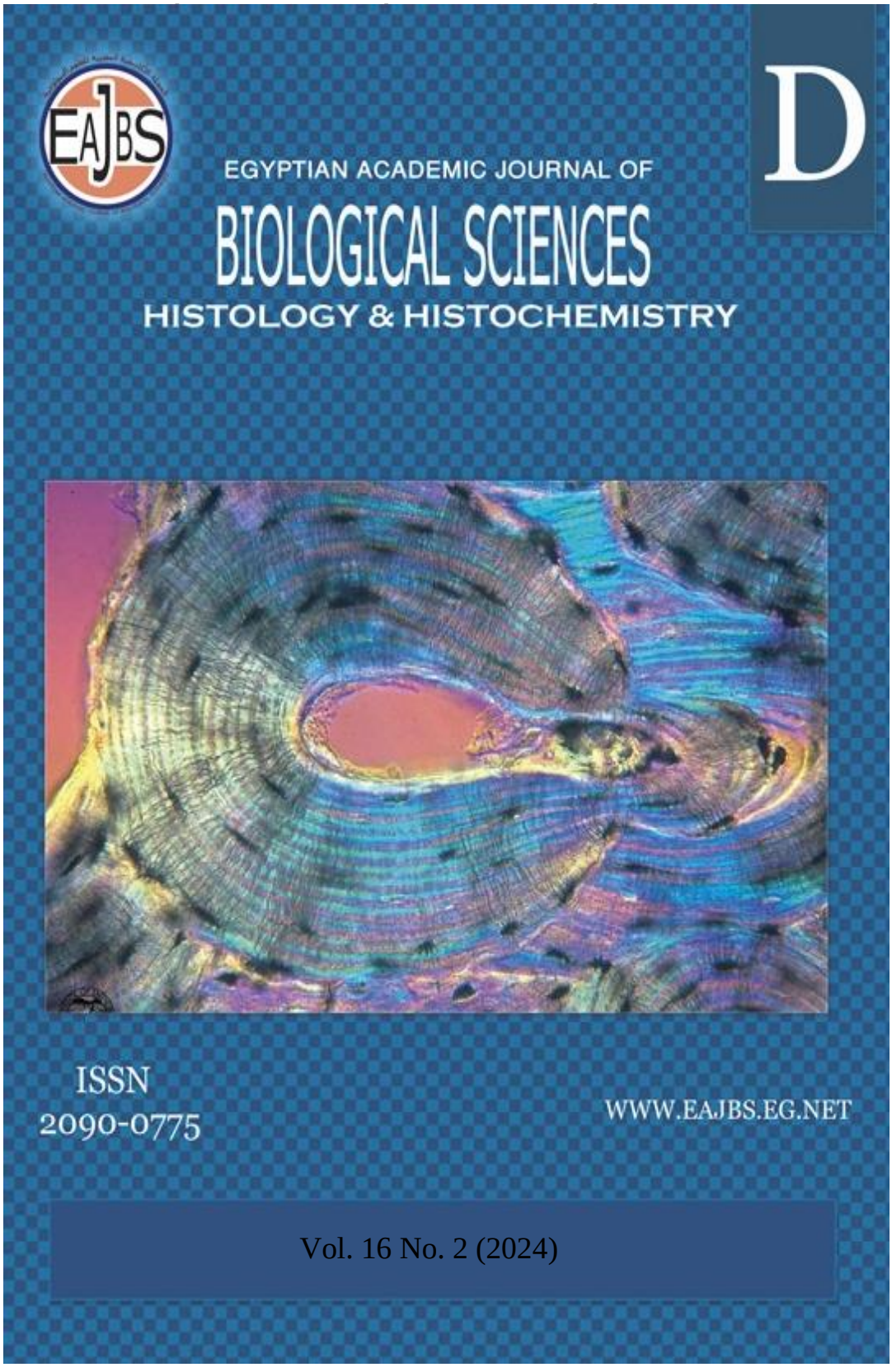

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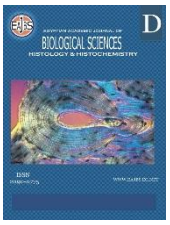

Histomorphometric Comparison of The Male Reproductive System of The Wild Brown Rat (*Rattus norvegicus*) and the White Wistar Rat

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ABSTRACT

Reproduction in wild animals is not well studied, which explains the limited information available on the male reproductive system of the brown rat (*Rattus norvegicus*). This work presents a histomorphometric comparison between the male reproductive system of the brown rat, which is poorly investigated, and the white Wistar rat, which is well-explored as a laboratory strain. Cross-sectional and longitudinal sections were made of the testes, epididymis, vas deferens, and seminal vesicles, and these sections underwent various histomorphometric analyses. The results indicate a structural similarity between the two species in the epididymis and the testes, which exhibit large seminiferous tubules. However, morphometric analysis reveals differences in the surface area of the seminiferous tubules between the two species, as well as variations in epithelial cell height, nuclear height, and supranuclear space height in the epididymis. For the vas deferens and seminal vesicle, the histological results of both species are almost identical; however, morphometric data indicate notable differences.

INTRODUCTION

The brown rat (*Rattus norvegicus*) is one of the most abundant mammals, with a nearly worldwide distribution (Galef, 2009; Puckett *et al*., 2016). They belong to the order Rodentia, which comprises over 40% of all mammal species (Wilson and Reeder, 2005). Many other species commonly referred to as rats belong to various families and genera within the superfamily Muroidea, which also includes gerbils, true mice, and hamsters; brown rats are members of the subfamily Murinae, known as Old World rats (Wilson and Reeder, 2005). Unlike the brown rat, which is largely unknown like most wild species (Derouiche *et al.*, 2023a), the white rat, or Wistar strain, is a well-established strain. This strain was developed by Donaldson in 1906 at the Wistar Institute (USA) from a stock belonging to the University of Chicago (Lindsay, 1979; Russel *et al.*, 1981). The Wistar rat is a non-inbred, versatile strain used across all disciplines of medical and biological research because it is well-studied both anatomically and physiologically, particularly regarding the male reproductive system (Stevens and Lowe, 1993). To better understand reproduction in *Rattus norvegicus*, we conducted this study, which is a comparative histomorphometric analysis of the male reproductive system between the brown rat and the white rat.

