with Neoprene 450 latex in 60 molds exhibited a sinuous trajectory and the length (cm): was mean, maximum and minimum, 1.35. 1.80 and 0.80 on the right and 1.32. 1.85 and 0.70 on the left, respectively. These values showed no significance at 5%.

Key-words:
Spermatic cord.
Mice.
Irrigation.
Vascularization.
Testis.

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Introduction

Mice, which belong to the order Rodentia, family Muridae, are considered to be laboratory animals and are the animals most frequently used in biomedical research (WILLIANS, 1976 apud SHIVELY, 1987, p. 508).

Because of the great importance of these mammals for research, and as a continuation of our studies, we carried out the present investigation in order to obtain more information about the anatomical elements detected in the spermatic cord of this species which, by being responsible for the maintenance of testicular thermoregulation and perhaps for the reduction of testicular arterial pressure, are also of fundamental importance for spermatogenesis.

Thus, we studied the spermatic cord of mice in terms of the histological aspects of the arterial and venous vessels and of intervascular tissues, and also measured the length of the segment of testicular artery running inside the cord.

With this information, we intend to continue our studies which are devoted not only to a better understanding of the

mechanisms responsible, in part, for mammalian reproduction, but also to the acquisition of indispensable information for the development of Comparative Anatomy, since this topic was part of the author's Master's thesis and represents an important research line currently being developed in the Department of Surgery of the Faculty of Veterinary Medicine and Zootechny of the university of São Paulo.

Materials and Methods

In the present study we used 33 pairs of spermatic cords obtained from adult mice of different ages supplied by the Animal House of the Department of Pathology, Faculty of Veterinary Medicine and Zootechny of the University of São Paulo.

For histology, we used 3 pairs of spermatic cords obtained immediately after animal sacrifice by excess ether inhalation. The cords were fixed in Bouin's for 48 hours and then dehydrated, cleared and embedded in paraffin according to standard techniques. Transverse 5-mm thick sections were obtained from the middle third of the spermatic cords and stained with hematoxylin-eosin, Verhoeff stain (elastic fibers), Gordon reticulin (reticular fibers), and picrosirius (collagen fibers).[1] Photomicrographs were obtained for some of the preparations for further documentation (Figure 1 to 7).

The length of the testicular artery fragment contained inside the spermatic cord was measured in 60 molds (Figure 8) corresponding to 30 testis pairs and their respective spermatic cords, obtained with Neoprene 450 latex (Du Pont do Brasil S.A. - Indústrias Químicas), diluted 2:1 in distilled water and stained with a specific pigment. To this end, after sacrifice by excess ethyl ether inhalation we opened the chest of the animals by removing the sternum and cutting close to the ribs in the costochondral joints. We then isolated and cannulated the thoracic aorta and, after heating the abdominal region with compresses soaked in water at 40°C, we injected the Neoprene 450 latex solution until it reached the testes. We then froze the animals for at least 24 hours and then thawed them in running water, we isolated the testes and the corresponding spermatic cords and submitted these parts to corrosion with 30% sulfuric acid for 48 to 72 hours in order to obtain molds of the testicular artery by washing with fine and controlled water streams. After straightening, but not stretching, these preparations, we fitted them into a 1-mm deep sulcus made in a 15-cm long wood ruler and measured the length of this vascular segment (Table 1). The data concerning these measurements were analyzed statistically by normal probability distribution (α= 5%).

Results and Conclusions

In mice, the components of the spermatic cord are enveloped by a fine capsule practically consisting of mesothelium, which represents the visceral lamina of the vaginal layer (Figure 1 and 2)

Immediately below the capsule and throughout the extension of the spermatic cord, there is a dense adipose tissue layer that completely surrounds all the components of the spermatic cord (Figure 1and 2).

Among the components of the vascular ensemble that form the spermatic cord there is a thin layer of loose intervascular connective tissue with some arterioles, venules and lymphatic vessels in which collagen fibers and some elastic and reticular fibers prevail (Figure 1, 3, 4, 5 and 6).

The testicular artery running inside the spermatic cord regularly presents a circular shape with little variation in caliber and is surrounded by the veins of the pampiniform complex (Figure 1, 4, 5 and 6).

