www.spermova.pe

SPERMOVA

Spermova 2022; 12(2): 189-196

Artículo original – original article

DOI. 10.18548/aspe/0010.25

A COMPARATIVE HISTOLOGICAL STUDY AMONG TESTICLES AND EPIDIDYMIS OF PETS (DOG AND CAT) AND WILD ANIMALS (GENET AND MONGOOSE)

Estudio histológico comparativo entre testículos y epididimos de animales domésticos (perros y gatos) y silvestres (ginetas y mangostas)

Louiza Derouiche*[1], Sameh Sahi [2,3], Yamina Zatra [4], Houria Ouennes [3], Karima Ghadbane [4], Amira Bouadeld [4]

[1] Higher School of Food Sciences & Agri-food Industries Algiers, Algeria.
[2] Chadli Bendjedid University, Department of Veterinary Sciences El Tarf, Algeria.
[3] Laboratory of Animal Productions, Biotechnologies and Health, Institute Agronomic and Veterinary Sciences, Souk-Ahras University, Algeria.
[4] Saad Dahleb Blida1 University, Faculty of Natural and Life Sciences, Algeria.

* Corresponding author: Louiza Derouiche. E-mail: derouiche_fatma@yahoo.fr

Recibido: 23/07/2022

Aceptado: 05/09/2022

Publicado: 31/03/2023

ABSTRACT

This paper study aims to compare morphologically and histologically the testes of the two predatory animals (mongoose and genet) with the two pets (cat and dog). The research is based on direct observations, preparation of histological sections, an accurate measurement of the seminiferous tubules diameters and epididymal ducts ones. For this purpose, the testes and epididymis have been removed and quickly fixed. The results of the histological comparison showed that the size is more pronounced with dogs than in other species; significant differences were found between wild and domestic species. This study requires further investigation regarding the great differences between selective breeding and implies the possibilities of using biotechnologies, such as artificial insemination, to increase their multiplication especially that of those exposed to extinction.

Keywords: Pets-epididymal-canals; Histology; Testis; Wild-animals.

RESUMEN

Este trabajo de estudio tiene como objetivo comparar morfológica e histológicamente los testículos de los dos animales depredadores (mangosta y gineta) con las dos mascotas (gato y perro). La investigación se basa en observaciones directas, preparación de cortes histológicos, mediciones precisas de los diámetros de los túbulos seminíferos y de los conductos epididimarios. Para ello, los testículos y el epidídimo se han extraído y fijado rápidamente. Los resultados de la comparación histológica mostraron que el tamaño es más pronunciado en perros que en otras especies; significant differences were found between wild and domestic species. This study requires further investigation regarding the great differences between selected breedings and implies the possibilities of using biotechnologies, como la inseminación artificial, para aumentar su multiplicación, especialmente la de las expuestas a la extinción.

Palabras clave: Mascotas-canales-epididimales; Histología; Testículos; Animales-salvaje

INTRODUCTION

Life development of men passed by several steps, such as domesticating wild species, which represents an important period of the development of human societies. Among the very first domesticated animals (Ollivier, 2017) that went from wild animals to pets, the dog Canis lupus familiaris, although it belongs to the same wolf race Canidae family, was domesticated, that is the best witness of the great development of farming. Next, men were rather able to domesticate also the cat Felis silvestris catus.

Despite domesticating and adoption of several animals for our own benefit, several others are stillwild, including the genet "Genetta genetta" and the mongoose "Herpestes ichneumon", belonging to the same breeds of dogs and cats, that of the Carnivora. These two species have always kept their wild character, translated into damage caused to farm animals, leading to death while they are protected (IUCN, 2010).

Animals' reproduction inside farms is better than in the nature, because, it has been said that wild animals may reproduce much better inside farms where they are well fed and well overseen by veterinary care throughout their lives (Khamas and al., 2014). Moreover, being aware of the reproductive biology of these species is very important for a correct management of their breeds, especially in its basic aspects, so that many studies could be carried out performed using them as a biological model for the physiology of reproduction's, especially species in danger of extinction (Mehanna and al., 2018).

The increased interest in breeding dogs and cats and their use as models for other canids and felids demand research to improve reproductive techniques. Among them, testicular cryopreservation stands out. Testicular cryopreservation enables the maintenance of reproductive capacity and allows the establishment of germplasm banks for several species of commercial value or at risk of extinction. Furthermore, it enables the transport of genetic material among different regions. It is noteworthy that this biotechnology represents the only possibility of preserving the fertility of prepubertal animals that have died, so it has great importance in the propagation of the genetic material of animals (Silva, 2022).

We try to find out if there are differences in histology, morphometry and similarities between several species. This study aims to provide more information on the intraspecific variation of male reproductive organs for two wild animals (genet and mongoose), using domestic animals (cat and dog) as a model. To know about diversity in various organs and physiological and behavioural measures of these species is a prerequisite for the control of reproduction as well as the use of biotechnologies for the preservation of these species, especially those that are in danger of extinction.

MATERIALS AND METHODS

The animals

Adult testicles of four different species were used for this experiment. This is a prospective study carried out within the Pathological Anatomy Department of the Issad Hassani university hospital of Beni Messous in Algiers, Algeria. The details are shown in table (1):

Table 1. Animals studied in our experiment.