MATERIALS AND METHODS

Biological Material:

This work is part of a comparative experiment aimed at comparing the white Wistar rat and the brown rat (*Rattus norvegicus*) in terms of histological and morphometric characteristics. We dissected adult samples from both species to obtain the male reproductive organs: testes, epididymis, vas deferens, and seminal vesicles.

Histological Technique:

Our samples underwent the standard histological technique, which allows for microscopic observations after specific staining. This process involves several steps, primarily detailed in Martoja and Martoja (1967) and Vilar *et al.* (2017), summarized as follows:

Fixation: This step preserves cellular and tissue structures in a state as close to life as possible. The fixative used is 10% formalin, with the specimens submerged in a volume 60 times greater than their own.

Inclusion: This process consists of four stages:

Dehydration: This aims to remove water from the tissues to be replaced by paraffin, which is hydrophobic. The cassettes containing the organs are placed in five baths of increasing concentrations of alcohol: 70° (1 bath), 96° (2 baths), and 100° (2 baths), each for 1 hour.

Clearing: This step eliminates all traces of alcohol and allows for impregnation with butanol, a solvent for paraffin. The specimens are successively placed in two baths of butanol for 1 hour each.

Infiltration with Paraffin: This step involves replacing butanol with paraffin. The specimens are placed in two baths of paraffin heated to 58°C for one hour each.

Block formation: The specimens are removed from the infiltration medium and placed in molds (special metal bars called leukart bars) containing melted paraffin, and poured into slightly preheated molds at 45°C. The cassette is placed on the mold, and the block is only removed after complete cooling on a cold plate, followed by freezing at -4°C for sectioning.

Sectioning with a Microtome: First, the block is mounted on the microtome holder, set to 20 µm to trim excess paraffin. When the specimen appears in the cutting plane, the scale is adjusted to 5 µm to obtain thin sections in the form of ribbons. These ribbons are spread on cleaned glass slides, and using a diamond-tipped engraving pen, the organ's details are inscribed on the corresponding slide.

Staining: The purpose of staining is to enhance the visibility of various cellular and tissue components. This is achieved using hematoxylin-eosin staining. Hematoxylin solutions contain hematin and a metallic mordant (aluminum or iron salts), responsible for staining the nucleus blue-purple; eosin stains the cytoplasm pink, with varying intensity based on the acidophilicity of different elements.

Mounting: Finally, mounting involves preserving the stains using Eukitt (Merck, Darmstadt, R.F.A), which facilitates adhesion between the slide and coverslip. After mounting, the slides are dried on absorbent paper and examined under a photonic microscope (Optika B 235, Italy).

Morphometric Study:

To compare the sizes of the seminiferous tubules, epididymes, vas deferens, and seminal vesicles in our samples, measurements were taken from histological sections of the animals. Images were captured using a digital camera (HIROCAM, 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 areas of the seminiferous tubules and epididymes, as well as the contours of the epididymes, vas deferens, and seminal vesicles, were measured using the image

analysis and processing software "Axio Vision 4.6.3.0," developed by Carl Zeiss.

RESULTS

We performed cross-sectional and longitudinal sections (Derouiche *and al.,* 2023b) of the various components of the male reproductive system (testes, epididymis, vas deferens, seminal vesicle) of the white rat and the brown rat, on which we conducted hematoxylin-eosin staining. The results obtained were observed using an optical microscope at different magnifications.

Testes:

Observation at Low Magnification (Gx10):

We observed a series of seminiferous tubules that collect the products of the seminiferous epithelium. The seminiferous tubules consist of a central lumen lined by seminiferous epithelium containing germ cells. In the white rat, observations of histological sections (Fig. 1) show that the seminiferous tubule has a large diameter, with a minimally reduced lumen containing spermatozoa. The different seminiferous tubules are organized and separated from each other by interstitial spaces. Morphometric analysis reveals that the surface area of the tubules is 71,086.5 ± 849.6 µm², and the lumen area is 12,768.6 ± 339.3 µm².

In *Rattus norvegicus*, observations of histological sections (Fig. 1) indicate that the seminiferous tubules are large with a wide lumen containing spermatozoa, organized closely together. Morphometric analysis showed that the surface area of the tubules is 73,046.6 ± 1,148.7 µm², and the lumen area is 15,582.4 ± 463.3 µm².

