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Anatomy and Histology of the Male Reproductive Tract and Spermatogenesis Fine Structure in the Lesser Anteater (*Tamandua tetradactyla*, Myrmecophagidae, Xenarthra): Morphological Evidences of Reproductive Functions

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With 5 figures and 1 table

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Summary

The anatomy and histology of the male genital tract of the lesser anteater were studied. Fine details of spermatozoa regarding their genesis and morphology were also studied in six adult specimens. The testes lie in the pelvic cavity. The deferent duct emerges from the epididymis and opens into the ejaculatory duct, which drains into the membranous urethra. Accessory glands (prostate, seminal vesicle and bulbourethral gland) are histologically similar to those described in other mammals. The short penis presents an urethral orifice, while the corpus spongiosum becomes thinner at the end indicating the absence of a histologically defined glans. The seminiferous epithelium shows: (1) Sertoli cells with deep nuclear indentations, (2) spermatogonia with crusty-like chromatin, (3) spermatocytes at different stages of maturation and (4) three morphologically distinct stages of spermatid differentiation according to nuclear shape, acrosome development and chromatin condensation. Sperm heads appear oval. The length of the spermatozoa averages 67.33 ± 1.60 μm. Two specimens with inactive spermatogenesis were azoospermic. Their testes and epididymis presented sizes smaller than those with active spermatogenesis. These studies together with others in anteaters may contribute to successful breeding in conservation programmes.

Introduction

Xenarthra's reproductive tract exhibits several peculiar features. Among them, the occurrence of internal testes (Kaudern, 1914; Grassé, 1955) and the extraordinary shape of the spermatozoa's head in males (Cetica et al., 1998) stand out. The presence of polyovular follicles and urogenital sinus in females (Cetica et al., 2005) is also another unusual characteristic. There are several methodologies for assisted reproduction (e.g. means of sperm preservation or the detection of the stage of the oestrous cycle by vaginal cytology) that would be interesting to apply to these species.

The lesser anteater (*Tamandua tetradactyla*), one species of Xenarthra, belongs to the family of the anteaters (Mymercophagidae). This family constitutes an important group for conservation, because some species, as the giant anteater (*Myrmecophaga tridactyla*), present a 'vulnerable' status according to the International Union for Conservation of Nature (IUCN; Superina et al., 2010).

Although the first anatomical studies of the male genital system in *T. tetradactyla* were carried out about 100 years

ago (Kaudern, 1914; Grassé, 1955), histological data are still scanty. Some important issues like the histological details of the glands and ducts remain unknown. Transverse sections of the penis were illustrated by Kaudern (1914); however, the arrangement of the erectile tissues remains unclear. No work has been focused on the penile functional morphology, and nothing is known about the roles and behaviour of erectile tissues.

Even though species with internal testes are unusual among mammals, no studies about testes have been carried out in the lesser anteater yet. Furthermore, nothing is known about the fine structure of the cells of the seminiferous epithelium.

In spite of the continuous spermatogenesis suggested for the giant anteater (Bartmann et al., 1991), rest and rut periods were reported by Grassé (1955) for the lesser anteater. This indicates that further studies are required. For other xenarthrans, like the common long-nosed armadillo (*Dasypus novemcinctus*), testicular cycles with changes in size, either with continuous spermatogenesis along the year (Mc Cusker, 1985) or with seasonal spermatogenesis (Torres et al., 1983), have been demonstrated. Finally, there is a fragmentary knowledge about spermatology (i.e. cell shape and size) in this family. Only the morphology and morphometry of the male gametes were commented for the lesser anteater (Hay et al., 1994), and no illustrations were provided.

Characterizing the morphology of the reproductive tract and the male gamete is critical to develop and optimize techniques for assisted reproduction. With this scope, we studied the anatomical and histological characteristics of the male reproductive tract of the lesser anteater (fine structure of the seminiferous epithelium together with some morphological sperm features). This information would contribute to protocols for applying reproductive biotechnology to diverse programmes related to its conservation.

Material and Methods

Taking into account the vulnerable status of the species, a main difficulty of this survey was to obtain well-preserved samples to accomplish fine morphological studies. Such conditions were met by four dead *T. tetradactyla* supplied by Argentinian zoos (see Table 1): Roque Saenz Peña Zoo, Province of Chaco (male 1); Mendoza Zoo, Province of Mendoza (male 2); Rosario Zoo, Province of Santa Fe (male 3); and Florencio Varela Zoo, Province of Buenos Aires (male 4).