ANIMAL	NUMBER of pairs	SPECIES	REGION
Cat	*06*	*Felis catus*	Veterinary clinic in Dely-Brahim, Algeria
Dog	*06*	*Canis lupus familiaris*	Veterinary clinic in Kouba, Algeria
Mongoose	*01*	*Herpestes ichneumon*	Recovered dead from the forest in Souk-Ahras, Algeria
Genet	*01*	*Genetta genetta*	Recovered dead in Belezma national park in Batna, Algeria

The histological study

Dealing with these samples was carried out through a series of mandatory successive steps, the purpose of which is to obtain fine cuts ready to receive the staining of interest. The procedure used is inspired from that developed by Martoja and Martoja (1967). The details are shown in table (2).

Dehydration and Impregnation

The testicles and epididymis were placed in kerosene blocks, according to old histological techniques (dehydration in alcohol baths in an increasing way, and Kerosene inclusion). To carry out the dehydration, we used a series of Ethyl alcohol baths of increasing degrees (50%, 70%, 80%, 90%, 100%), for 2 hours for each bath to avoid disorganization of the structures.

The last bath is a Xylene bath to complete the dehydration and prepare the impregnation of the organ with kerosene because Ethanol and kerosene are not miscible. Immediately after the Toluene baths, the organs were immersed in three successive Kerosene baths, each lasting 2 hours at 60°C; this is the impregnation. The second and third baths contain pure Kerosene, while the first consists of half Kerosene and half Toluene.

Inclusion

For the inclusion operation, the organs were placed in molds that will receive the Kerosene. The respective cassettes, identifying each sample, were placed on the surface of the molds. Kerosene was poured inside the molds until the sample is completely immersed. Then the device is placed on a cooling plate of the apparatus (-10°C to -15°C) until the block becomes solid.

Table 2. Steps for preparing samples for staining.

STEP	PRODUCT USED	DURATION	OBJECTIVE
1	100% Formalin	Min. 12 h	Fixing
2	Running water	Rinse	Removing the fixative
3	60% Ethanol, (2 baths)	1 h (x2)	Dehydration
4	75% Ethanol, (2 baths)	1 h (x2)	
5	100% Etanol (2 baths)	1 h (x2)	
6	Toluene (2 baths)	1 h (x2)	Inclusion
7	Parrafine liquid at 56°C	12 h min.	

Cutting, Colouring, and Observation

Sections of 3 µm were made in the microtome and then rehydrated and stained with Haematoxylin and Eosin, Congo Red, Masson's Trichrome. Before staining, the rehydration was performed in a reverse sequence to that of dehydration.

Connexion is the operation that consists of preserving the staining with the help of Eukitt (Merck, Darmstadt, r.f.a) which allows the adhesion between the slide and the coverslip. After connexion, the slides are dried on absorbent paper and finally observed. To draw histograms and to compare the sizes of the seminiferous tubules and epididymides of our samples, measurements were made on histological sections of the animals studied. The images were captured by a digital camera (hero cam, ma88-500, BME lab and science, st. Paul, USA) connected to a photonic microscope (Optika b 235, Italy) via TS view software (microscopes America, cumming, ga, USA).

The surface area, diameter (minor and major axis), perimeter for seminiferous tubules and epididymides at G×10, and epididymides contours at G×40 were measured using image analysis and processing software "Axio Vision 4.6.3.0" developed by Carl Zeiss company.

In our morphometrical study on 3 animals of each species, and for each section, 50 measurements were made for surface area, diameter, and perimeter magnified at G×10 magnification; for the seminiferous tubules as well as 20 measurements magnified at G×40 magnification to measure the contour of the epididymides andthe heights of the main cells of the epididymis.

Statistical analysis

The results obtained are presented in the form of means ± MSE.

-Arithmetic mean (x) of individual values:

$$x = \frac{\sum_{i=1}^{n} xi}{n}$$

Σx: Sum of individual values

n: number of values

-Standard error of the mean (SEM):

$$\text{SEM} = \frac{\delta}{\sqrt{n}} \quad \delta = \sqrt{\frac{\sum(xi-x)}{n-1}}$$

δ : Standard deviation, xi: Individual value

-Correlation coefficient r:

$$r = \frac{p}{\delta x \delta y} p = \frac{1}{n} \sum xiyi - xy$$

$$(\delta x)^2 = \frac{1}{n}\sum(xi-x)^2 (\delta x)^2 = \frac{1}{n}\sum(yi-y)^2$$

xi and yi: individual values compared x and y: average of individual values compared.

-Statistical validity:

The statistical validity of the differences is calculated by Student's test using the statistical software. The difference between two compared means is statistically significant if the probability "p", read as a function of the number of degrees of freedom (d.d.l. = n1 + n2 - 2) is equal to or less than 5%.

RESULTS

Our study is based on the comparison of the male genitalia of the cat "*Felis catus*" and the mongoose "*Herpestes ichneumon*" with two other species, the genet "*Genetta genetta*" and the dog "*Canis lupus familiaris*"

Morphological study

Before making the histological sections, we made a morphological comparison of the testicles. We noticed that the size of the dog's testicles was higher than that of the three other species, namely the mongoose, the cat, and the genet, and the same is true for the morphology of the testicles (Figure 1).

Figure 1. Testicles of the dog (1), the cat (2), the mongoose (3) and the genet (4).