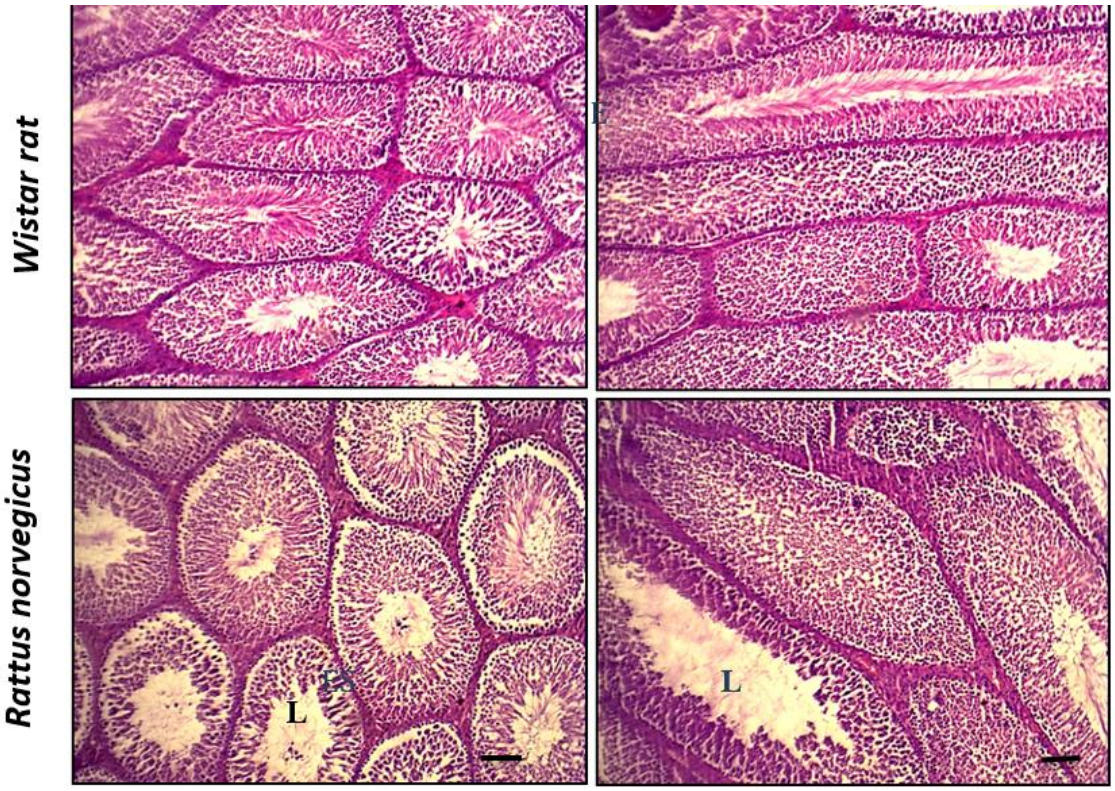

Fig. 1: The structural aspect of the testis shown by longitudinal sections (right) and cross sections (left) in the two studied species, observed at G×10. Scale bar: 100 µm. L: Lumen.

Observation at Medium Magnification (Gx40):

The surfaces of the seminiferous tubules are composed of two distinct populations of cells: Sertoli cells and germ cells of various shapes and sizes, with an arrangement that varies from the periphery to the lumen according to their maturity. The spaces between the seminiferous tubules are occupied by blood vessels and clusters of Leydig cells. The peritubular cells line the

seminiferous tubules, conforming to their rounded shape.

We observed that in the white rat, the seminiferous tubule has a large diameter and a wide lumen containing few spermatozoa, along with germ cells at different stages of maturation (Fig. 2). In the brown rat, the seminiferous tubule is large and features a lumen lined by cells at various stages of maturation, with fewer spermatozoa located near the lumen of the tubule (Fig. 2).

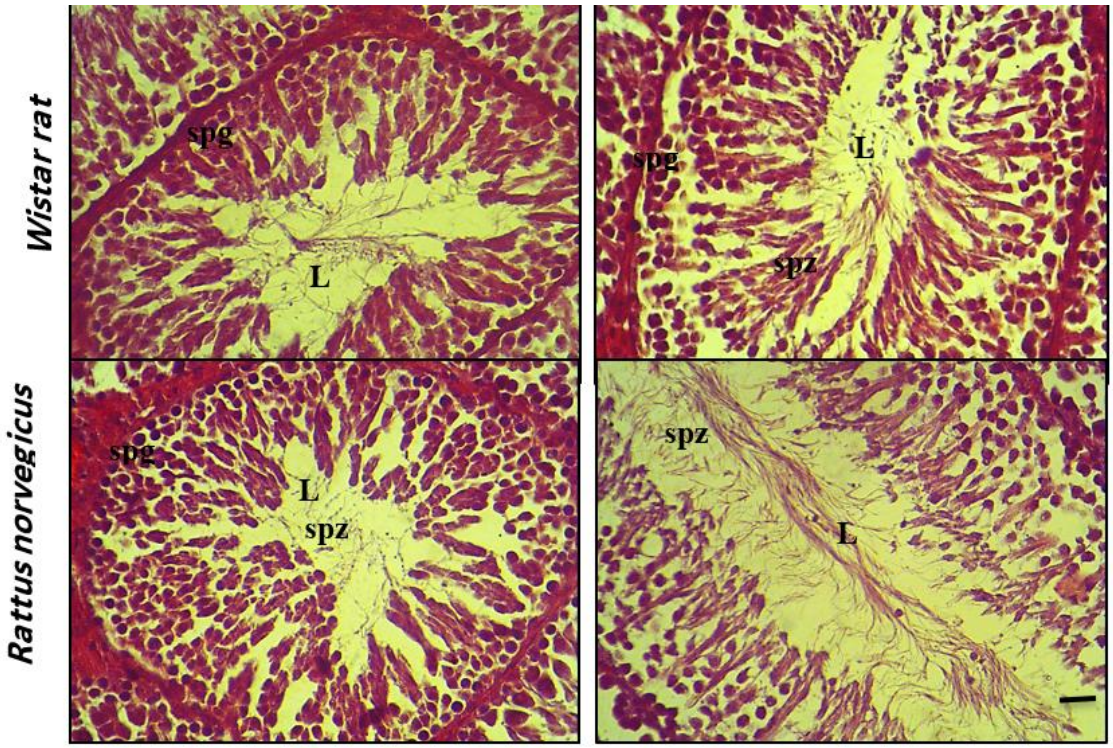

Fig. 2: Structural aspect of the seminiferous tubule shown by longitudinal sections (right) and cross sections (left) in the two studied species, observed at G×40. Scale bar: 50 µm. L: Lumen, spg: Spermatogonia, spz: Spermatozoa.

Observation at High Magnification (Gx100):

In both species, we can easily observe the various stages of spermatogenesis occurring in a centripetal manner along the walls of the seminiferous tubules. Small spermatogonia are located near the basement membrane. Larger primary and secondary spermatocytes have voluminous nuclei. Smaller spermatids are situated towards the interior of the tubules. Mature spermatozoa fill almost the entire lumen of the tubules with their flagella. Sertoli cells are characterized by a clear nucleus and a more advanced position within the epithelium, sometimes adhering to the basement membrane. The nucleus of the Sertoli cell occupies a basal position, and the cytoplasmic extensions of Sertoli cells surround all categories of germ cells (Fig. 3).