Reproductive tracts were dissected and submitted to macroscopic observation, identification and the pertinent measurements. Tracts were preserved by immersion in 4% formaldehyde. Testicular volumes were calculated using the Hansen formula (Hansen and With, 1952) [V (volume) = L (length) × W^2 (width) × 0.52). After dissection, the tracts were marked serially for identification of corresponding histological sections (Fig. 1c). Samples from the different regions were dehydrated and embedded in paraffin. Microtome sections of 5-μm thickness were stained with haematoxylin-eosin (H-E), Crossman trichrome or periodic-acid-Schiff (PAS) reaction for identification of glycoconjugates containing hydroxyl groups. Microscopic details were observed and digitalized with a Leica optic microscope connected to a Leica camera DFC300 (Leica Microsystem, Wetzlar, Germany). Images were taken using Leica Application Suite, version 3.6.0, Leica Microsystem.

For fine structure studies, transmission electron microscopy (TEM) was carried out. A small piece of testicular parenchyma was excised and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 6.9) at room temperature for 2 h and post-fixed in 0.1% OsO_4. The samples were embedded in Maraglas, and serial sections were picked up in single-hole grids and stained with uranyl acetate and lead citrate. Electron micrographs were obtained with a Zeiss EM–109T electron microscope (Carl Zeiss Inc., Oberkochen, Germany).

For sperm morphology studies, samples were obtained from the cauda epididymis of males 3 and 4. The epididymis was removed and then minced in phosphate-buffered saline medium; the suspension of spermatozoa was centrifuged for 10 min at 600 rpm, the supernatant was discarded, and a small amount of the suspension was placed on a slide. The linear dimensions of the length and width of the head, tail length, midpiece length and total sperm length of 150 spermatozoa were measured from photographs obtained by the silver nitrate staining method (Cetica et al., 1993). An estimate of the chromatin volume was obtained multiplying the average nuclear thickness calculated in 30 different points (acrosomal, equatorial segment and post-acrosomal region), by the average area of the sperm head measured in micrographs of five spermatozoa arising from sperm fixed, embedded and observed by TEM as previously described (Cetica et al., 1993, 1997). Area measurements were made from light microscopy photographs using the Adobe Photoshop CS3 software (Adobe Systems Inc., San Jose, CA, USA).

In addition, external genitalia of two live animals from Bioparque Temaikén (males 5 and 6, see Table 1), Province of Buenos Aires, were photographed for morphological descriptions. Furthermore, these animals were scanned using a Medison SA 600 ultrasound scanner (7.5 MHz convex; Samsung Medison Co., Seoul, South Korea) for internal morphological traits.

According to the configuration of the seminiferous epithelium, all animals were classified as sexually mature

Table 1. Morphometry of the genital tract of the studied specimens of *Tamandua tetradactyla*

			Testes				Epididymis	Penis	
Male no.	Weight (kg)	Season of the study	Lenght (cm)	Width (cm)	Depth (cm)	Volume (cm^3)	Diameter (cm^3)	length (cm^3)	Spermatozoa
1	4.1	Summer (12 February 1999)[a]	2.0	1.0	0.8	1.0	0.4	2.0	No
2	4.0	Winter (1 August 1999)[a]	2.0	1.3	0.8	1.8	0.4	2.6	No
3	5.2	Winter (21/07/1999)[a]	3.5	2.0	1.5	7.3	0.6	NA	Yes
4	4.0	Autumn (4 April 2009)[a]	2.7	1.8	1.3	4.5	0.6	3.6	Yes
5	4.5	Summer (18 February 2010)	3.1	1.9	NA	5.8	NA	3.3	NA
6	4.5	Summer (18 February 2010)	3.6	2.4	NA	10.8	NA	3.5	NA

NA, not available.
Testes and epididymes dimensions are expressed as media of the left and right testis and epididymus.
[a]Date of dead.

(i.e. presence of Sertoli cells, spermatogonia, spermatocytes and in some cases spermatids). Additionally, the specimens' weight corresponded to adults (Wetzel, 1985).

Results

Anatomy and histology of the genital tract in *Tamandua tetradactyla*

The general anatomy of the male genital tract of the lesser anteater is shown in Fig. 1a–c. The testes are found in the pelvic cavity, joined medially to each other by a thin layer of visceral peritoneum, between the bladder and the rectum (Fig. 1a). The kidneys are located next to the testes and cranially to them. Renal veins were observed coming off the caudal vena cava at an acute angle, denoting the caudal position of the kidneys in this species (Fig. 1b, see inset).

Testes are ovoid structures, elongated in a cephalocaudal direction. Both are approximately of the same size (Fig. 1a-c). They are lined by a well-developed layer of connective tissue, corresponding to the tunica albuginea. The rete testis constitutes a system of interconnected channels extending from the mediastinum to the tunica albuginea. From the seminiferous tubules to the rete testis, there is a transitional epithelium extended from the typical testicular stratified germinal type (Fig. 2a) to a single layer of cuboidal cell type. Efferent ducts, which lead from the rete testis to the epididymis, emerge from the testicular cranial pole becoming highly convoluted before opening into the epididymis.

