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Morphological Description of Unusual Urinary Tract in the Female of a Rodent, *Galea spixii* (Wagler, 1831)

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Abstract. - The *Galea spixii* are rodents belonging to the subfamily Caviinae and family Caviidae. They live in the semiarid Caatinga vegetation of Brazilian Northeastern. Currently, they are on the red list of endangered species by the International Union of Conservation of Nature. Due to the importance and the need for more knowledge on the species, we aimed to describe morphologically, the urinary organs in females of *G. spixii* adults, from scientific breeding, by detailed anatomical and histological description. The urinary organs of females consist of a pair of asymmetric kidneys (right and left) constituted by medullar and cortical region, with renal corpuscles and proximal and distal convoluted tubules, beyond collector tubules. The ureters began in the renal pelvis in the hilum region, and followed caudally until the oval-shaped urinary bladder. The ureters and urinary bladder was lined by transitional epithelium, and presented muscular and serous layers. The urethra was divided into two portions: pelvic urethra lined by squamous epithelium and clitoral urethra with its ostium opening at the top of the clitoris, forming a pseudo-penis. At the clitoris, the urethral glands were present at the base, but were not present at the apex. Finally, further studies during development, particularly during sexual differentiation in order to explain the origin of clitoral urethra and a possible intrauterine masculinization of the female are required.

Key words: Clitoris, kidneys, ureters, urethra, urinary bladder.

INTRODUCTION

The *Galea spixii* are rodents belonging to the subfamily Caviinae and family Caviidae. They live in the semiarid Caatinga vegetation of northeastern Brazil (Oliveira *et al*., 2008), where they are, constantly used as an alternative source of protein for inhabitants of this region (Santos *et al*., 2014).

In Brazil, they are bred in captivity for preservation of the species, which is on the Red List of endangered species by the International Union of Conservation of Nature (IUCN, 2013). In addition, they are used as experimental models for research on reproductive biology (Rodrigues *et al*., 2013).

Among the several studies using *G. spixii* as an experimental model, we highlight those related to the role of the female reproductive biology. These studies have revealed that this species has polyestrous continuous cycle, with the female maintaining pregnancy (approximately 48 days) even devoid of environmental conditions and favorable food supply (Larcher, 1981); during pregnancy develops a kind of inverted choriovitelinic placenta (Oliveira *et al.*, 2008, 2012), and each pregnancy generates from two to four cubs (Oliveira *et al*., 2008). Furthermore, Santos *et al*. (2014) studied reproductive organs of the females and found that urethra seems to have its external orifice at the top of the clitoris, rather than the vestibule of the vagina, commonly found in domestic mammals (International Committee on Veterinary Gross Anatomical Nomenclature, 2012).

The urinary organs have great importance for the homeostatic control, regulating pH, osmotic balance, excretes of metabolites (Hickman *et al*., 2004), elimination of the wasteful chemicals from body, selective reabsorption (Latif *et al*., 2013), and can presents different morphological arrangements in animals from different habitats (El-Gohary *et al*., 2011). Then, due to the importance and need for more knowledge on the species, we aimed to describe morphologically the urinary organs in females of *G. spixii* adults, from scientific breeding,

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by detailed anatomical and histological description.

MATERIALS AND METHODS

Urinary organs of 10 *G. spixii*, females, adults, from the Multiplication Center for Wild Animals at Federal Rural University of Semi-Arid, Mossoró, Rio Grande do Norte, Brazil, with authorization from the Brazilian Institute of Environment and Renewable Resources (IBAMA) at 1478912/2011 were used. The study was authorized by the Ethics Committee of the School of Veterinary Medicine and Animal Science, University of Sao Paulo, Sao Paulo, Brazil (protocol 2400/2011).

The urinary organs were dissected for macroscopic description *in situ* and subsequently extracted from the abdominal cavity, together with the reproductive organs for *ex situ* analysis. For macroscopic photodocumentation Olympus SP-810UZ digital camera 14.0 Mp and magnifying glass Lambda LEE-3 005 252 were used.