Histo-morphometry

Structural aspect and morphometryof the seminiferous tubes

At the magnification Gx4 ion; In "Felis catus" and "Canis lupus familiaris": Seminiferous tubules are fused with a central lumen (Figure 2). In "Herpestes ichneumon" and "Genetta genetta": the seminiferous tubules are scattered with an invisible lumen. The extra-tubular space is occupied by interstitial tissue (Figure 2).

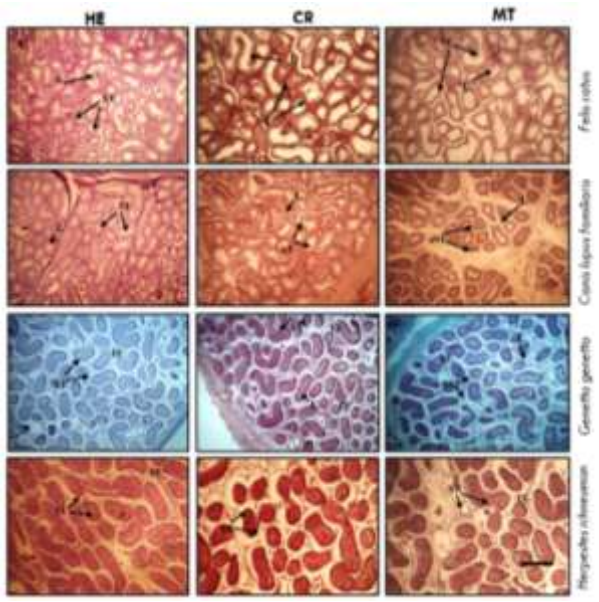

Figure 2. The structural aspect of the seminiferous tube in *Felis catus, Herpestes ichneumon, Canis lupus familiaris* and *Genetta genetta* at Gx4 magnification. Stained with Haemalun Eosin (HE), Congo Red (CR), Masson's Trichrome (MT), Scal bar: 200µm. ST: Seminiferous tube, L: Lumen, IT: Interstitial tissue.

At Gx10 magnification; in "*Felis catus*" and "*Canis lupus familiaris*": We observed a network of ducts that collect the products of the seminiferous epithelium. The seminiferous tubules consist of a central lumen sometimes occupied by spermatozoa, it is bordered by a seminiferous epithelium, the seminiferous tubules are fused, between the tubules we found spaces occupied by interstitial tissue (Figure 3).

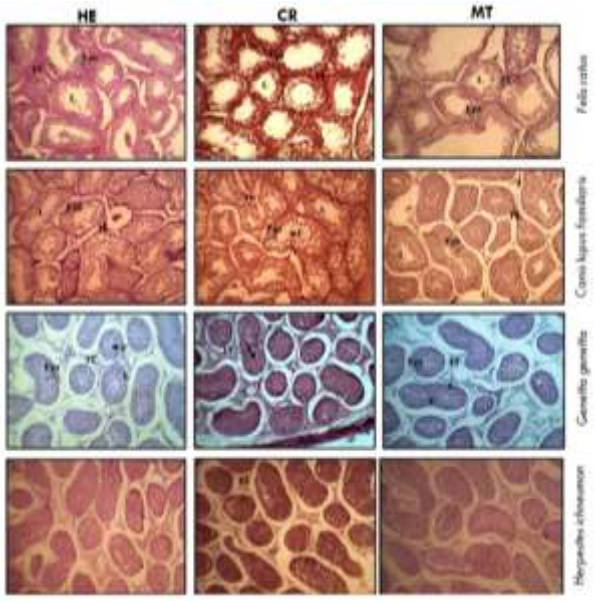

Figure 3. The structural aspect of the seminiferous tube in *Felis catus, Herpestes ichneumon, Canis lupus familiaris* and *Genetta genetta* at Gx10 magnification. Stained with Hemalun Eosin (HE), Congo Red (CR), Masson's Trichrome (MT), Scal bar: 100µm. Ept: Epithelium, L: Lumen, IT: Interstitial tissue, F: Flagella.

The morphometric study showed that the surface area of these tubes in cats and dogs was 57704.5 ± 1464.2; 33482,8 ± 818,5 respectively and the lumen of these tubes is 16251± 871.6 ùm2; 5513 ± 247,3 respectively (Figure 4).

We noted that the seminiferous tubules are large in both cats and dogs and are fused in an anarchic manner. A visible lumen poor of spermatozoa, which is large in the cat. The statistical study showed that there is a highly significant difference between these two species studied in favor of Felis catus, the difference is 72.34%; p=0.0000 for the surface of the tubes and 66.07%; p=0.0000 for the lumen of these tubes.

In Herpestes ichneumon and Genetta genetta: the surface area of these tubes is 21728.4±524.3; 20191.1± 1335.4 µm2 respectively, the lumen is (364.7± 13.7; 9366.3± 1054.8) µm2 (Figure 4: A, B) respectively. An invisible lumen full of spermatozoa, which is large in Genetta genetta. The presence of a large envelope surrounding the seminiferous tubules was also noticed.

In Figure 4, the surface area of the envelope is 54530.9±1262.8 µm2 in *Genetta genetta* and 40548,4801 ± 1007,11657 µm2 in *Herpestes ichneumon*. There is no significant difference between the two species studied for the surface area since the difference is only 7,6%; p= 0,2831, whereas, for the surface area, the difference is statistically highly significant for the lumen of these tubes (-96.1%; p=0.000000) and (-25.9031%, p=0.0000).