The epididymides are demarcated into a convex caput that covers the upper pole of the testes, connected by a corpus to a caudal prominence which projects from the caudal border of the testes (Fig. 1c). They are positioned on the internal side of

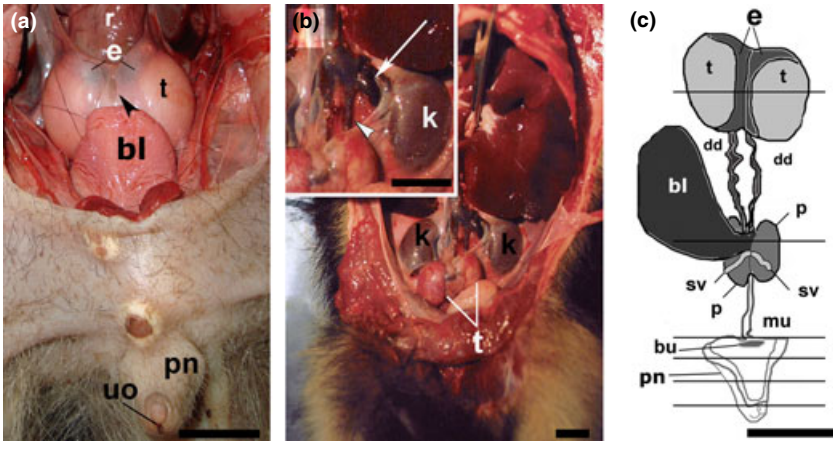

Fig. 1. Anatomical gross features of the male genital tract of *Tamandua tetradactyla* (ventral view). (a) Significant gross features of the genital tract, arrowhead marks the visceral peritoneum that connects both testes. (b) Position of the testes in the pelvic cavity. Inset: detail illustrating the left renal vein (arrow); arrowhead marks the left testicular vein. (c) Schematic drawing of the genital tract showing the sites where histological sections were made. Scale bars: 2 cm. Abbreviations: r, rectum; bl, bladder; k, kidney; t, testis; e, epididymis; dd, duct deference; p, prostate; sv, seminal vesicle; bu, bulbourethral glands; pn, penis; uo, urethral orifice; mu, membranous urethra.

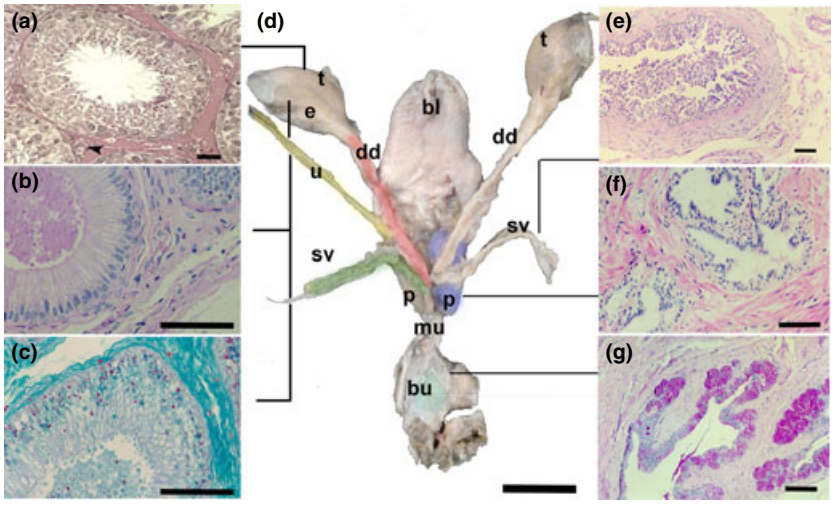

Fig. 2. Anatomy and histology of the testis (a, d), epididymis (b–d) and accessory sex glands (d–f) of *Tamandua tetradactyla*. (a) Cross section of the testis showing seminiferous tubules, with spermatids lying around the lumen ready to be released and interstitium (arrowhead marks Leydig cells). H-E staining. (b) Cross section of the epididymis showing PAS-positive granules in the lumen. PAS staining. (c) Epididymis with active secretion. Crossman trichrome staining. (d) Dissected genital tract denoting glands (dorsal view). (e) Seminal vesicle. H-E staining. (f) Prostate. H-E staining. (g) Bulbourethral gland showing PAS-positive cell mucus secretion. PAS staining. Scale bars: 20 μm (a), 100 μm (b, c), 3 cm (d), 50 μm (e–g). Abbreviations: t, testis; e, epididymis; dd, duct deference; u, ureter; bl, bladder; p, prostate gland; sv, seminal vesicles; bu, bulbourethral glands; mu, membranous urethra.

the testes. As a consequence, epididymides remain in contact with each other (Fig. 1a,c). The epididymal duct of the caput is lined by a pseudostratified epithelium with tall columnar and basal epithelial cells surrounded by a layer of smooth muscle cells (Fig. 2b,c). The lumen increases in diameter through the corpus, while the epithelium decreases in height. The ducts of the caudal region are greatly distended, lined by a low epithelium with flattened nuclei.