Right and left kidneys were measured with scales and precision calipers to check the measurements of weight, length, width and thickness. Analysis of variance was performed using Instat program to obtain mean and standard deviation and mean comparison test was Student-Newman-Keuls (SNK) to analyze biometric measurements, due to the coefficient of variation 15% <CV <30%, considering level of significance $p < 0.05$.

For microscopic analysis, the urinary organs and the clitoris (fixed in 10% formalin solution) were collected. Then, these organs were dehydrated in increasing alcohol series (70% to 100%) and diaphanized in xylene for later inclusion in paraffin blocks, from which, sections of 5µm under Leica RM2165 microtome were obtained. Then, the tissue sections were placed on slides for subsequent histological staining with hematoxylin/eosin (HE) and Masson's trichrome (MT). The microscopic photodocumentation was performed under a BX61VS Olympus photomicroscope.

RESULTS

Macroscopic

Macroscopically, a pair of kidneys (right and left), with their ureters, urinary bladder and urethra (Fig. 1a) with its external orifice opening in the top of the clitoris (Fig. 1c) were found. Both kidneys (left and right) were placed in the abdominal cavity, cranially associated with adrenal glands and the caudal vena cava. The right kidney was dorsum caudally positioned from the liver, while the left kidney was dorsum caudally positioned from greater curvature of the stomach. In addition, topographically, we showed that the right kidney was more cranially positioned than the left kidney.

The right kidneys weighed 1.304 ± 0.203 g and the left kidney weighted 1.199 ± 0.274 g. The length of the right kidney was 2.03 ± 0.28 cm and the left 1.86 ± 0.17 cm. The width was 1.31 ± 0.07 cm for the right kidney and 1.17 ± 0.07 cm for the left kidney. The thickness was 0.41 ± 0.02 cm for the right kidney and 0.39 ± 0.05 cm for the left kidney. All measures showed statistical difference between the right and left kidneys to Student-Newman-Keuls (SNK) $p < 0.05$.

After longitudinal incision in the sagittal plane, kidneys with a distinct cortical region, a medullar region and a renal pelvis, which was facing the medial side of the organ were found (Fig. 1b).

The shape of the kidneys was similar to a bean, where it was possible to locate cranial and caudal pole, dorsal and ventral surface and medially directed hilum, where it was observed in each kidneys, an artery and a renal vein and their ureters, extending caudally until reaching the urinary bladder. The urinary bladder had oval format, from which came the urethra. This urethra had two portions: a pelvic portion, which ventrally accompanies the vaginal tube and clitoral urethra with its ostium opening at the top of the clitoris.

Microscopic

Microscopically, the kidneys (right and left) were composed by the renal capsule of connective tissue, a cortical region, just below the capsule and a medullar region inside the organ, beyond the renal pelvis, medially directed to the renal hilum. In the cortex, we found groups of tubules radially arranged, which consisted of collector tubules and straight portions of nephrons forming pars radiata and other groups of twisted tubules, consisting of