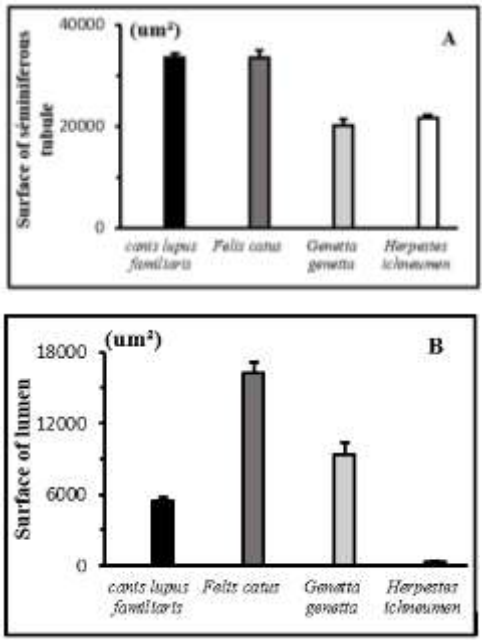

Figure 4. Tissue morphometry of the seminiferous tubules, *Canis lupus familiaris, Felis catus, Genetta genetta, Herpestes ichneumon*, A: Seminiferous tubule surface, B: Lumen surface.

In *Felis catus* and *Canis lupus familiaris*, the seminiferous epithelium delimiting a central lumen shows that the epithelium consists of a thin peripheral tunica fibrosa and several layers of cells. The most mature cells are located close to the lumen while the least mature cells are close to the extern tunica. They correspond to four generations of cells that have started their evolution. The size and shape of the cells vary from the periphery to the lumen about according to the size and appearance of the nucleus. The tissue occupies a small area and contains large cells either isolated or in clusters, closely related to the blood and lymphatic capillary networks and linked to the seminiferous tube (Figure 5).

In Herpestes ichneumon and Genetta genetta: spermatozoa occupy the lumen. The seminiferous epithelium contains cells at different stages of maturation distributed along the epithelium in an unorganized manner, the interstitial tissue occupies a large area and contains large cells either isolated or in clusters (Figure 5).

At the magnification G×100, in Felis catus and Canis lupus familiaris: The seminiferous epithelium is made up of cells that have reached different stages of differentiation, in particular, the characteristics of the cells, from the periphery to the lumen are successively observed as:

- Small and oval, with a large nucleus (spermatogonia).
- Slightly larger than the above and circular, with a large round nucleus containing chromatin arranged in coarse clusters or fine filaments (spermatocytes).
- Similar in size to the above, with a relatively smaller and denser nucleus and a homogeneous, finely granular cytoplasm, and their position is close to the light, these are spermatids.

- Elongated, teardrop-shaped cells with a flagellum on the lumen side of the tube and containing an equally long, slightly curved nucleus of uniform coloration. The cells described are Sertoli cells (Figure 6).

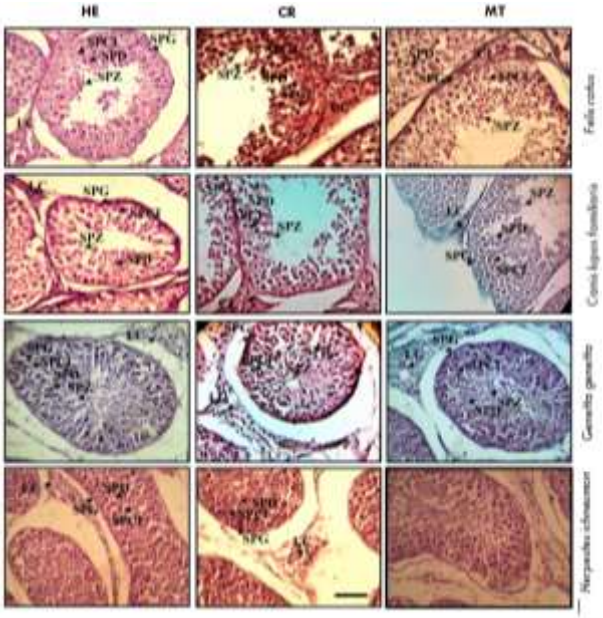

Figure 5. Structural aspect of the seminiferous tube in *Felis catus, Herpestes ichneumon, Canis lupus familiaris* and *Genetta genetta* at Gx40 magnification. Stained with Hemalun Eosin (HE), Congo Red (CR), Masson's Trichrome (MT), Scal bar: 50µm. LC: Leydig cell, SPG: Spermatogonia, SPCI: Spermatocyte I, SPD: Spermatid, SPZ: Spermatozoa.

