Morphometry of leydig cells in the collared peccary (*Tayassu tajacu*)

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Abstract

This work aimed to determine total and individual volume of Leydig cells, leydigosomatic index and the number of Leydig cells per testis and per gram of testis in the collared peccary (*Tayassu tajacu*). Testes were collected from sexually mature collared peccaries, destined for commercial slaughter. Total and individual volumes of Leydig cells were 2.02 ml and 1,202.74 x 10^{-12} ml, respectively. The leydigosomatic index was 0.022%, and the number of Leydig cell per testis and per gram of testis was 1.7 billion and 92.12 million, respectively. These results show that morphometric characteristics of Leydig cells in collared peccaries are similar to average results observed for most of the mammalian species studied.

Key words:
Collared peccary.
Tayassu tajacu.
Leydig cells

Introduction

The collared peccary (*Tayassu tajacu*), similar to other species of the Brazilian fauna, has been commercially exploited for the supply of restaurants and supermarket networks offering the consumption of meat from wild animals. This demand has increased recently, due to rising concerns of the population with healthier food habits. A number of groups has thus dedicated to the investigation of the reproductive physiology of these animals, to provide ways of improving the techniques of rearing and breeding of these species.

Spermatogenesis in collared peccaries has been investigated by Costa, Henry and Paula[1]. The results of the study showed that average diameter and height of the seminiferous epithelium are 274.94 mm and 66.33 mm, respectively, and that 16% of the testicular parenchyma is occupied by intertubular compartment, whereas the remaining 84% are occupied by seminiferous tubules. The eight stages of the seminiferous epithelium cycle and their frequency, and characteristics of different cell populations (Sertoli cells, A spermatogonia, pre-leptotene and pachytene spermatocytes I, and round spermatids), were also investigated. The authors concluded that spermatogenesis yield in the collared peccary was in general 64.0%, and that each Sertoli cell supports in average 20.89 germ cells.

Although spermatogenesis in collared peccaries is well characterized, no studies were found about the morphometric characterization of Leydig cells in the species. This work aimed to characterize the volumetric proportions of elements composing the intertubular tissue, to determine total and individual volume of Leydig cells, number of Leydig cells per testis and per gram of testis and the leydigosomatic index in adult collared peccaries.

Material and Method

Ten adult collared peccaries, from the commercial abattoir Pro-Fauna (Iguape, SP, Brazil), were used in this study. The abattoir

is certified by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA).

After slaughter, the animals were weighed and testes were collected. The epididimum was separated and one testis was frozen for posterior analysis of the space occupied by the albuginea and mediastinum. The testicular artery of the other testis was cannulated for perfusion for 10 to 15 min with 0.9% saline containing 125 UI/l heparin, and for approximately 20 min with 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.4. The vials with the perfusion solution were placed 120 cm above the samples, to allow for a pressure of 80 mmHg. [2] After fixation, each testis was weighed and fragments of approximately 10.0 x 5.0 x 2.0 mm were collected and stored under refrigeration (5ºC) until used.

The testis samples were dehydrated in alcohol solutions of increasing concentration, and included in glycol methacrylate-based plastic resin. Four ìm-thick sections were prepared using a rotative microtome with a glass blade, and stained as routine with toluidine blue containing 1% sodium borate.

The volumetric proportion of elements of the testicular parenchyma was evaluated under light microscopy using a square 10 mm/100 sq micrometric mesh containing 110 points, with a magnification of 1,000x. The points positioned over seminiferous tubules, the nucleus or the cytoplasm of Leydig cells, or the stroma, were scored in 20 random fields, making a total of 2,200 points per testis.

The average diameter of the nucleus of Leydig cells was determined by the analysis of 20 cells/animal, using a linear 10 mm/100 micrometric mesh. The nuclear volume of Leydig cells was calculated with the formula $4/3pr^3$, where r is the average nuclear radius observed. The volume of Leydig cells was determined from the nuclear volume and its proportion relative to the total cell volume. The leydigosomatic index, or the percentage of total body weight represented by Leydig cells, was estimated by the following formula: (percentage of body weight represented by the two testes) x (percentage of testes represented by Leydig cells) / 100.

Data were analyzed with the Excel for Windows software, and the results were expressed as mean ± standard deviation, according to Sampaio. [3]

Results

The collared peccaries used in this study were 11 or 12 months old. As shown in table 1, mean body weight ranged between 18.7 and 23.4 g, and testis weighed 15.4 to 25.0 g. The testicular albuginea and mediastinum weighed in average 3.0 and 0.7 g, respectively. The net weight of testes, which corresponds to the functional portion (testicular parenchyma), was estimated by subtracting the weight of albuginea and mediastinum from total testis weight. All results in this study were based on this value.

The results showed that seminiferous tubules of collared peccaries occupy about 84% of testes net weight, whereas the interstitial compartment corresponds to about 16% of that weight, distributed between Leydig cells (10%) and the stroma (6%), which includes blood and lymphatic vessels and connective tissue.