The segment of testicular artery has an internal layer consisting of endothelium and is accompanied by a well-delimited internal elastic membrane (Figure 5). The middle layer, formed of thick smooth muscle, is supported by a rich and orderly network of reticular fibers (Figure 6), and the external layer, the adventitia, consists of dense connective tissue containing collagen,

elastic and few reticular fibers (Figure 3, 4, 5 and 6), which continue with the loose intervascular connective tissue and with the adventitia of the testicular veins.

The testicular veins, which form the pampiniform complex, are arranged around the testicular artery, which is separated by the adventitias, which in turn continue and appear to be formed of dense connective tissue with the participation of elastic fibers and some reticular fibers (Figure 3, 4, 5 and 6). These veins have irregular lumens, have no valves, and have a well-developed middle layer formed of smooth muscle fibers resting on a fine and irregular network of reticular fibers (Figure 1 and 6).

The vas deferens covered with mesothelium is located at the periphery of the spermatic cord and is accompanied by deferential vessels and nerves (Figure 7).

The Neoprene 450 latex molds show that the segments of the testicular artery contained in the spermatic cord present no divisions, have a sinuous trajectory and are arranged in a disorderly manner, so that no type of harmonious arrangement can be characterized (Figure 8).

The measurements of the Neoprene

Table 1
Length (cm) of the segment of the testicular artery inside the spermatic cord of the mouse, obtained by the straightening of Neoprene 450 latex molds. São Paulo, 2000

Number	Right	Left
1	1.30	1.50
2	0.80	0.70
3	1.40	1.20
4	1.80	1.85
5	1.60	1.40
6	1.15	1.30
7	1.30	1.00
8	1.20	1.05
9	1.15	1.30
10	0.80	0.90
11	1.50	1.60
12	1.35	1.20
13	1.60	1.80
14	1.30	1.60
15	1.60	1.45
16	1.15	1.10
17	1.20	1.10
18	1.70	1.40
19	1.50	1.30
20	1.70	1.50
21	1.40	1.50
22	1.60	1.70
23	1.40	1.50
24	1.20	1.10
25	1.10	1.30
26	1.35	1.10
27	0.90	0.80
28	1.50	1.20
29	1.50	1.60
30	1.60	1.70
Mean	1.355	1.325

Table 2
Mean values (cm) of the right and left testicular artery segments inside the spermatic cords of some species, obtained with Neoprene 650 and 450 latex molds. São Paulo, 2000

Species	Testicular artery		Authors
	Right	Left	
Thoroughbred Horse	130.30	129.40	STERMAN (1988)
Horses of undefined race	102.90	105.80	SANTOS (1990)
Pêga breed mule	71.34	68.78	NORONHA (1996)
Donkey	58.2	66.3	FOZ (1997)
Nelore cattle	244.70	250.80	VIANA (1986)
European cattle	289.20	280.20	MARÇAL (1988)
Murrah Buffaloes	135.30	136.30	MACHADO (1992)
Wool-less sheep	260.00	259.00	BORELLI et al. (1989)
Corriedale Sheep	150.40	149.60	CARVALHAL (1990)
Bhuj Brazilin Goats	134.60	137.00	COSTA (1987)
Swine	155.40	156.80	TONIOLLO (1988)
Mongrel Dogs	42.30	44.40	BARROS (1995)
Mongrel Cats	8.16	7.83	MENEZES (1995)
Agoutis	15.85	15.72	BORELLI et al. (1999-a)
Guinea pigs	19.89	19.81	BORELLI et al.(2000)
Cavies	14.57	13.22	BORELLI et al.(1999-b)
Golden Hamsters	3.97	3.88	GONÇALVES (1996)
Mice	1.355	1.325	Current paperl

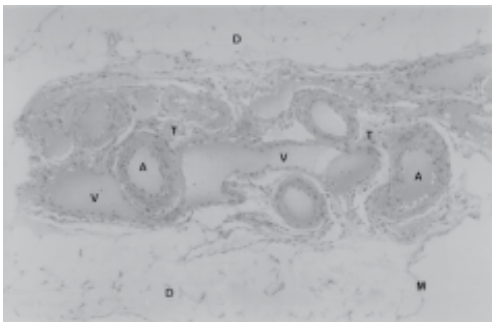

Figure 1
Photomicrograph of the spermatic cord of a mouse showing the testicular artery (A), testicular veins (V), adipose tissue (D), loose intervascular connective tissue (T), and mesothelium (M). (34X – hematoxylin-eosin)