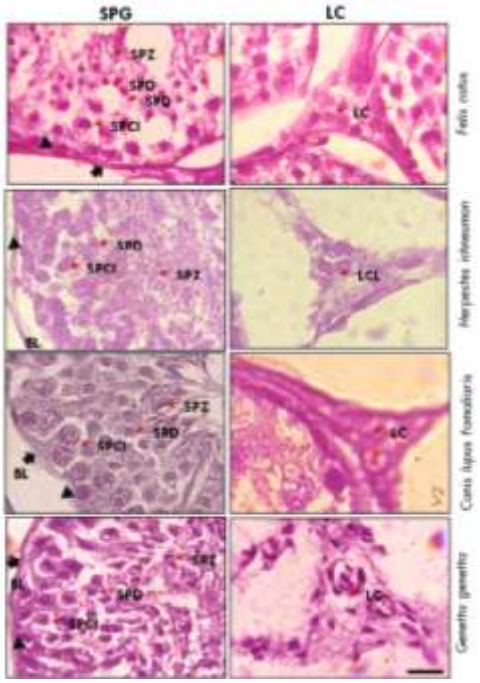

Figure 6. The structural aspect of the seminiferous tube in *Felis catus, Herpestes ichneumon, Canis lupus familiaris* and *Genetta genetta* at Gx100 magnification. Stained with Hemalun Eosin (HE), Congo Red (CR), Masson's Trichrome (MT), Scal bar: 10µm. LC: Leydig cell, SPG: Spermatogonia, SPCI: Spermatocyte I, SPD: Spermatid, SPZ: Spermatozoa, BL: Basal lamina.

The histologic aspect of the epididymis

At the magnification Gx10, in Felis catus and Canis lupus familiaris: The epithelium of the epididymis is of the pseudostratified cylindrical type and the cells are provided with long stereocilia. The epididymis is surrounded by a circular layer of smooth muscle cells. The lumen is sometimes occupied by spermatozoa. In Genetta genetta: The epithelium is pseudostratified cylindrical with the presence of stereocilia at the apical pole, the extra-tubular space is occupied by smooth muscle cells (Figure 7).

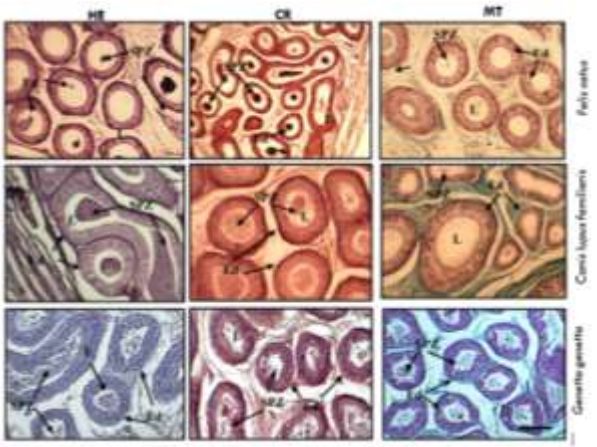

Figure 7. The structural aspect of the seminiferous tube in Felis catus, Herpestes ichneumon, Canis lupus familiaris and Genetta genetta at Gx100 magnification. Stained with Hemalun Eosin (HE), Congo Red (CR), Masson Trichrome (MT), Scal bar: 100µm. SPZ: Spermatozoa, Ed: Epididymal duct, L: Lumen.

The surface area of the epididymis in cats and dogs is 7356.7± 545.8; 39628.6±1995.2) µm2 respectively, that of the lumen is (8138.56±332.3; 14227.42±973.2) µm2 (Figure 8: A, B) so there is a highly significant statistical difference between these two species in favor of Felis catus and Canis lupus familiaris, the difference is 438.7%; p=0.000000 for the surface area of the tubes and 74.8%; p=0. 000000 for epididymal lumen in favor of the dog.

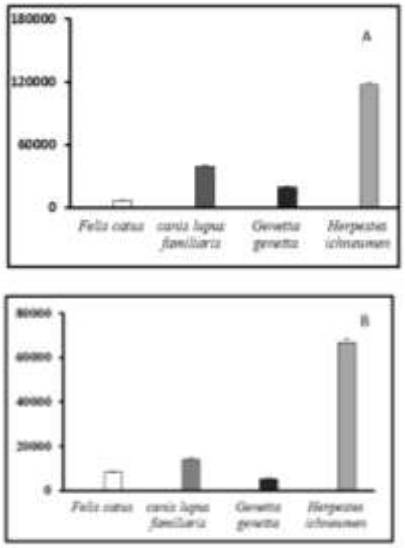

Figure 8. Epididymis surface (A), epididymis lumen (B), of the 4 species studied, *Felis catus, Canis lupus familiaris, Genetta genetta, Herpestes ichneumon*.

In Herpestes ichneumon and Genetta genetta: The surface area of the epididymis is high in the mongoose (116379.7±2800.4) µm2, compared to the genet (34.7±1995.2) µm2, the same is true for the lumen, which is visible and sometimes full of spermatozoa (Figure 8: A, B). Thus, the statistical study shows a highly significant difference between these two species in favor of Herpestes ichneumon. The difference is 482.6%; p=0.0000 for the surface of the tubes and 1165.5%; p=0.0000 for the epididymis lumen.

In all four species studied, the epididymal canal wall consists of an epithelium of constant height, which is not scalloped and rests on a muscular connective tissue layer via a basement membrane. The circular lumen contains numerous spermatozoa. We also observed cells resting on the basal lamina in the deep part of the epithelium, and prismatic principal cells with stereocilia at the apical pole, and a chorion containing circular smooth muscle fibers (Figure 9).