The deferent ducts are long (4.25–4.49 cm in length), convoluted, star shaped in cross section, lined by a columnar pseudostratified epithelium and emerge from the cauda epididymis (Fig. 1c). The deferent ducts have a typical lamina propria and are surrounded by two thin layers of smooth muscle and a fibroelastic adventitia. The ductus deferens open into the ejaculatory ducts, which drain into the membranous urethra (Fig. 2d), at the *colliculus seminalis*.

The urethra is lined by a polymorphic epithelium (Fig. 3g–i). It is divided into two segments: the membranous urethra (2.8 cm in length), which leads from the bladder to the base of the penis, and the star-shaped penile urethra (4.6 cm in length; Fig. 3g–i), which runs along the length of the penis on its ventral surface.

The seminal vesicles (1.8–2.3 cm in length) are elongated, thin and coiled glands located dorsally to the prostate gland (Fig. 2d). These glands are composed of alveoli lined by cuboidal or columnar epithelial cells (Fig. 2e). The lumen is filled with secretory droplets that are mainly acidophilic. The alveoli drain into the main collecting ducts, which open into the ejaculatory duct (Fig 2d) caudally to the openings of the ductus deferens.

The prostate gland (2.4–2.8 × 1.0–1.4 × 0.4–0.7 cm) consists of a bilobed gland situated dorsally to the membranous urethra (Fig. 2d). It is composed of alveoli that are lined with cuboidal to columnar epithelial cells (Fig. 2f). The alveoli drain into a main collecting duct that runs through the urethral tissue for a short distance and then joins the duct on the opposite side to form a common duct that ends into the membranous urethra.

The bulbourethral or Cowper glands are a pair of flattened ovoid bodies (0.9–1.2 × 0.7–0.8, 0.5–0.6 cm) situated on the ventral surface of the penile urethra (Fig. 2d). The internal surface is divided by broad connective tissue partitions into numerous small alveoli. The epithelial lining is composed of simple cuboidal cells with nuclei characterized by a PAS-positive secretory product near the apical surface (Fig. 2g). The glandular ducts arise from the medial surface of the glands into the penile urethra ventrally on each side of the midline. Each gland is smoothly encapsulated by connective tissue and striated fibres of the bulbocavernosus muscle.

The penis is directed backwards, located ventrally and very close to the anus without a noticeable perineum (Fig. 3a–d). In the specimens studied, its length and diameter were approximately 2.0–3.6 and 1.6–2.0 cm, respectively (Table 1, Fig. 3a–c). The organ is conically