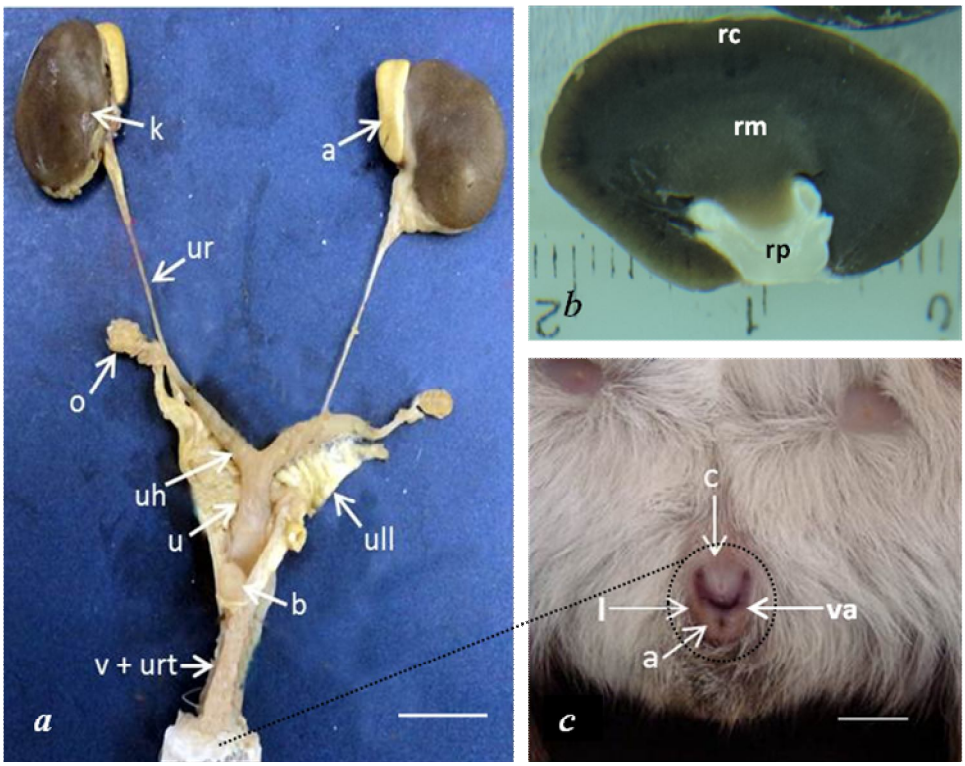

Fig. 1. Photography of urogenital apparatus in female of *G. spixii*. a: kidney (k), adrenal (a), ureter (ur), ovary (o), uterine horns (uh), uterus large ligament (ull), body of uterus (u), urinary bladder (b), vagina+ pelvic urethra (v+urt). b: hemi-kidney showing renal cortex (rc), renal medulla (rm) and renal pelvis (rp). c: magnification of clitoris (c) vulvar labia (l) anus (a) and vagina (va). Bars: 1cm

renal corpuscles and numerous proximal and distal convoluted tubules forming the twisted pars. The filtrate from the nephron passes to the collector tubules through the papillary ducts. The renal corpuscles were composed by Bowman's capsule, a glomerulus and the urinary space. In the medullar region, the segments of the collector tubules and loops of Henle were present (Fig. 2a-d).

The circular-shaped ureter was divided into distinct layers, where the innermost was the proper lamina, whose mucosa was composed of fold-shaped transitional epithelium, which lines the inside of the organ, and dense modeled connective tissue with elastic fibers. Externally from the proper lamina were the inner circular muscular layer and the outer longitudinal muscular layer. The serous layer (adventitial) was composed by not modeled connective tissue, which was lining externally the organ (Fig. 2e-f).

The urinary bladder was composed of a proper lamina with fold-shaped transitional epithelium and not modeled dense connective tissue with a presence of blood vessels. Externally from the proper lamina was the submucous layer, which was composed of not modeled dense connective tissue and a thin muscular layer. The muscular layer was composed of longitudinal and circular fibers. Coating the organ was the serous layer (adventitial), which was composed of not modeled dense connective tissue (Fig. 2g-h).

The pelvic urethra was composed of a proper lamina with its mucosa lined by squamous epithelium and urethral glands circulating inside the organ, beyond not modeled dense connective tissue and venous sinuses. Externally from the proper lamina were inner muscular layer with longitudinally-arranged fibers and an outer muscular layer with circular muscular fibers. Externally coating the organ was the serous layer (adventitial), formed by not modeled dense connective tissue