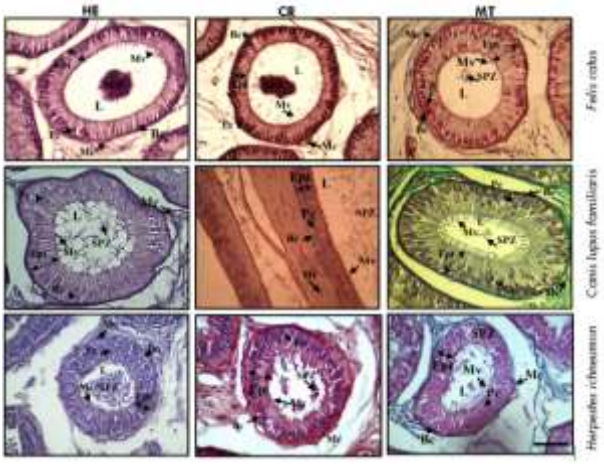

Figure 9. The structural aspect of the epididymis in Felis catus, *Canis lupus familiaris* and *Genetta genetta* at Gx40 magnification: (a, b, c). Stained by Hemalun Eosin (HE), Congo Red (CR), Masson Trichrome (MT), Scal bar: 50µm. SPZ: Spermatozoa, Pc: Principal cell, Mc: Muscle cells, L: Lumen, Mv: Microvilli, EPT: Epithelium, Bc: Basal cell, Ept: Epithelium.

In *Felis catus* and *Canis lupus familiaris*: The heights of the epithelium and the supra-nuclear zone are respectively (33.9 ± 0.6; 40.28 ± 1.8) and (19.3 ± 0.5; 28.6 ± 0.8) in dogs and cats. (Figure 10: A, B), so it can be seen that the dog cells are higher and the differences are 18.78%; p= 0.002241 for epithelial height and 48.12%; p=0. 000000 for the height of the supa-nucleus.

We noticed that the principal cells had long stereocilia is Canis lupus familiaris with a full sperm lumen with a higher epithelial height and principal cells resting on the basal lamina up to the apical pole and basal cells resting on the basal lamina.

DISCUSSION

Testicular cryopreservation enables the maintenance of reproductive potential, the creation of germplasm banks and the transport of genetic material between different regions. This biotechnology represents the only possibility of preserving the fertility of prepubertal animals that have already died or that need to undergo gonadotoxic treatments. Despite advances in the use of cryopreserved testicular fragments, protocols that can be used in the clinical routine of dogs and cats have not yet been established. Due to the great importance of the topic, the objective of this review is to provide an overview of the subject, approaching the main works on testicular cryopreservation in dogs and cats (Silva, 2022).

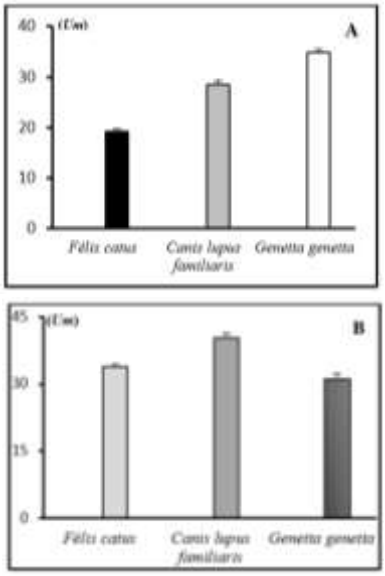

Figure 10. The height of the epididymis epithelium (A), the height of the epididymis nucleus (B), of the 3 species studied, *Felis catus, Canis lupus familiaris, Genetta genetta*.

The need for promotional projects for the preservation of endangered species in Algeria was the main source of motivation to carry out this study. To get to know these species well, we've chosen to lead a comparative histological study between the testicles and epididymis of wild animals (genet and mongoose) and domestic those of domestic ones (dog and cat). This study showed comparable histological characteristics overall. Some differences were observed and described. From an anatomical point of view, the histology of the testis and epididymis, is comparable to that found in the four species, but concerning the size of the gonads of these different animals studied, we noticed that the dog's testicle comes first, then the mongoose, and lastly the genet after the cat. This means that there is a conformity between the weight of the testis and the body weight of the animal.

Our results differ from the results of Franca and Godinho (2003), who found that the average weight of the testes of the cat is 1, 2 g and that there is no significant correlation between the weight of the testes and the body weight (r = 0.36), on the other hand, they found out that there is a similarity between the two testes (p>0,05). In fact, there is a positive correlation between body weight, testicular weight and plasma testosterone concentration (Berger and al., 1982). Comparing the histological aspects of the four testes of these species studied, we found that the seminiferous tubules of Félis silvestris catus and Canis lupus familiaris are fused together with a larger size, colored green with Masson's Trichrome, this space is reduced. Whereas, in the mongoose and genet, the seminiferous tubules are scattered, dispersed and separated

from each other by an interstitial tissue (the green coloring is very attractive) in the form of a network, the latter taking up a large space, which is full of Leydig cells the tubes are small in relation to the first ones. The seminiferous tubules are surrounded by envelopes located between the seminiferous tubule and the interstitial tissue. These testicular envelopes are larger in Genetta genetta by contribution to Herpestes ichneumon.

On the other hand, in Genetta genetta and Herpes ichneumon, it is difficult to identify the type of germ cells, with a seminal tube lumen full of spermatozoa, which means that they are in the reproductive period. Garcia-Tomas and al. (2009a) and Garcia-Tomas and al. (2009a) reported that spermatogenesis varies according to species, environment and management, which should be taken into consideration in studies.