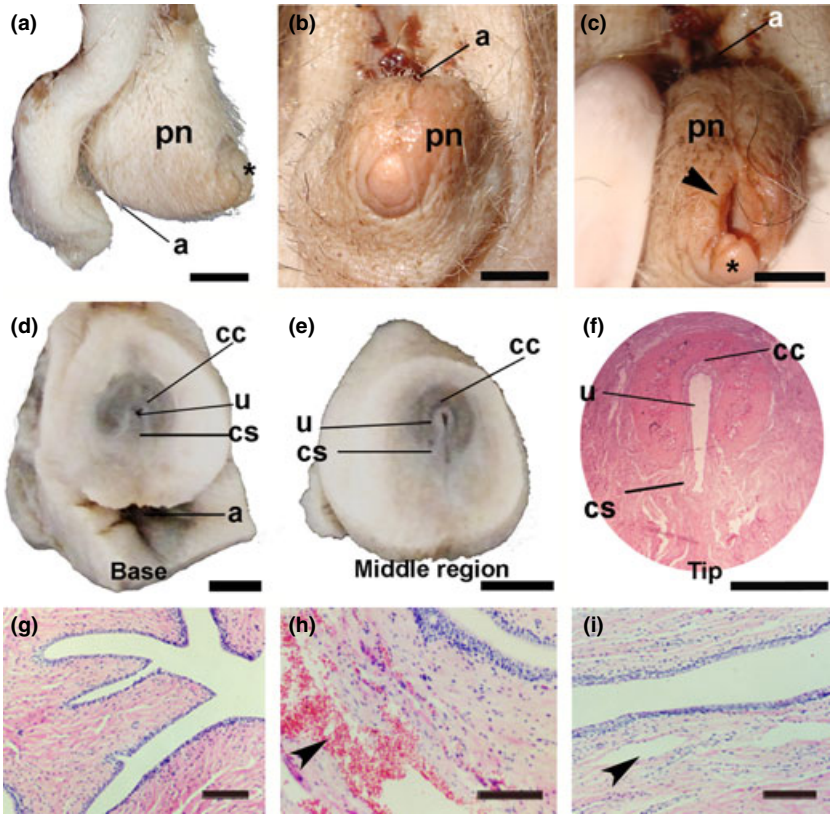

Fig. 3. External genitalia and penis histology of *Tamandua tetradactyla*. (a) Penis in lateral view. (b) Penis in front view showing tip (*). (c) Penis in front view showing urethral orifice (arrowhead). (d, e) Transverse sections of the penis at the base (d) and middle portion (e). (f) Histological transverse section at the penis tip. H-E staining. (g) Penile urethra showing star-shaped lumen. H-E staining. (h) Proximal section of the penis showing cavernous sinus (arrowhead). H-E staining. I. Distal section of the penis showing small cavernous sinus (arrowhead). H-E staining. Scale bars 1 cm (a–f), 100 μm (g–i). Abbreviations: pn, penis; a, anus; cc, corpus cavernosum; cs, corpus spongiosum; u, urethra.

shaped with a central fold (0.8 cm in length) that ends in the external urethral orifice next to the summit (Fig. 3c). In histological cross sections, the bulk of the penis was formed by dense connective tissue. The erectile tissues (corpus cavernosum and spongiosum) were centrally located at the base of the penis and more dorsally located in the middle portion. The corpus cavernosum is horseshoe shaped and surrounds the small corpus spongiosum except at penile ventral part (a general view of the penile structures is shown in Fig. 3d–f). The tunica albuginea that surrounds the corpus cavernosum is continuous, with no clear limit, with the dense connective tissue of the bulk of the penis. The corpus spongiosum surrounds the urethra and is present along the penile length, becoming thinner and weaker at the upper body (Fig. 3d–i). As a consequence, its distal end is not expanded to form a true glans penis. No skin receptacle or prepuce was observed. The erectile tissues are constituted of caverns lined by endothelium and surrounded by dense irregular connective tissue with very few smooth muscle cells (Fig. 3f–i).

Testis histology and spermatogenesis fine structure

The testicular parenchyma is formed by the seminiferous tubules and the interstitium (with Leydig and stromal cells; Fig. 2a). The seminiferous epithelium is composed by Sertoli cells and four or five concentric layers of germ cells (Fig. 4a). Unexpectedly, periodic indentations of the basal membrane inside the seminiferous tubules were observed. Sertoli cells show an ovoid nucleus, mostly close to the basal membrane, with indentations that extend into the nucleoplasm deep down the long axis (Fig. 4b). The chromatin of this cells is dispersed within the nucleus and presents small and flat heterochromatin 'clumps' attached to the nuclear envelope (Fig. 4b,c); the nucleolar complex is not apparent, a fact that was also reported in other mammalian species. In these cells, lipid droplets can be observed in the cytoplasm (Fig. 4b,c). Next to the Sertoli cells, B-like spermatogonia containing crusty-like chromatin are lying over the basal membrane. Their round nucleus is characterized by dark chromatin clumps mostly attached to the nuclear envelope (Fig. 4c). Spermatocytes at different stages of maturation