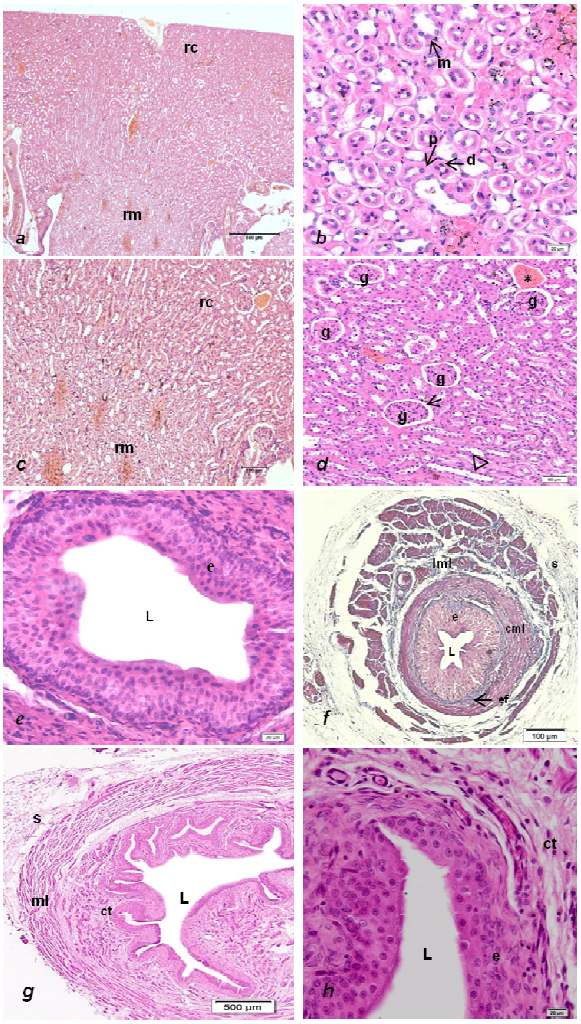

Fig. 2. Photomicrography of kidneys (a-d), ureters (ef) and urinary bladder (g-h) in female of *G. spixii*. a: renal córtex (rc) and renal medulla (rm). Bar: 500µm. HE. b: proximal (p) and distal (d) convoluted tubules and cells of dense macula (m). Bar: 20µm. HE. c: renal cortex with glomerules (rc) and renal medulla. Bar: 100µm. HE. d: glomerules (g), blood vessel (*), urinary space lined by Bowman's capsule (arrow) and collector duct (arrowhead). Bar: 50µm. HE. e: transitional epithelium (e) and lumen (L). Bar: 20µm. HE. f: lumen (L) transitional epithelium (e), elastic fibers (ef) in the submucous layer, circular muscular layer (cml), longitudinal muscular layer (lml) and serous layer (s). Bar: 100µm. TM. g: lumen (L), not modeled dense connective tissue (ct), muscular layer (ml) and serous layer (s). Bar: 500µm. HE. h: lumen (L), transitional epithelium (e) and not modeled dense connective tissue (ct). Bar: 20µm. HE.

(Fig. 3a-b). On the other hand, clitoral urethra in its initial portion at the base of clitoris had similar morphology to that pelvic urethra, however, with visible decrease in muscular layer and transition between urethra with mucous gland to urethra where the glands were absent. Large amount of venous sinuses were present in this region. Furthermore, externally, the organ was lined by epidermis with hair follicles and sebaceous glands (Fig. 3c-d). The apical portion of the clitoral urethra was internally lined by squamous epithelium and no urethral glands were found in the mucosa. Tiny muscular layer and large amount of connective tissue was found. Externally the clitoris was lined by keratinized squamous epithelium continuous with the skin, which presented sebaceous glands at the base of hair follicles (Fig. 3e-f).

DISCUSSION

It has been well established that species that live in different environments need anatomical and physiological mechanisms that prioritize the economy of body water. This ability occurs through the production and concentration of the urine, which is directly related to the structure of the kidney and other urinary organs, which are developed according to several environments factors involving interspecific morphological differences (El-Gohary *et al*., 2011). In this sense, we demonstrated significant morphological data of the urinary tract, which were not found in other previous works, and these data are related to some questions, as discussed below.