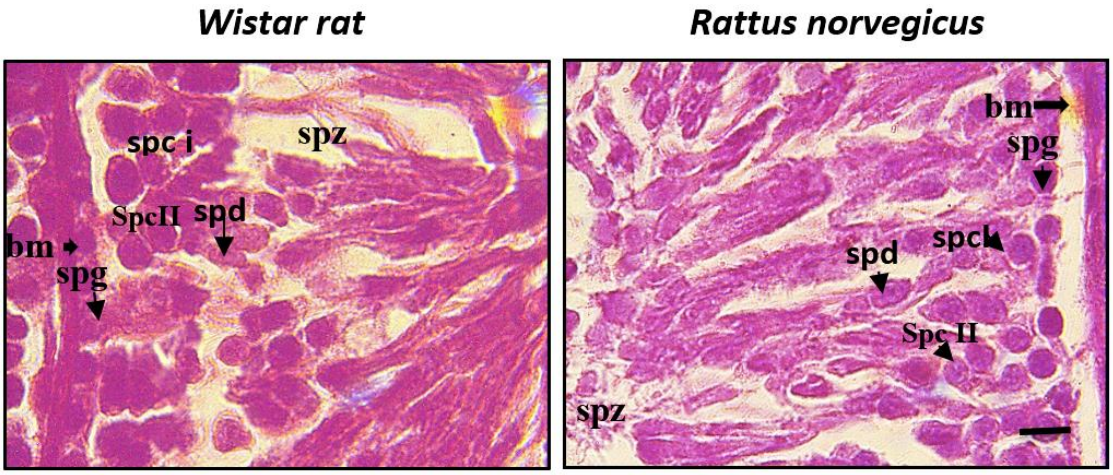

Fig. 3: Structural aspect of the seminiferous tubule in the two studied species, observed at G×100. Scale bar: 10 µm. spg: Spermatogonia, spd: Spermatid, spz: Spermatozoa, spc I: Spermatocyte I, spc II: Spermatocyte II, bm: Basement membrane.

In both species, Leydig cells are located near the walls of the seminiferous tubules. We observed Leydig cells in the testicular interstitial space, characterized by a branched plasma membrane. The nucleus and cytoplasm are hypertrophied, with a regular contour and a rounded shape located at the center of the cell, containing a substantial mass of decondensed chromatin. We noted that the size of Leydig cells is significant in both rats (Fig. 4).

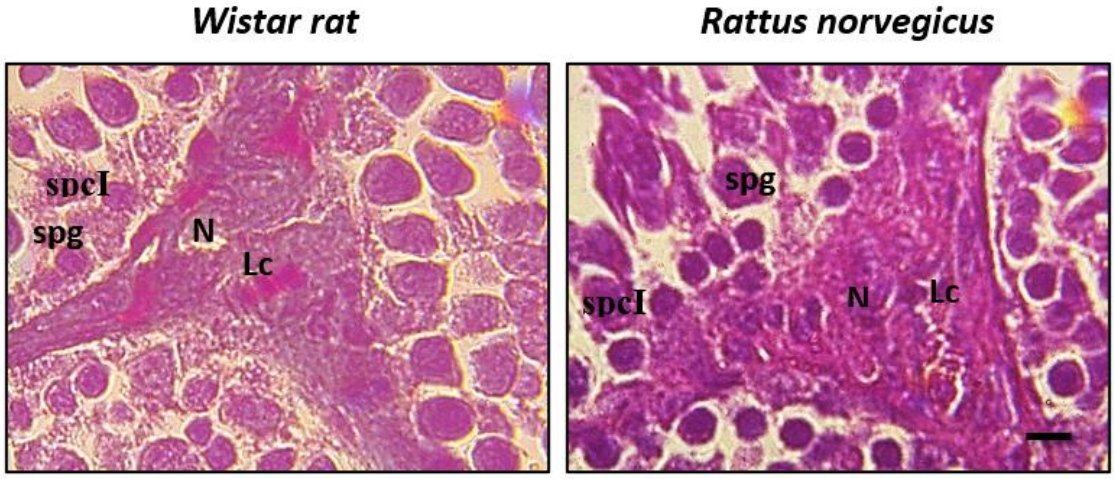

Fig. 4: Structural aspect of Leydig cells in the two studied species, observed at G×100. Scale bar: 10 µm. spg: Spermatogonia, Lc: Leydig cell, N: Nucleus, spc I: Spermatocyte I.

Epididymis:

Observation at Low Magnification (Gx10):

In both the white rat and the brown rat, the epididymis is composed of medium-sized tubules separated by connective septa and surrounded by a thin muscular wall. The epididymal tubule features a wide lumen containing numerous spermatozoa (**Fig. 5)**. Morphometric analysis revealed that in the white rat, the surface area of the tubules is 72,728.9 ± 6,204.6 µm², and the lumen area is 56,218 ± 3,464.7 µm². In contrast, in the brown rat, the surface area of the tubules is 62,900.7 ± 2,246.1 µm², and the lumen area is 45,844.7 ± 2,046 µm².