The interstitial tissue of the testis located in the intertubular compartment is important for the nutrition of the cells of seminiferous tubules, transporting hormones and androgen production. The spaces between the seminiferous tubules of the testes are filled with connective tissue, blood and lymph vessels, and Leydig cells or interstitial cells, the main components of this compartment (Junqueira and Carneiro, 2013).

The distribution of germ cells in the seminiferous epithelium (spermatogonia, spermatocytes I, spermatocytes II, spermatids) is well organized in the seminiferous epithelium in Felis catus and even in Canis lupus familiaris. On the other hand, in Genetta genetta and Herpestes ichneumon, it is difficult to identify the type of germ cells, the latter have an elongated nucleus, the light becomes invisible because it is full of spermatozoa which means that they are in the breeding season. According to Mehanna and al. (2016), in cats, interstitial cells and Leydig cells are more abundant compared with other species and substantially fill the space intertubular; they have a polyhedral shape with a large spherical nucleus and evident nucleolus; which resembles the Leydig cells present in the Pampas cat. While, The Sertoli cells are less frequent, distinguished by a more elongated shape and pyramidal cell with irregular contours and extending from the basement membrane to the tubule lumen and appear spherical to the oval nucleus, nucleolus with noticeably stained.

Silva and al. (2009) describe the Leydig cells in domestic cats showing varied dimensions with polyhedral shape, vacuolated cytoplasm, clear nucleus, nucleolus evident.

In canids, interstitial cells and Leydig cells are present in intertubular spaces, but to a lesser extent than observed in cats, along with the connective tissue; have polyhedral shape with a large spherical nucleus and evident nucleolus (Diagone, 2009). Caldeira (2007) reports on morpho functional analysis of the testis and spermatogenesis process of the crab-eating fox (Cerdocyon thous, Linnaeus, 1766) that spermatogenesis is a synchronous and regular process of differentiation and cell division, whereby a spermatogonia gradually differentiates a highly specialized haploid cell, the sperm.

The testicular envelopes are larger in Genetta genetta compared to Herpes ichneumon. The distribution of germinal cells in the seminiferous epithelium (spermatogonia, spermatocytes, spermatids) is well organized in Felis silvestris catus and Canis lupus familiaris. These results are similar to those of Franca and Godinho (2003) who observed well-organized germ cells at different stages of spermatogenesis in the cat's testis. Yasser and al. (2012) reported in chinchilla rabbits that the height of the epithelium of the seminiferous tubes decreases from the first to the 4th week and remains constant until the 7th week then increases again, about the onset of spermatogenesis.

The latter is probably associated with cell death, which increased with age. According to histological analysis of the testes of hoary fox Lycalopex vetulus (Lund, 1842), the seminiferous tubules are formed by the columnar epithelium consisting of spermatogenesis cells and Sertoli cells, surrounded by a basement membrane, separated by interstitial tissue (Mehanna and al., 2018).

However, the Leopardus colocolo male individuals have the testicle constituted by a capsule called tunica albuginea, seminiferous tubules with stratified epithelium well developed with alternating presence and absence of light, evident Sertoli cells, and intertubular compartments or developed and interstitial vascular tissue, with Leydig cells (Mehanna and al., 2016).

According to Bacha and Bacha (2003), combinations of spermatogenesis cells of the epithelium development occurring inside a seminiferous tubule, in which these cellular stages occupy a portion of the tubule. The wall of the epididymal canal in all four species consists of the epithelium of constant height, not scalloped, resting via a basement membrane on a connective-muscular layer, with a circular lumen containing numerous spermatozoa. We also observed cells resting on the basal lamina in the deep part of Epithelium, and main prismatic cells presenting at the apical pole of long stereocilia and a chorion containing circular smooth muscle fibers. Knowing that the epididymis in mammals is considered a very important segment in extra-testicular sperm via. In Canis lupus familiaris, the height of the epithelium varies depending on the epididymal segment considered, the epithelium being higher in the region of the head and lower in that of the tail.

On the other hand, the size of the epididymis is narrow at the level of the head and wider at the level of the tail. An internal circular smooth muscle layer, increasing in thickness from the head to the tail, and an external longitudinal layer, visible from the body, surrounding the epithelium and the basal lamina.

The maturation and storage of sperm are its main functions. Further, the epididymis provides a "biochemical environment" in which, the sperm undergoes morphological and physiological changes, affecting its functional maturation passing through several regions of the epididymal (Schimming and al., 2002). In this sense, these latter presents morphological data epididymal ducts of dogs near to the hoary fox, where the epididymal duct is lined by pseudostratified columnar epithelium with a cell population consisting of principal cells, basal and apical, present in all regions.

Hoshino and al. (2002) showed, in a morphometric study of epididymal ducts of domestic cats, the same pattern of cell structures of the Leopardus colocolo epididymal epithelium. The epididymal duct showed pseudostratified columnar epithelium, standing on a delicate basement membrane and integrated by myoid cells. In the domestic cat (Felis catus) the terminal part of

the epididymis continue in the vas deferens where the epithelial continues to be pseudo stratified with small cell stereocilia (Diagone, 2009). As well as the pampas cat (Leopardus colocolo), whose vas deferens showed a thick layer of smooth muscle, a rounded mucosa without longitudinal folds and pseudostratified columnar epithelium with the presence of stereocilia.