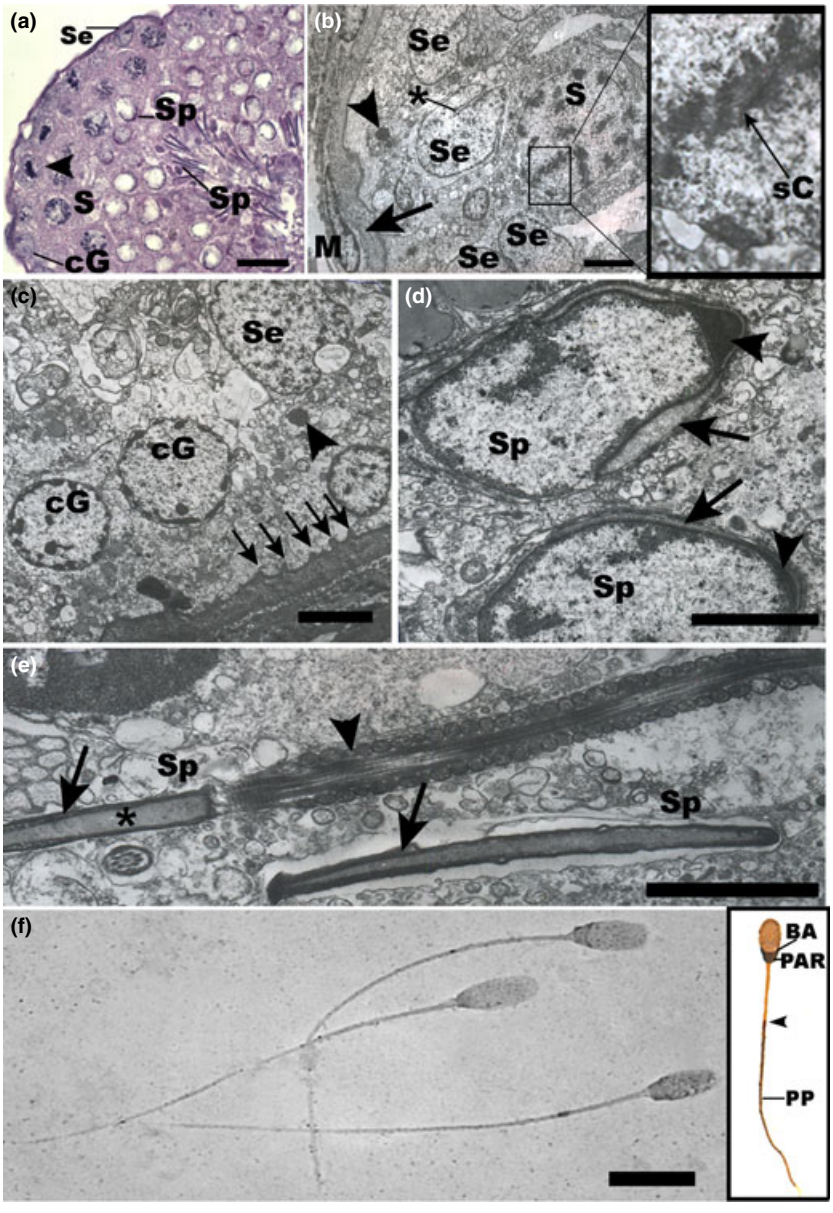

Fig. 4. Histology (a) and fine structure (b–e) of the seminiferous epithelium and spermatozoa's morphology (f) of *Tamandua tetradactyla*. (a) Seminiferous tubule in transverse section, arrowhead marks spermatogonial mitosis. PAS staining. (b) Sertoli cells with nuclear indentations (*) and lipid droplets (arrowhead), spermatocyte (inset shows the synaptonemal complexes) and myoepithelial cell at the basal membrane (arrow). (c) Sertoli cell with lipid droplets (arrowhead) and crusty spermatogonia cells. Arrow marks basal membrane indentations. (d) Spermatids with oval-shaped nucleus and proacrosomic dense material (arrowhead) extending laterally, acrosomic sac (arrow). (e) Elongated spermatids with nucleus capped by a well-developed acrosome (arrow), homogeneous chromatin (*) and midpiece with noticeable mitochondria (arrowhead). (f) Left, spermatozoa in phase contrast; right, spermatozoa stained with silver nitrate. Arrowhead marks end of middle piece. Scale bars: 10 μm (a); 3 μm (b–e); 10 μm (f). Abbreviations: Se, sertoli cell; S, spermatocyte; sC, synaptonemal complexes; M, myoepithelial cell; cG, crusty spermatogonia; Sp, spermatids; AB, acrosome boundary; PAR, post-acrosomal region; PP, principal piece.

can be recognized, and the pachytene stage could be clearly identified because well-defined synaptonemal complexes were observed (Fig. 4b, see inset). In the adluminal compartment, round spermatids are found with a single large granule nearby the nucleus signalling the beginning of the Golgi phase of the acrosome development. Round spermatids show little clumps of heterochromatin all over the nucleus (data not shown). In other seminiferous tubules, spermatids with oval-shaped nucleus were observed, with proacrosomic dense material extending laterally and cover-

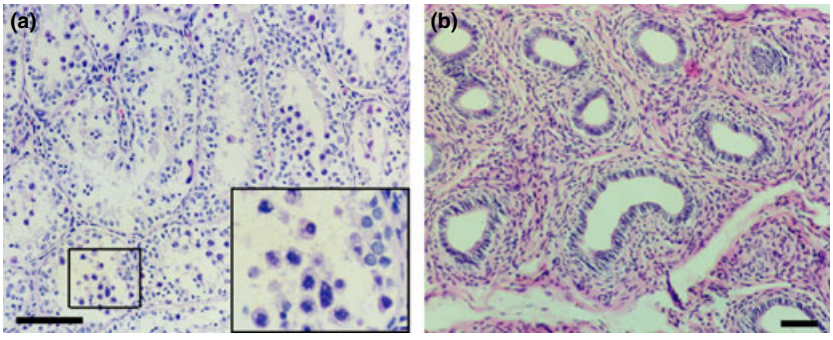

Fig. 5. Testis (a) and epididymis (b) of an inactive individual (male 1). (a) Cross section of an inactive testis showing partial degeneration of seminiferous tubules. Inset shows free cells in lumen with apoptotic figures. H-E staining. (b) Cross section of the epididymis of an individual with inactive gonads showing no spermatozoa in the lumen. H-E staining. Scale bars 100 μm.

ing a small nuclear region. This is the beginning of the cap phase of spermatids (Fig. 4d). In some tubules, a second layer of spermatids is observed, showing an elongated nucleus capped with a well-developed acrosome (Fig. 4e). The chromatin is homogeneous and grey stained, indicating an advanced stage of maturation (Fig. 4e).