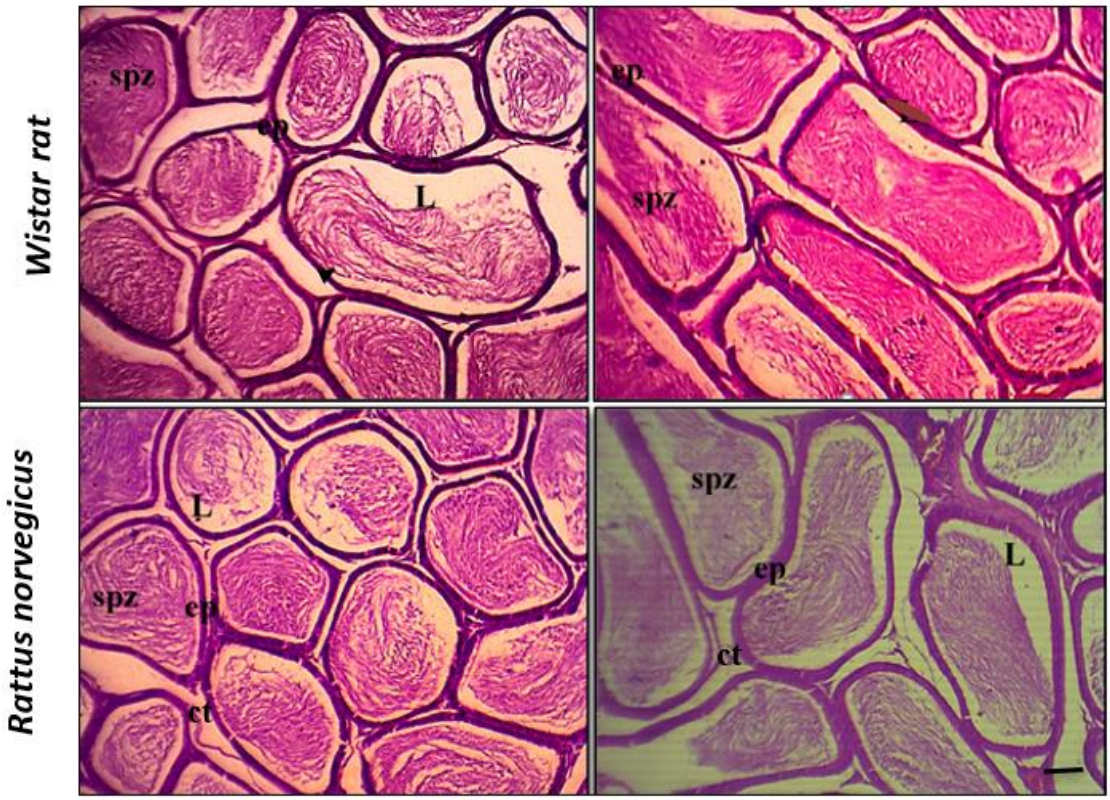

Fig. 5: Structural aspect of the epididymis in the two studied species presented through longitudinal sections (right) and transverse sections (left), observed at G×10. Scale bar: 100 µm. L: Lumen, spz: Spermatozoon, ep: Epithelial cell, ct: Connective tissue.

Morphometric results showed a statistically significant difference between the brown rat and the white rat, with a difference of -15% (p = 0.022136) for the surface area of the epididymis and -19% (p = 0.005677) for the lumen of the epididymis.

Observation at Medium Magnification (Gx40):

In the white rat, the lumen of the epididymal ducts is wide, the epithelial cells are tall and rest on a basement membrane with an absence of apical microvilli; the lumen contains numerous spermatozoa (Fig. 6). The morphometric study showed that the height of the epididymal cells is 36 ± 1.3 µm, the height of the nucleus is 6.9 ± 0.2 µm, and the height of the supranucleus is 24.7 ± 1.3 µm.

In the brown rat, the epididymal ducts are slightly less voluminous, and irregularly shaped, with fewer spermatozoa in their wide lumen; the epithelial cells are shorter and rest on a folded basement membrane (Fig. 6). The morphometric study showed that the height of the epididymal cells is 14.5 ± 0.3 µm, the height of the nucleus is 4.1 ± 0.1 µm, and the height of the supranucleus is 8.6 ± 0.2 µm.

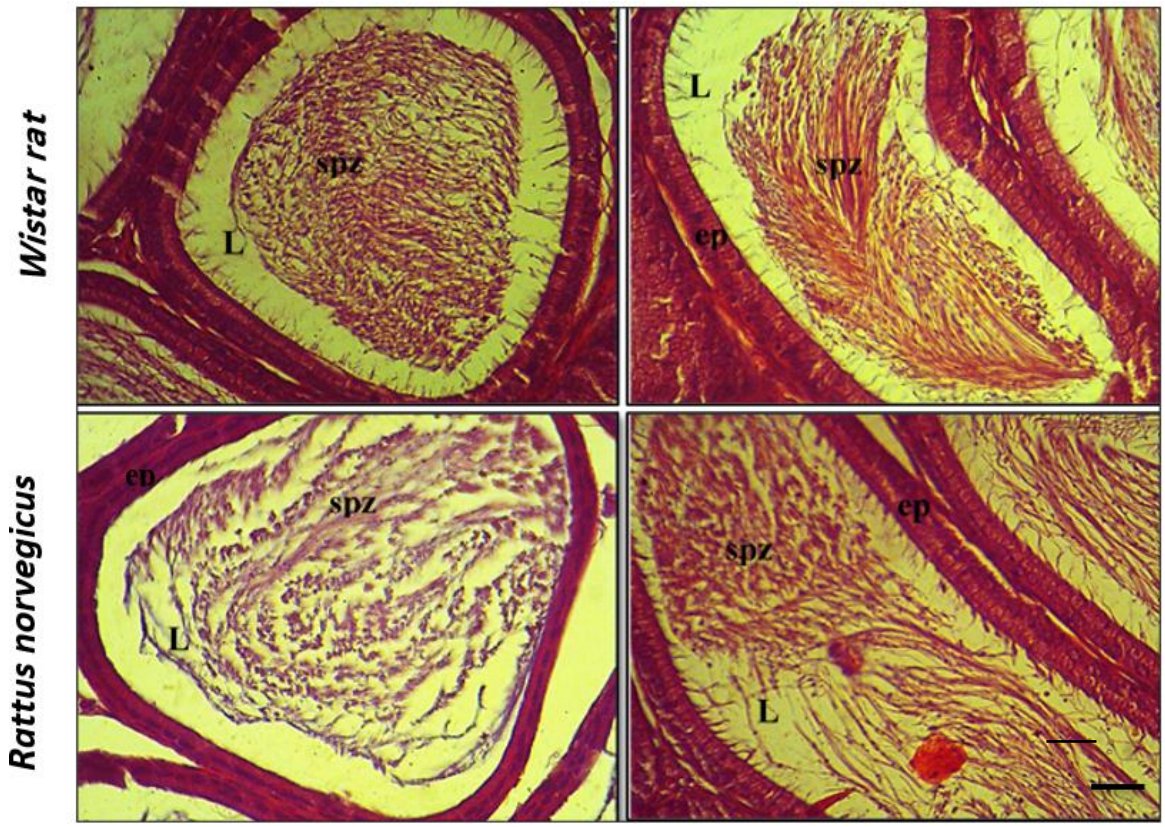

Fig. 6: Structural aspect of the epididymis in the two studied species presented through longitudinal sections (right) and transverse sections (left), observed at G×40. Scale bar: 50 µm. L: Lumen, ep: Epithelial cell, spz: Spermatozoa.

Morphometric results revealed a statistically significant difference between the two studied species, with a difference of 150% (p = 0.000000) for epithelial height, 70% (p = 0.000000) for nucleus height, and 191% (p = 0.000000) for supranuclear height.

Observation at High Magnification (Gx100):

The epididymis of the white rat is composed of a simple stratified epithelium, with the nuclei of the epithelial cells visible. The epithelial cells are tall with a well-developed supranuclear space, and the lumen is wide, containing spermatozoa. In the brown rat, the epithelial cells are tall but have a less developed supranuclear space, and the lumen also contains spermatozoa (Fig. 7).