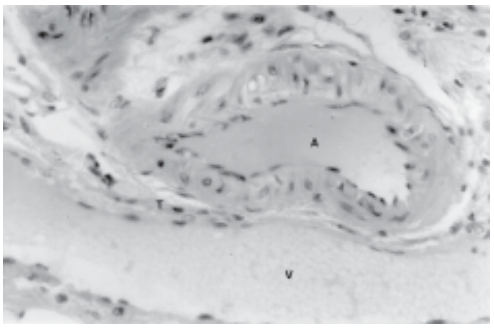

Figure 3
Photomicrograph of the spermatic cord of a mouse showing the testicular artery (A), testicular veins (V), and loose intervascular connective tissue (T). (140X – hematoxylin-eosin)

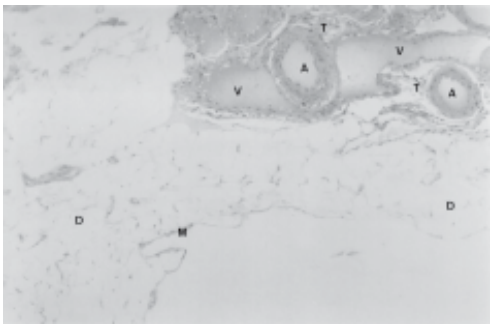

Figure 2
Photomicrograph of the spermatic cord of a mouse showing the testicular artery (A), testicular veins (V), adipose tissue (D), loose intervascular connective tissue (T), and mesothelium (M). (34X – hematoxylin-eosin)

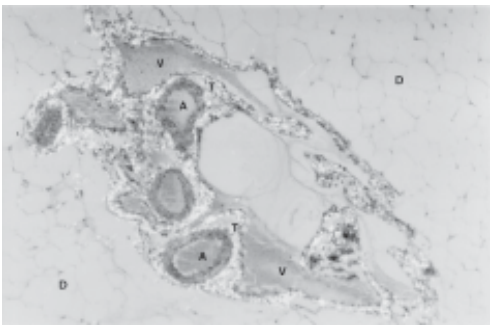

Figure 4
Photomicrograph of the spermatic cord of a mouse showing the testicular artery (A), testicular veins (V), loose intervascular connective tissue (T), and adipose tissue (D). (34X – with a polarizer - Picrosirius)

450 latex molds of the testicular artery segments contained in the spermatic cords of mice revealed mean, maximum and minimum values of 1.355, 1.80 and 0.80 cm on the right and 1.325, 1.85 and 0.70 cm on the left, respectively.

Statistical analysis of the data showed no significant difference at the 5% level between the right and left artery segment inside the spermatic cord.

Discussion

By examining histological sections of the different segments of the spermatic cord of mice, animals frequently used in experimental research (WILLIANS, 1976 apud SHIVELY, 1987, p. 508), we observed that the cord is surrounded by a fine layer of connective tissue lined with the mesothelium, which represents the visceral lamina of the vaginal layer and forms the funicular capsule, also identified in other rodent species[2,3,4,5]. This capsule covers a thick layer of adipose tissue, as reported by all authors who studied this subject in rodents[2,3,4,5,6,7,8,9]. In rodents, this adipose pannicle that contours the funicular structures is assumed to have not only the protective function commonly attributed to the connective capsule, but also to act as a thermal insulating structure, as suggested by some investigators who detected adipose tissue partially[10,11,12,13,14,15,16,17] or totally[18] enveloping the funicular vessels. It is also our understanding that the presence of adipose tissue in a subcapsular position and even intermingled with funicular vessels, which is

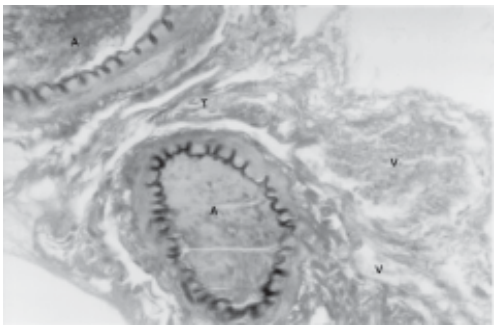

Figure 5
Photomicrograph of the spermatic cord of a mouse showing the testicular artery (A), testicular veins (V), and intervascular connective tissue (T). (140X – Verhoeff)