CONCLUSION

This study attempts to provide more information on the morphological aspects of the testes of the different selected species. It appears that the histological appearance and distribution of the seminiferous tubules of wild animals are different from those of domestic animals. Although the number of germ cells produced during spermatogenesis is affected by hormones, such as gonadotropins or androgens, these structural histological differences necessarily reflect functional differences in the male reproductive system which may also reflect differences in the rate and timing of reproduction, which are key factors in animal behavior.

It is essential to try to illustrate the contribution of the anatomy of the reproductive system in the knowledge of these species to be used. Similarly, it would be interesting to see the possibilities of using biotechnologies, especially artificial insemination to increase their multiplication. Our aim is that this research is the starting point for other more profound studies on a large population, taking into account the impact of the different influential factors.

ACKNOWLEDGMENT
The authors would like to acknowledge the staff of the Belezma national park in Batna and the veterinarians of the veterinary clinics in Dely- Brahim and Kouba in Algiers.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
SS: Literature search, drafting the article; YZ: Data analysis, statistical análisis; HO: Revising it critically for important content; KG, AB: Experimental studies; LD: Experimental studies, final approval of the version to be submitted.

REFERENCES

- Bacha WJ, Bacha LM. Male reproductive system, 335–336. In: Ibid. (Eds), Atlas Colorido de Histologia Veterinária. 2a ed, Roca, São Paulo, 2003.
- Berger M, Jean-Faucher CH, De-Turckhein M, Veyssiere G, Blanc MR, Poirier JC, Jean C. Testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in plasma rabbit from birth to adulthood. Correlation with sexual and behavioural development". Acta Endocrinol, 1982; 99 (3): 459-465.
- Caldeira BC. Assessment of morphofunctional testis and spermatogenesis process of the crab-eating fox (Cerdocyon thous, Linnaeus, 1766) adult. Master's thesis, Viçosa: Federal University of Viçosa, 2007.
- Diagone KV. Histology and morphometry of adult domestic cats testicles. Faculty of Agricultural and Veterinary Sciences, Unesp, Jaboticabal, São Paulo, 2009.
- Franca LR, Godinho CL. Testis morphometry, seminiferous epithelium cyclelength and daily sperm production in domes-tic cats. Biol. Reprod, 2003; 68: 1554–1561.
- Garcia-Tomas M, Sanchez J, Piles M. Postnatal sexual development of testis and epididymis in the rabbit: Growth and maturity patterns of macroscopic and microscopic markers. Theriogenol, 2009b ; 71, 292–301.
- Garcia-Tomas M, Sanchez J, Piles M. Post-natal sexual development of testis and epididymis in the rabbit: Variability and relationships among macroscopic and microscopic markers. Animal Reproduc, Scie, 2009a. ; 110: 347–355.
- Hoshino P, Nakaghi LSO, Pacheco MR, Lui JF, Malheiros EB. Morphometry of the seminiferous tubules and epididymal ducts of dogs and cats from neutering surgery. Biotemas, 2002; 15 (1):97–110.
- IUCN. Red list categories prepared by the IUCN species survival comission, gland. Switzerland. Markers. Animal Reproduc. Sci, 2010; 110: 347–355.
- Junqueira LC, Carneiro J. Basic Histology. 12th Ed Rio de Janeiro: Guanabara Koogan, 2013; 412-424.
- Khamas W, Al-Tikriti M, Albayati M, Tkalcic S, Eng C. Histological Description of the Testis, Epididymis and Ductus Deferens of the Northern Great Grey Kangaroo (Macropus giganteus giganteus). J Cytol Histol, 2014; 5: 287.
- Martoja R, Martoja M. Introduction to animal histology techniques. Eds Masson et cie, Paris. 1967, 343.
- Mehanna M, Ferreira A, Ferreira LS, Adelina P, Regina CR. Morphology of the testes and epididymal ducts in the Pampas cat Leopardus colocolo (Molina, 1782). Animal Morphophysiology. Pesquisa Veterinária Brasileira, 2016; 36 no.10.
- Mehanna M, Ferreira A, Luís S, Ferreira A, Rodrigues RC, DA Paz M, Thaís O. Histology of the testis and the epididymal ducts from hoary fox. Lycalopex vetulus (LUND, 1842). Biosci. J., Uberlândia, 2018; 34, n 6, p. 1697–1705.
- Ollivier M. Reconstructing and understanding the history of dog domestication through paleogenetics". Archéozoologies, 2017; 148: 50–55.
- Schimming BC, Vicentini CA, Tirapelli LF, Tirapelli DPC. Morphological examination of the epididymal duct in the dog. Braz. J. Vet. Res. Anim. Sci, 2002; 39 (2):61-65.
- Silva CAO, Perri HV, Koivisto MB, Silva AM, Carvalho RG, Monteiro CMR. Histologic and morphometric aspects of domestic cats testicles (Felis catus). Braz. Vet. Res, 2009; 29 (4):321-316.
- Silva LDMd. Canine and Feline Testicular Preservation. Animals, 2022, 12, 124. https:// doi.org/10.3390/ani12010124
- Yasser AA, Mahmoud MAE, Gamal KMA. Histologica and histomorphometric changes of rabbit testis during postnatal development. Res. J. Vet. Sci, 2012; 5 (2): 42-50.