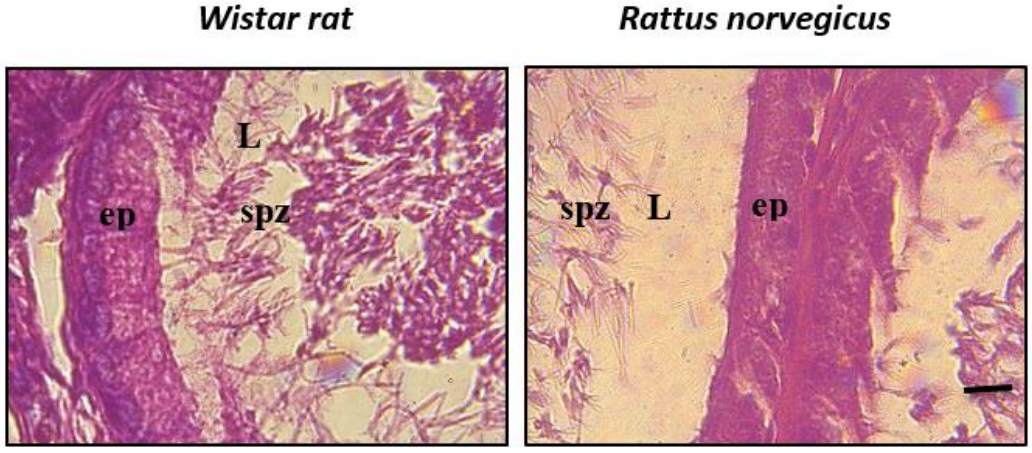

Fig. 7: Structural aspect of the epididymis in the two studied species, observed at G×100. Scale bar: 10 µm. spz: Spermatozoa, L: Lumen, ep: Epithelial cell.

Vas deferens:

Observation at Low Magnification (Gx10):

The serosa of the white rat is thin, but the muscular wall is very developed, with very reduced epithelial folds and the lumen is narrow, containing no spermatozoa. In the brown rat, the serosa covering the vas deferens is less dense, the muscular wall is developed, and the connective tissue of the chorion separating it from the epithelium is narrow. The epithelium is well-developed, the lumen is wide, and it contains spermatozoa (Fig. 8).

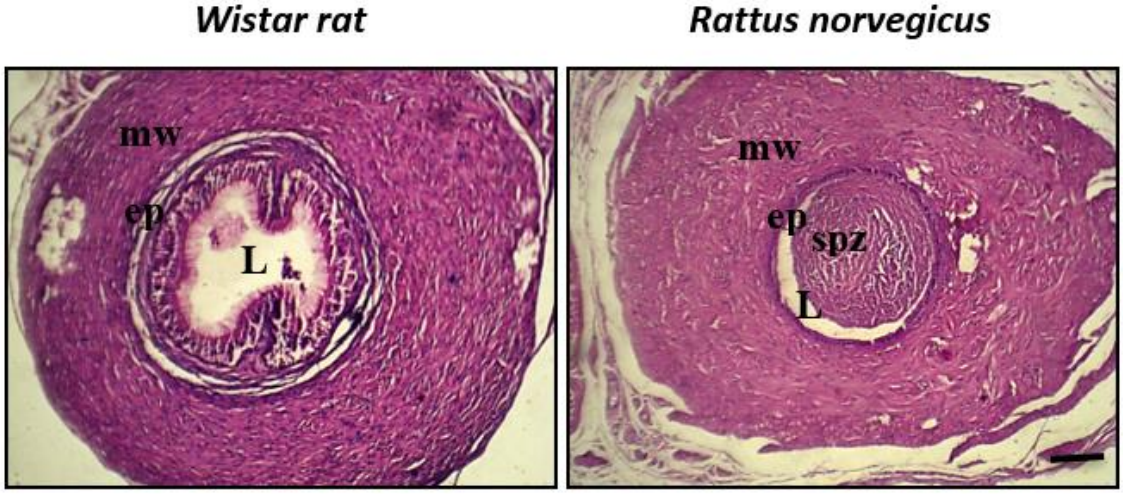

Fig. 8: Structural aspects of the vas deferens in the two studied species, observed at G×10. Scale bar: 100µm. L: Lumen, spz: Spermatozoa, ep: Epithelial cell, mw: Muscular wall.

Observation at Medium Magnification (Gx40):

In the white rat, the epithelial cells are tall with the presence of apical microvilli, while the apocrine secretion vesicles are not visible. The connective tissue of the chorion consists of closely packed bundles of connective fibers (Fig. 9). The morphometric study showed that the height of the vas deferens cells is 13.5 ± 0.3 µm; the height of the nucleus is 3.9 ± 0.2 µm; and the height of the supranucleus is 6.9 ± 0.3 µm.

In the brown rat, the epithelium is narrow and intersperses with the lumen, which is very wide and contains spermatozoa. The microvilli border the apical ends of the principal cells. The cells are tall and narrow, with an oval nucleus in a basal position (Fig. 9). The morphometric study showed that the height of the vas deferens cells is 57.9 ± 2.6 µm; the height of the nucleus is 10.3 ± 0.7 µm; and the height of the supranucleus is 38.7 ± 1.8 µm.

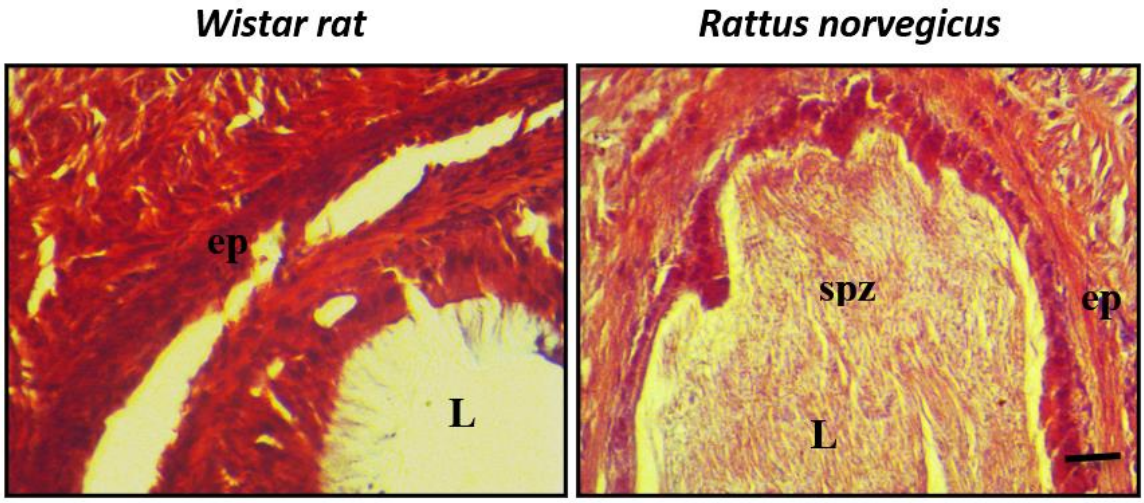

Fig. 9: Structural aspects of the vas deferens in the two studied species, observed at G×40. Scale bar: 50 µm. L: Lumen, ep: Epithelial cell, spz: Spermatozoa.