Sperm morphology

The sperm heads are oval shaped, with a central tail insertion (Fig. 4f). The mean and standard deviations for the linear dimensions obtained for the length and width of the sperm heads were 9.50 ± 0.66 and 4.65 ± 0.34 μm, respectively (Fig. 4f). The acrosome covers a large portion of the head, accounting for 70.3% of the total head length (Fig. 4f). The tail length was 59.18 ± 1.78 μm, and midpiece length was 14.67 ± 0.76 μm (Fig. 4f). The average of the total length was 67.33 ± 1.60 μm (Fig. 4f). The TEM study showed that the nuclear thickness of the sperm head averaged 0.23 ± 0.07 μm and increased from the tip to the base. The sperm nuclear volume was estimated to be 9.98 ± 0.63 μm^3.

Morphological variations of the genital tract between individuals

Males 1 and 2 presented no spermatozoa in the epididymis and showed a disorganized seminiferous epithelium, with abundant apoptotic figures and presence of free cells in the lumen (Fig. 5a), which lacked late spermatogenic cell types (i.e. round and elongated spermatids). Testes of these specimens were classified as inactive, and testicular volumes were lesser respect to males 3, 4, 5 and 6 (Table 1). Finally, epididymal diameters in males 1 and 2 were lesser compared to males 3 and 4 (Table 1). The epididymis' histology showed lower lumen diameter in the tubules and rich intertubular connective tissue in males 1 and 2 (Fig. 5b) with respect to males 3 and 4.

Discussion

In this article, we describe for the first time, the histology of the male reproductive tract and the fine structure of the spermatogenesis in the lesser anteater (*T. tetradactyla)*. Our histomorphological studies allowed a correct recognition of the accessory glands and a detailed description of the penile structure in this species. The spermatozoon was illustrated. Additionally, while we were assessing the reproductive condition, reproductively inactive individuals were found.

Testes in primitive mammals (except for marsupials) are placed in the abdominal cavity next to the kidneys (condition of testicondia; Werdelin and Nilsonne, 1999). As illustrated in the present study (Fig. 1a,b), testes in the lesser anteater, even when they are internal, are found more caudally into the pelvic cavity. This condition, different from testicondia, was reported for other groups of xenarthrans like armadillos and sloths (Grassé, 1955). Joined testes, which were found in the lesser anteater, are also present in the giant anteater (Bartmann et al., 1991) and the sloth *Bradypus torquatus* (Dos Santos Martins, 2003). However, in the latter, testes are asymmetrically disposed (one positioned more cephalical than the other; Dos Santos Martins, 2003). On the other hand, testes in armadillos are not joined one to another (Grassé, 1955), and further studies are needed to see whether joined testes is a shared feature of all species of anteaters and sloths (order Pilosa).

Our anatomical observations of the male accessory sexual glands are in agreement with previous reports for this species (Kaudern, 1914; Grassé, 1955). As described in the giant anteater (Bartmann et al., 1991) and sloths (Dos Santos Martins, 2003), the presence of a *colliculus seminalis* was observed in this species. The histological characterization of the male accessory sexual glands (prostate, seminal vesicle and bulbourethral glands) in the lesser anteater showed no important differences from those of other mammals (Banks, 1986).

The short and conical-shaped copulatory organ of the lesser anteater is comparable to that found in the giant anteater (Bartmann et al., 1991) and sloths (Dos Santos Martins, 2003). Because of the shortness of the penis in this species, copulation could be explained by a shallow penetration. This is in agreement with the proposal of Bartmann et al. (1991) for the giant anteater. Additionally, as previously proposed, the hymen in females of the lesser anteater might not be perforated during copulation (Rossi et al., 2011).

By our histological analyses, some clues of the erection and intromission mechanisms could be ascribed: (1) the arrangement of erectile structures of the penis (i.e. the corpus cavernosum and the corpus spongiosum) vary depending on the species: in the lesser anteater, the corpus cavernosum forms a unit, which is largest proximally and extends laterally covering partially the ventrally placed urethra, while the corpus spongiosum is poorly developed. The stretched disposition of the corpus cavernosum and the urethra suggests a protection of the latter and the maintenance of the lumen aperture during intromission in this species. (2) Smooth muscle elements are not well developed in the lesser anteater. At a large number of species, the septum that circumscribes the areolas of the corpus cavernosum contains smooth muscle elements (Stanley and Hillemann, 1960; Cook, 1965; Nickel et al., 1981; Banks, 1986; Wrobel and Bergmann, 2006). The penile erectile tissue, specifically the cavernous smooth muscle cells, plays a key role in the erectile process (Dean and Lue, 2005). Because corporeal smooth muscle cells control the vascular event leading to erection, the presence of few smooth muscle cells in the erectile tissues of the lesser anteater can be expected to affect a good erectile response. (3) Additionally, according to Wrobel and Bergmann (2006), based on the predominance of erectile tissue over the connective tissue and *vice versa*, penises can be classified into three types, namely the vascular type, where the caverns predominate; the fibroelastic type, where the connective tissue prevails; and the intermediate type, which is in-between the previous two types. The penis of the lesser anteater was suitably considered in 'fibroelastic type penis'. In the cross sections, it was observed that dense connective tissue occupies most of the penis. This great development of dense connective tissue also limits the erectile process. However, as the exact length of the penis during the erectile process can only be demonstrated in live animals, more studies are yet to be performed to clarify the strategy for transferring gametes from one individual to the other in this and the in rest of anteaters species.