The cytoplasm represents about 88% of the volume of Leydig cells in collared peccaries, and the nucleus occupies the remaining 12% of the volume (Table 1). Table 1 also shows average diameter and volume of nuclei, as well as cytoplasm volume, in collared peccaries Leydig cells.

The individual volume of Leydig cells ranged between 882.8 and 1,449.2 $x10^{-12}$ ml, resulting in a variation of 0.9 to 3.2 ml for the total volume of Leydig cells in the testicular parenchyma (Table 2). The number of Leydig cells per testis and per gram of testis was around 1.7 billions and 92 million cells, respectively, and the mean leydigosomatic index was 0.02% (Table 2).

Discussion and Conclusion

Age, average body weight and

testicular weight (Table 1) were in accordance with results obtained for sexually mature animals.[1] The average weight of the testicular albuginea and mediastinum were 16.2% ± 2.1% of the testicular weight. A study by Paula and Navarro[4] showed slightly higher results (18.5%). These differences are frequently seen, and may be due to technical difficulties in completely isolating and collecting the mediastinum, resulting in occasional overestimation of the results.

The weight of the testicular parenchyma could be directly converted into volume, since density of the testis is close to 1.0. [1,5] The results thus show that adult collared peccaries have, in average, a volume of 18.6 ± 4.1 ml of testicular parenchyma (Table 1).

The components of the testicular parenchyma presented volumetric proportions as shown in table 1. These results are in agreement with previous studies by Costa, Henry and Paula[1], with methods similar to those used in the present work. Although the composition of the testicular parenchyma is basically the same in different mammals, the proportion of its components may vary among species.[6] It is not unusual to observe reports showing large variations of volumetric proportion within a same species, when comparing different studies; these discrepancies are generally related to differences in the race, age and mainly methodology used by different groups. [7]

The endocrine portion of the mammalian testis is represented by the Leydig cells, which together with connective cells, leukocytes, and blood and lymph vessels constitute the intertubular space or tissue. The distribution and relative proportion of these components differ among mammal species. They are responsible for mechanisms that maintain the levels of testosterone, the main product of Leydig cells, two or three times higher in the interstitial fluid than in the testicular blood vessels, where in turn it is 40 to 250-fold higher than in peripheral blood.[8,9]

The volumetric proportion of Leydig cells in the testicular parenchyma (Table 1) was about 65.2% of the intertubular space. In wild boars, Leydig cells constitute only 6.3% of the testicular parenchyma.[10] Although the collared peccary does not belong to the Suidae family, as the wild boar does (*Sus scrofa scrofa*), it is important to compare the two species due to the scarcity of data about spermatogenesis in the other member of the Tayassuidae family, the white-lipped peccary (*Tayassu pecari*).

Steroids are mainly produced by Leydig cells in mammals, but the large variation on the proportion of testis occupied by these cells observed when different species are compared has not yet been explained. These differences are clearly seen when animals such as the guinea pig[11] (2.0%) and the capybara[12] (32.9%), for instance, are compared.

The nucleus in Leydig cells has excentrical position in the cytoplasm, and is generally round or oval, but may also have a polygonal shape. When the cell is near to vessel walls or to the tunica propria of the seminiferous tubules, the nucleus adjusts to the elongated pattern of the cell and presents an elliptic shape.[13] The existence of a layer of heterochromatin closely associated to the nuclear envelope is a universal characteristic of Leydig cells. [14]

In Leydig cells of collared peccaries, as shown in table 1, the nucleus occupies a proportion similar to that of other species, about 10 to 15% [15], but less than half the value reported for wild boars [10] (30%). As for the proportion of the cell represented by the cytoplasm, the difference is inverted, and whereas in collared peccaries it is 87.7%, in wild boars the cytoplasm corresponds to about 70% of the cell. [10] These differences are discussed below, in the context of the individual volume of Leydig cells.

The mean nuclear diameter of Leydig cells (Table 1) was very similar to that reported for wild boars[10] (6.0 mm). These results allowed the determination of the mean nuclear volume of Leydig cells (Table 1). Since the nuclear volume is estimated using the formula for the volume of a sphere, the difference in nuclear diameter observed

Table 1 - Testis and Leydig cell morphometry, and volumetric rate of the testicular parenchyma components of adult collared peccaries (mean ± standard deviation)

PARAMETERS	n = 10
Testicular weight (g)	22.2 ± 4.8
Testicular albuginea + mediastinum weight (g)	3.75 ± 0.7
Testicular parenchyma volume (ml)	18.6 ± 4.1
Volumetric rate (%)	
Seminiferous tubules	83.6 ± 2.7
Intertubular tissue	16.4 ± 2.7
Leydig cells	10.7 ± 3.0
Stroma	5.7 ± 0.8
Leydig cells	
Cell proportion occupied by nucleus (%)	12.3 ± 1.5
Cell proportion occupied by cytoplasm (%)	87.7 ± 1.5
Nuclear diameter (μm)	6.52 ± 0.3
Nuclear volume (μm³)	146.47 ± 22.44
Cytoplasm volume (μm³)	1056.27 ± 183.89

when cells from collared peccaries and wild boars are compared implicates higher values for the former species. Most mammal species investigated show nuclear volumes between 150 μm^3 and 250 μm^3 [15,16], so that although collared peccaries have higher nuclear volume in Leydig cells than wild boars, these results are lower than those of other species.