The morphometric results showed a statistically significant difference between the two studied species: -65% (p=0.000000) for epithelial height, -93% (p=0.000000) for nuclear height, and -30% (p=0.000086) for supranuclear height.

Seminal Vesicle:

Observation at Low Magnification (Gx10):

The seminal vesicles are lined with a fibro-muscular wall, and the connective tissue separating it from the epithelium consists of a few separate connective fibers. The epithelium develops epithelial folds that converge towards the center of a very wide lumen filled with secretion; the epithelium is pseudostratified cylindrical and composed of tall cells with oval-shaped nuclei in a basal position. The cytoplasm is eosinophilic and has a significant supranuclear region (Fig. 10). In the white rat, the fibro-muscular wall of the seminal vesicles is very developed and thick, with a wide and dense connective axis of the epithelial folds interrupting a very large lumen in which secretion is absent. The shape of the nuclei of the epithelial cells has become oval. In the brown rat, the fibro-muscular layer is less developed, and the epithelial folds are very long, extending into a very wide lumen filled with abundant secretion.

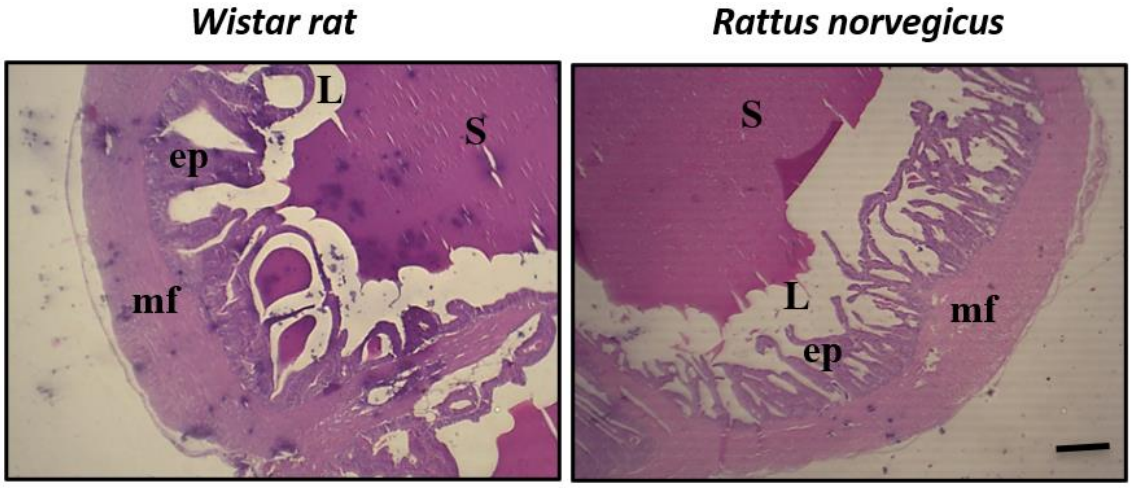

Fig. 10: Structural aspects of the seminal vesicle in the two studied species, observed at G×10. Scale bar: 100 µm. L: Lumen, ep: Epithelial cell, S: Secretion, mf: Muscle fiber.

Observation at Medium Magnification (Gx40):

In the white rat, the volume of the epithelial cells is significant; they are very closely packed and exhibit reduced height and a greatly diminished supranuclear area. The fibro-muscular wall of the seminal vesicles is developed (Fig. 11). The morphometric study showed that the height of the seminal vesicle cells is 11 ± 0.3 µm, the height of the nucleus is 4.3 ± 0.2 µm, and the height of the supranucleus is 5.2 ± 0.2 µm.In the brown rat, the epithelial cells are larger; they are also very closely packed and show significant height and a high supranuclear area. The fibro-muscular wall of the seminal vesicles is developed (Fig. 11). The morphometric study indicated that the height of the seminal vesicle cells is 15.6 ± 0.4 µm, the height of the nucleus is 5.1 ± 0.2 µm, and the height of the supranucleus is 8.4 ± 0.4 µm.

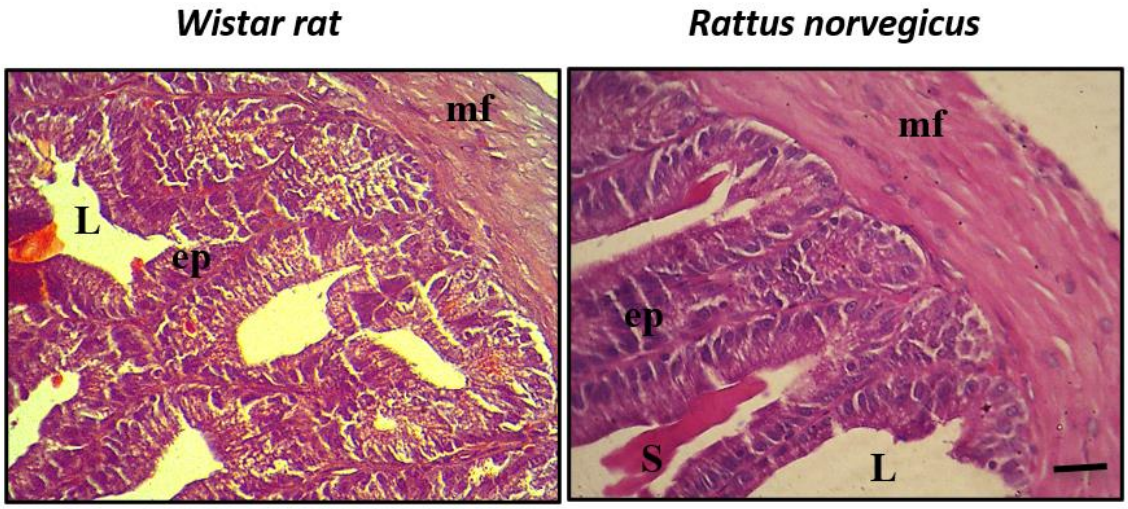

Fig. 11: Structural aspects of the seminal vesicle in the two studied species, observed at G×40. Scale bar: 50 µm. L: Lumen, ep: Epithelial cell, S: Secretion, mf: Muscle fiber.