The urinary tract of female *G. spixii* was basically composed of two kidneys (left and right) and their ureters, which empty into the urinary bladder and the urethra, responsible for eliminating urine to the outside. These results agree with the description for domestic mammals such as cattle, horses, pigs and dogs (Schaller, 1999; International Committee on Veterinary Gross Anatomical Nomenclature, 2012), as well as for laboratory animals as lagomorphs *Oryctolagus cuniculus* (Popesko *et al*., 2002) and Caviidae rodents *Cavia porcellus* (Cooper and Schiller, 1975), and other tetrapods vertebrate (Hickman *et al*., 2004). Weight differences between left (smaller) and right

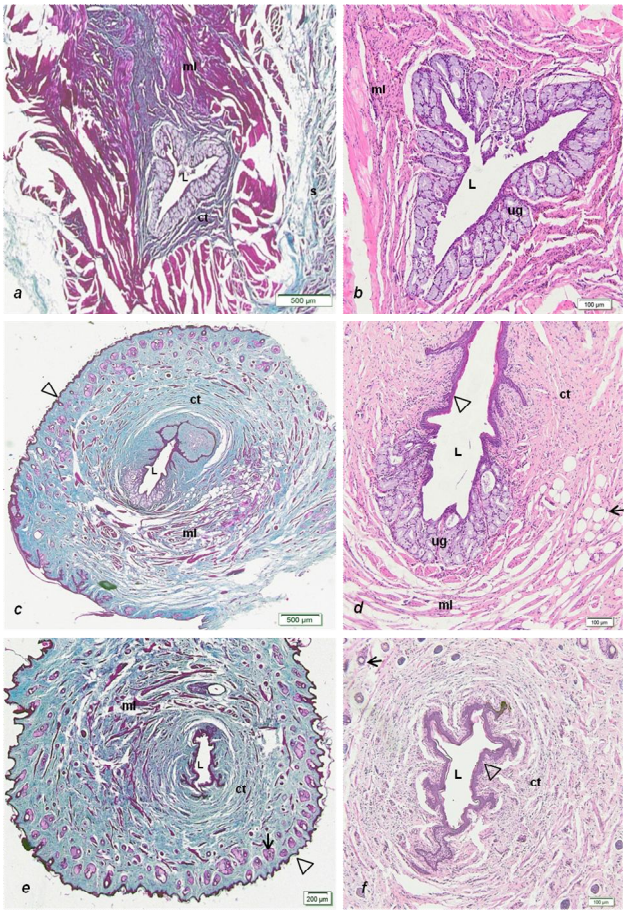

Fig. 3. Photomicrography of pelvic urethra (a-b) and clitoral urethra at base (c-d) and at apex (e-f) of organ in female of *G. spixii*. a: lumen (L), connective tissue (ct), muscular layer (ml) and serous layer (s). Bar: 500µm. MT. b: lumen (L), urethral glands (ug) and muscular layer (ml). Bar: 100µm. HE. c: lumen (L), connective tissue (ct), muscular layer (ml) and epidermis (arrowhead). Bar: 500µm. MT. d: lumen (L), squamous epithelium (arrowhead) with urethral glands (ug), connective tissue (ct), venous sinuses (arrow) and muscular layer (ml). Bar: 100µm. HE. e: lumen (L), connective tissue (ct), muscular layer (ml), sebaceous glands of hair follicle (arrow) and epidermis (arrowhead). Bar: 200µm. MT. f: lumen (L), epithelium (arrowhead), connective tissue (ct) and hair follicle (arrow). Bar: 100µm. HE

(greater) kidneys as described by Onyeanusi *et al.* (2009) in both Wistar Rat and African Giant Rat were found. These authors also observed that the mean kidney weight in the male was higher than that of the female.