The cytoplasmic volume, in addition, shows much larger differences when cells from collared peccaries and wild boars are compared. The cytoplasmic volume of Leydig cells in collared peccaries (Table 1) is almost four-fold higher than that of wild boars[10] (283 mm^3), but is within the average volumes reported for most animals, that ranges between 600 mm^3 and 2000 mm^3. [15] These differences may be due to the small proportion represented by the cytoplasm in Leydig cells of wild boars, as previously mentioned.

Although Leydig cells in collared peccaries occupy 17.0% more of the intertubular space than in wild boars, the testicular weigh in the suid is nearly three-fold higher[10]. The total volume of Leydig cells in the testes of collared peccaries (Table 2) is therefore lower.

Since the proportion of testes occupied by Leydig cells and their volume show considerable variation among species, the total number of cells is also expected to vary largely among different mammals. The comparison of different species, however, is difficult due to the large variation of testicular weight, and is made possible by converting the number of Leydig cells per testis into the number of Leydig cells per gram of testicular parenchyma. The parameter becomes does independent of the size of the animals or of their testes. In most mammals, the results show 20 to 60 million Leydig cells per gram of testis [8]. In suids, these values range from 60 to 90 million cells. [17,18] Wild boars, therefore, show values higher not only than that of collared peccaries (Table 2), but than that of the other members of the family. This may represent a compensatory mechanism for the small volume of Leydig cells in that species when

Table 2 - Individual and total Leydig cell volume, Leydig cells number per testis and per gram of testis and Leydigosomatic index in adult collared peccaries

Animal	Individual Leydig cell volume (x 10^{-12} ml)	Total Leydig cell volume (ml)	Leydig cells number per testis (x10^6)	Leydig cells number per gram of testis (x10^6)	Leydigosomatic Index (%)
1	1,295.72	1.31	1,012,75	55.95	0.0142
2	1,312.30	3.22	2,453.59	99.14	0.0355
3	1,352.95	3.20	2,363.80	118.48	0.0348
4	1,223.41	0.93	761.78	81.41	0.0097
5	882.81	1.43	1,623.92	106.14	0.0150
6	1,449.17	1.41	970.17	50.93	0.0142
7	999.24	2.18	2,178.24	109.18	0.0248
8	1,404.76	1.64	1,166.15	53.25	0.0174
9	1,162.95	2.61	2,241.49	119.87	0.0335
10	944.10	2.27	2,404.55	126.89	0.0254
mean	1,202.74	2.02	1,717.65	92.12	0.0225
sd	199.28	0.80	683.11	29.52	0.0097

compared to other animals. [10]

Results presented in table 2 show that Leydig cells represent around 0.023% of the body weight in collared peccaries (leydigosomatic index). The leydigosomatic index in the wild boar, estimated from the data reported by Almeida [10] is 0.02%. Although the wild boar has in average about 65 million more Leydig cells per gram of testis than the collared peccary, the proportion of body weight occupied by these cells is similar in the two species. These results suggest that the production of steroids in each species is more related to the individual capacity of Leydig cells in secreting the hormones than to differences in the proportion, volume or number of these cells in the testis. [11] According to Zirkin et al. [19], this capacity is closely related to the amount of smooth endoplasmic reticulum in the Leydig cell.

We conclude that the morphometric parameters of Leydig cells investigated in the present study are similar to those reported for most of the other mammalian species.

Morfometria das células de leydigem catetos (*Tayassu tajacu*)

Resumo

Objetivou-se com esta pesquisa determinar o volume total e individual das células de Leydig, o índice leydigossomático e o número de células de Leydig por testículo e por grama de testículo em catetos. Utilizaram-se testículos de 10 catetos sexualmente maturos, destinados ao abate

Palavras-chave: Caititu. *Tayassu tajacu*. Células de Leydig.

comercial. O volume total e individual das células de Leydig foi 2,02ml e 1202,74 x 10^{-12}ml, respectivamente. O núcleo e o citoplasma ocuparam, respectivamente, 12,3% e 87,7% de cada célula de Leydig. O índice leydigossomático foi de 0,022%, enquanto que o número de células de Leydig por testículo e por grama de testículo foi, respectivamente, 1,7 bilhões e 92,12 milhões de células. Concluiu-se que os parâmetros morfométrios estudados para as células de Leydig de catetos estão inseridos na média relatada para a maioria das espécies de mamíferos.

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