The morphometric results demonstrated a statistically significant difference between the two studied species: -29% (p=0.000000) for epithelial height, -17% (p=0.000158) for nuclear height, and -38% (p=0.000000) for supranuclear height.

DISCUSSION

Our study was conducted on the male reproductive system of the white rat and the brown rat, aiming to compare them histologically and morphometrically. In comparing the testes of the two studied species, we found that the testes of both the white rat and the brown rat exhibit the same structural appearance, with stratified seminiferous epithelium and several germ cells at different stages of spermatogenesis. These results are similar to the findings from the comparison of *Gerbillus tarabuli* (a wild species) and *Mus musculus* (a laboratory strain) conducted by Brahim Djeffal and Ben Moussa (2018), which found that the histological structure of the testes presents the same general aspect in both *Gerbillus tarabuli* and *Mus musculus*.

According to Beaumont and Cassier (1998) and Welsch (2002), small-sized spermatogonia are easily found near the basement membrane; larger spermatocytes I and II have voluminous nuclei, while smaller spermatids are located towards the interior of the tubules. Mature spermatozoa are located in the lumen of the seminiferous tubules, characterized by their flagella. This organization has been detected in both of our species. We observed that there are five cellular layers in the wall of the seminiferous tubules: spermatogonia,

spermatocyte I, spermatocyte II, spermatids, and spermatozoa. These results have been previously observed in Meriones (*Meriones lybicus* and *Meriones crassus*) and in the sand rat (*Psammomy obesus*) (Belhoucine *and al.*, 1996 a and b; Menad *and al.*, 2017).
Structural analyses of the sections show the same histological appearance of the epididymides in both the white rat and the brown rat. The epididymis contains principal cells, apical-nucleus cells, clear cells, basal cells, and an epithelial lining. This cellular composition has been reported in many mammals (Hamilton, 1975; Goyal, 1985; Amann, 1987; Robaire and Hermo, 1988). Morphometric observations of the epididymis have shown that the epithelial cells are taller in the white rat compared to the brown rat, insert into a folded basement membrane, and present a larger supranuclear space with a larger nucleus. These differences may be due to seasonal variations that lead to structural modifications of the epididymis, as indicated in the works of Menad *and al.* (2017; 2014), which mention that during the breeding season, they observed a strong expression of all the epithelial cells.

The hematoxylin-eosin staining of the vas deferens allowed us to observe the nuclei of the epithelial cells, which appear blue-violet and round, with a very clear lumen at the center. Similar to the two previously described organs, there is a structural similarity between the vas deferens of the two species. A similar result was observed in the sand rat (Gernigon-Spychalowicz *and al.*, 1994; Gernigon-Spychalowicz, 1995) and in gerbils (Belhocine *and al.*, 1996 a, b). This is a duct with a muscular wall formed by an outer longitudinal layer and an inner circular layer, and a pseudostratified epithelium made up of tall, narrow cells equipped with apical microvilli, resembling the principal cells of the epididymis. Morphometrically, in the brown rat, the epithelial cells are narrower compared to the white rat, possessing an oval nucleus in a basal position with an elongated supranuclear space. These results are similar to those of Beu *and al.* (2009) and Serre *and al.* (1998) regarding the shape of the epithelium in the brown rat and hamster, where they demonstrated species-specific differences.
The morphology of the seminal vesicles varies between species. In humans, horses, and rats, they are sac-shaped, while in pigs and bulls, they are compact and multilobulated (Badia *and al.*, 2006). Some rodents exhibit a branched tubular structure (Mollineau *and al.*, 2009). In Saharan gerbils, the seminal vesicles are paired and tortuous, as observed in all rodents. The same observations made for the Saharan gerbil (*Gerbillus tarabuli*) have been noted in the small gerbil (*Gerbillus gerbillus*), the sand rat (*Psammomys obesus*) (Gernigon-Spychalowicz *and al.*, 1994; Gernigon-Spychalowicz, 1995), and the meriones (*Meriones crassus* and *Meriones libycus*) (Belhocine, 1998 and 2008). Our histomorphometric analysis shows that the height of the epithelial cells in the seminal vesicle differs between the two species; the white rat exhibits a reduced supranuclear zone, and the fibro-muscular wall of the seminal vesicles is more developed, with the nuclei of the epithelial cells being oval in shape. These differences between the two species may be attributed to variations in their biotope, as previously reported in other species (Belhocine, 1998; Gernigon-Spychalowicz *and al.*, 1994; Gernigon-Spychalowicz, 1995; Schindelmeiser *and al.*, 1988).

CONCLUSION

At the conclusion of this work, which involved a comparative histomorphometric study of the male reproductive system of the white Wistar rat and the brown rat (*Rattus norvegicus*), we were able to demonstrate that the structure of the testes, as revealed by histological images, appears to present the same constituent elements among most closely related rodents taxonomically. Our results describe a seminiferous epithelium in both species

that is stratified and contains several germ cells at different stages of development, which are similar in both animals. However, the diameter of the seminiferous tubules and the width of their lumens are larger in the brown rat. Despite these morphometric differences, the histological structure of the epididymis is quite comparable between the white rat and the brown rat. It is important to note that the morphology of the seminal vesicles varies among species.

Declarations:

Ethical Consideration: The Helwan University Institutional Animal Care and Use Committee for Laboratory Animals (HU-IACUC/Z/SR0604-43) gave its approval to the experiment. The Zoology Department at the Faculty of Science at Helwan University has received permission.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author contribution: Each author took part in the design of the study, contributed to data collection, and participated in writing the manuscript. The manuscript is neither being published nor being considered for publication elsewhere until a decision is reached by this journal.

Data availability statement: The collection of data developed and/or assessed throughout the present work is available through the corresponding author upon reasonable request.

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