In contrast with the anatomical observations made by Kaudern (1914), who described a tiny glans for the lesser anteater, no defined glans was observed in our histological study. Also, for the giant anteater, Bartmann et al. (1991) described the pigmented tip of the penis as a glans by anatomical studies. Among xenarthrans, a true histological glans has been described only for the large hairy armadillo *Chaetophractus villosus* (Affanni et al., 2001). A preputial opening in the site of the urethra ending has also been described in the giant anteater (Bartmann et al., 1991). As our present observations indicate the absence of a glans, and consequently the absence of a true prepuce, we consider the term urethral opening more adequate (instead of preputial opening) for the lesser anteater.

Testis histology and spermatogenesis fine structure

The main ultrastructural aspects of the cells of the seminiferous epithelium were described in this work, this information constitutes the basis to understand the process of spermatogenesis of the lesser anteater. In Xenarthra, the fine structure of the cells of the seminiferous epithelium has been reported only for the common long-nosed armadillo *Dasypus novemcintus* (Nagy and Edmonds, 1973; Weaker, 1977). The spermatogonia presently found were similar to that described as B spermatogonia by Weaker (1977). Sertoli cells present typical aspect with deep nuclear indentations. The general aspects of the process of spermiogenesis can also be derived from this work. The typical phases of development could be established for the spermatid cell according to nuclear shape and chromatin condensation. The description of the normal configuration of the seminiferous epithelium was necessary to confront with the one found in some individuals with incomplete spermatogenesis. Finally, protrusions of the basal lamina pointing towards the nuclei of the Sertoli cells were described only in pathological conditions in humans (Haider et al., 1986),

Sperm morphology

Sperm shape was consistent with the shape previously reported by Hay et al. (1994) for this species, but tail length was significantly shorter in our studies. Total sperm length (67 ± 1.60 μm) was similar to the average eutherian spermatozoa (69.23 ± 4.13 μm), except rodents (101 ± 3.17 μm, Roldan et al., 1992). We base our observations on several measurements of mature and well-preserved spermatozoa from two individuals. Additionally, we provide illustrations to support these data. As a consequence, we consider that the size of the male gamete of this report is the correct one. Sperm morphology in the lesser anteater is similar to that found in *Bradypus tridactylus*, but the spermatozoon is longer than that found in the sloth (33.2 ± 0.75 μm; Peres et al., 2008). In other xenarthrans, like the armadillos, the shape and size of the spermatozoa were similar to those of the phyloge-

netically basal species (the genus *Dasypus*; Cetica et al., 1998; Moller-Krull et al., 2007). This was different from those of the more derived ones (*Priodontes maximus*, *Cabassous unicinctus*, *Chaetrophractus vellerosus*, *Chaetrophractus villosus, Zaedyus pichiy, Euphractus sexcinctus*; Cetica et al., 1998; Moller-Krull et al., 2007), which present extremely thin and large spoon-like sperm heads (Cetica et al.,1998).

Morphological variations of the genital tract between individuals

Notorious differences in the size of the genital tract organs were observed. Specimens with active gonads had bigger testicular volumes and larger epididymal diameters than those with inactive gonads, probably suggesting different mating or heat periods. This observation is in agreement with the sketches showed by Grassé (1955). The histological study of the testes of the lesser anteater exhibited complete spermatogenesis in the specimens with active gonads. Abnormal spermatogenesis was present in specimens with inactive gonads. This was correlated with variation in testicular size and presence of spermatozoa in these individuals (see Table 1). In agreement with our observations in the lesser anteater, significative variations of testicular size and weight have also been reported in other xenarthrans, like the common long-nosed armadillo (Mc Cusker, 1985). To establish whether the lesser anteater has a reproductive seasonality, an increased number of individuals in their natural state must be studied.

As showed in the present work, the lesser anteater has intrapelvic testes, a feature that is shared with other xenarthrans; some of the studied specimens of the lesser anteater exhibit a gonadal regression that is reflected on other structures of the reproductive tract. We expect that our results will contribute to guide further studies on the reproductive systems of this species and this family, aimed to improve successful breeding in conservation programmes.

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