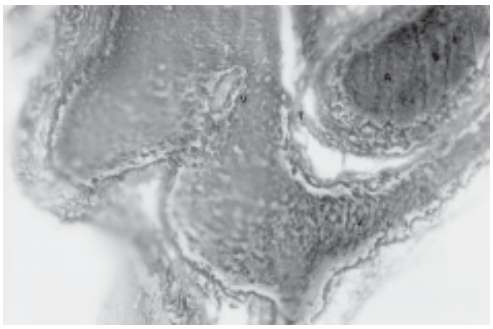

Figure 6
Photomicrograph of the distal portion of the spermatic cord of a mouse showing the testicular artery (A), testicular veins (V), and intervascular connective tissue (T). (140X – Gordon reticulin)

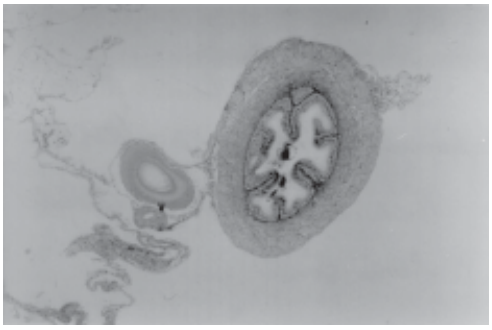

Figure 7
Photomicrograph of the spermatic cord of a mouse showing the ductus deferens (D);, and vasa deferentia (V). (34X – hematoxylin-eosin)

very possibly related to the mechanism of testicular thermoregulation, occurs not only depending on species and strain, but also depending on the influence of environmental factors and, in the case of rodents, on the habit of these animals to pull back the testes into the abdominal cavity. These considerations are also based on the fact that some species or even strains present, immediately below the funicular capsule, other peculiar structures also probably directly related to the mechanism of testicular thermoregulation, such as smooth muscle tissue in horses[11,14,16,17] and even lymphatic vessels in mongrel dogs[19].

Among the funicular vessels of mice we observed a delicate layer of loose connective tissue in which we noted a predominance of collagen fibers and few reticular and elastic fibers. This loose connective tissue that is connected to the adventitias of the testicular artery and veins is not exuberant, in agreement with the characteristics identified in agoutis[3], in view of the intimate relationship existing between the testicular artery and veins. In these specimens, however, we did not observe the absence of intervascular connective tissue although the testicular vessels were found to be in perfect juxtaposition. This disagrees with information reported by other authors who also worked with rodents[2,3,4,5,9] and who reported that the testicular vessels are intimately related, with the possible absence of intervascular connective tissue.

Indeed, the proximity between the funicular segment of the testicular artery and the veins of the pampiniform plexus, which are devoid of valves and have a thin muscle layer, may represent in this species the determinant factor for venous return, since no other structures were detcted that might better clarify this function.

The testicular artery, in turn, shows a variable format in transverse sections of the spermatic cord due to its sinuous trajectory, well identified in the Neoprene 450 latex molds, although without characterizing any type of harmonious arrangement as reported for swine[12] and as pointed out by some authors who attribute a spiral shape[20] or a skein shape[21] to this vessel. In this respect, it was not possible to identify any frequency

Figure 8
Photograph of a Neoprene 450 latex mold corresponding to the segment of testicular artery contained in the spermatic cord of the mouse (3.4X)

of vascular arrangement as reported by Harrison and Weiner[22] for some rodents, since mice show an unpredictable behavior of this vascular segment, in agreement with observations made by other investigators who analyzed the arrangement of the segment of the testicular artery in the spermatic cord of rodents.[2,3,4,5]

Also, with respect to the testicular artery segment contained in the spermatic cord of mice, we observed a middle layer consisting of a thick circular smooth muscle layer resting on a regular network of reticular fibers and fine elastic fibers, an inner layer consisting of endothelium and a delicate layer of subendothelial connective tissue presenting a well-defined limiting internal elastic membrane accompanied by the adventitia, which was formed by connective tissue. We did not identify any subdivision of this arterial segment, as is the case in Thoroughbred horses[11] and horses of undefined race.[14] With the techniques used in the present study, we did not observe any evidence in mice of possible anastomoses between this arterial segment and the veins that form the pampiniform plexus, as reported by some investigators for various species (WENSING; DIJKSTRA, 1981 *apud* RERKAMNUAYCHOKE, W.; KUROHMARU, M.; NISHIDA, T., 1988, p.274; WENSING; DIJKSTRA; FRANKENHUIS, 1981 *apud* RERKAMNUAYCHOKE, W.; KUROHMARU, M.; NISHIDA, T., 1988, p.274; HEES et al. 1984 *apud* RERKAMNUAYCHOKE, N.; KUROHMARU, M.; NISHIDA, T. 1988, p.274) or as suggested by NOORDHUIZEN-STASSEN et al., 1985 *apud* RERKAMNUAYCHOKE, W.; KUROHMARU, M.; NISHIDA, T. 1988, p.274, to explain eventual mechanisms of transfer of chemical substances.