In addition, in our study, characteristics that go far beyond the anatomical conformation of urinary organs above described were found. Among these features, we highlighted the presence of a urethra divided into a pelvic and a clitoral portion, which trespasses the clitoris, and for this reason, we anatomically called: clitoral urethra. This clitoral urethra had its external urethral orifice opening at the top of this organ, as previously noticed in the same species (Santos *et al.*, 2014) and mice (Yang *et al.*, 2010), however the authors did not describe more detailed morphological data. This clitoral urethra has not been described in other Caviidae as *Cavia porcellus* (Cooper and Schiller, 1975; Popesko *et al.*, 2002; Banks *et al.*, 2010), *Cuniculus paca* (Reis *et al.*, 2011) and *Hydrochoerus hydrochaeris* (Pocock, 1922); and other rodents as Wistar Rat and African Giant Rat (Onyeanusi *et al.*, 2009).

A clitoris trespassed by the urethra is described in cases of extreme female masculinization, as in hyenas (*Crocruta crocruta*), which has an hypertrophied clitoris trespassed by the urethra, however, forming a urogenital sinus due to the absence of an external vaginal ostium (Yalcinkaya *et al.*, 1993; Glickman *et al.*, 2006).

The moles *Talpa europea* and *Candilura cristata* have a penile clitoris (Rubenstein *et al.*, 2003.), while the females of lemurs *Eulemur fulvus rufus* (Ostner *et al.*, 2003) and *Lemur catta* (Drea, 2007) and monkeys *Saimiri sciureus* (Branco *et al.*, 2010) have an hypertrophied clitoris, however, the authors have not cited a urethra trespassing this organ. Clitoral urethra could be related to the fact of these females present absence of a vaginal vestibule and periodic development of a vaginal closure membrane (Santos *et al.*, 2014).

For this reason, future studies will demonstrate the importance of masculinized characteristics in females of *G. spixii*. In this sense, some authors describe that female masculinization and lack of sexual dimorphism may be related to interspecific competition for feeding territory in *Eulemur fulvus rufus* (Ostner *et al.*, 2003) and hyenas *Crocruta crocruta* (Glickman *et al.*, 2006), due these females present a high amount of circulating androgens and for this reason they have a high aggressiveness. Some authors describe that

these masculinized characteristics of females originates in the embryonic period, due to the large exposure of the fetus to androgen hormones (Yalcinkaya *et al*., 1993; Whitworth *et al*., 1999; Place *et al*., 2002; Glickman *et al*., 2006; Conley *et al*., 2007; Drea, 2007; Yang *et al*., 2010).

Microscopically, kidneys with morphological constitution similar to other rodents as albino rat (Al-Samawy, 2012), *Cavia porcellus* and *Acomys russatus* and insectivores as *Paraechinus aethiopicus* (El-Gohary *et al*., 2011) were found, however, stereological studies will confirm possible differences in the number of glomerulus or nephrons and renal papillae, beyond the relation between cortical and medullar area, since El-Gohary *et al*. (2011) suggest that lower numbers of nephrons can be considered an adaptive aspect that favors the development of smaller volume of concentrated urine in species adapted to arid climates, and this fact contributes to success in water conservation. Al-Samawy (2012) describes that the kidneys are highly vascularized, compound tubular glands that function to maintain the composition of body fluids at a constant level and to remove excretory wastes. The kidneys also regulate the fluid and electrolyte balance of the body and are the site of production hormones as rennin and erythropoietin.

Regarding the other organs, histological constitution was similar to those found in other species of mammals (Samuelson, 2007), with the exception from the urethra, which has some peculiarities as the presence of structural differences between the pelvic and clitoral urethra. This differences are the squamous epithelium with numerous venous sinuses and urethral glands at the base, but with the absence of urethral glands in the apical portion of the clitoris, beyond the muscular layer, which is present in the pelvic urethra and in the base region of the clitoris but is barely visible in the apical region of the organ. Therefore, future studies will confirm whether the clitoris of *G. spixii* female could be erectile as in the case of the hypertrophied clitoris of hyenas *Crocruta crocruta* (Glickman *et al*., 2006), besides confirming the embryological origin of clitoral urethra and a possible intrauterine masculinization of female through embryological studies, and its function to species through studies of behavioral biology and other ecological studies.

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