With respect to the veins that form the pampiniform plexus, we should point out that the absence of valves in the mouse does not represent an uncommon peculiarity since no valves accompanying these vessels were observed in many other animals[2,3,4,5,9,10,12,13,16,17,23,24,25]. However, extremely fine veins consisting of an inner layer formed by endothelium and a fine middle layer have few smooth muscle cells supported by an irregular network of reticular fibers and are covered by an adventitia that is intimately connected with the intervascular connective tissue or with the adventitia of the testicular artery. It should also be emphasized that the testicular veins of the species studied here presented ample communications which, together with the positioning of the veins around the testicular artery, represent, in our opinion, the major factors for venous return.

The vas deferens, in turn, does not occupy a subcapsular position in the mouse, as is the case for cattle[18] and buffaloes of the Murrah breed[15] and is not located at a distance from the spermatic cord as observed in Thoroughbred horses[11], horses of udnefined race,[14] donkeys of the Pêga breed[16] and burros[17], but is located at the

periphery of the adipose pannicle that contours the funicular vessels, where it can be seen totally enveloped by mesothelium and partially enveloped by the adipose pannicle mentioned above, a fact usually observed in rodents.[2,3,4,5]

In contrast, the length of the segment of testicular artery contained in the spermatic cord of mice had mean, maximum and minimum values of 1.355, 1.80 and 0.80 cm on the right and 1.325, 1.85 and 0.70 on the left, respectively. These data show wide variation when we consider the extreme values, although the means obtained on the right side did not differ significantly at the 5% level from the left side, confirming the data observed in all species studied thus far (Table 2). Analysis of these data indicates that the dimension of the testicular artery segment contained in the spermatic cord cannot depend on the physical size of the animal (stature and body i8weight), but rather depends on species and strain, as suggested by Sterman[11] and Noronha.[16] However, at this time we cannot relate this fact to the climatic conditions under which the animals live, a subject that requires further studies.

Finally, we would like to point out that, due to the complexity of the process of testicular thermoregulation, further studies should be conducted on this species and others for a better understanding of the participation of the different components of the spermatic cord in this mechanism.

Resumo

Estudando os aspectos morfológicos de 33 pares dos funículos espermáticos do camundongo *(Mus musculus)*, adultos, observa-se histologicamente, em 3 pares, que os seus componentes acham-se envolvidos por delgada cápsula de mesotélio. Sob esta cápsula, contornando completamente o funículo, encontra-se densa camada de tecido adiposo. Seus componentes estão rodeados por tecido conjuntivo frouxo, predominando fibras colágenas entre reticulares e elásticas. A artéria testicular mostra calibre variável rodeada pelas veias testiculares, possuindo: túnica interna constituída por endotélio e membrana elástica limitante interna; túnica média formada por espessa camada de musculatura lisa sustentada por uma rede de fibras reticulares; túnica externa constituída por tecido conjuntivo denso, que se encontra contínuo com o tecido conjuntivo intervascular e as adventícias das veias. As veias testiculares formam o plexo pampiniforme, que cerca parcialmente a artéria, apresentando lumens amplos e irregulares, delgadas paredes, constituídas quase que exclusivamente de endotélio, desprovidas de válvulas e apresentando íntima relação com a artéria testicular. O ducto deferente encontra-se na periferia do tecido adiposo subcapsular, que o envolve parcialmente, e está acompanhado de arteríolas, vênulas, linfáticos e nervos. O modelo do segmento da artéria testicular obtido com Neoprene látex "450", em 60 moldes, exibe trajeto sinuoso e como comprimentos médio, máximo e mínimo em cm, respectivamente, 1,35, 1,80 e 0,80 à direita e 1,32, 1,85 e 0,70 à esquerda; valores estes que não apresentam diferenças estatisticamente significantes, ao nível de 5%.

Palavras-chave:
Funículo espermático.
Camundongo.
Irrigaç ão.
Vascularização.
Testículo.

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