THE MORPHOLOGY OF FEMALE GENITALIA IN *Galea spixii* (Caviidae, Caviinae)

MORFOLOGIA DA GENITALIA FEMININA EM Galea spixii (Caviidae, Caviinae)

Amilton Cesar dos SANTOS¹; Bruno Machado BERTASSOLI²; Diego Carvalho VIANA¹; Bruno Gomes VASCONCELOS³; Moacir Franco de OLIVEIRA⁴; Maria Angélica MIGLINO⁵; Antônio Chaves de ASSIS NETO⁵

Doutorandos, Faculdade de Medicina Veterinária e Zootecnia- FMVZ. Universidade de São Paulo, São Paulo, SP, Brasil;
Doutorando, Universidade Federal de Minas Gerais- UFMG. Belo Horizonte, MG, Brasil;
Professor Assistente A, Setor de Anatomia Animal, Instituto de Ciências da Saúde - ICS, Universidade Federal de Mato Grosso - UFMT, Sinop, MT, Brasil. brunog_vet@hotmail.com;
Professor Doutor, Universidade Federal Rural do Semiárido - UFERSA, Mossoró, RN, Brasil;
Professores Doutores, Faculdade de Medicina Veterinária e Zootecnia – FMVZ, Universidade de São Paulo, São Paulo, SP, Brasil.

ABSTRACT: The yellow-toothed cavy (*Galea spixii*) is a hystricomorph rodent of the Caviidae family. In Brazil, *G. spixii* are bred in captivity to provide an alternative protein source and to preserve the species. However, there is a lack of data on the animal's female genital organs. Current research describes the morphology of the species's adult female genital organs, regardless of the stage of its estrous cycle, and permits basic knowledge on its anatomy that will be a help for future projects in reproduction in captivity. Adult female genital organs of *G. spixii* comprise two ovaries with follicles at several developmental stages; uterine tubes whose epithelium and muscular layer thickness modify themselves throughout the isthmus, ampulla and infundibulum regions; double uterus and uterine horns with uterine glands that open to a single cervix which is linked to the vagina by the fornix; a variegated vaginal epithelium from different animals; a vulva with a clitoris trespassed by the urethra which features a lack of vaginal vestibule and the presence of a vaginal closure membrane. The morphology of *G. spixii* female genitalia has interesting characteristics such as the vaginal closure membrane and a clitoris trespassed by urethra that needs further studies. Other investigations on developmental biology could demonstrate a possible intrauterine masculinization in the *G. spixii* female.

KEYWORDS: Cavies. Reproduction. Rodents. Vaginal Closure Membrane.

INTRODUCTION

Yellow-toothed cavies (*Galea spixii*) are hystricomorph rodents weighing approximately 375-405 g. They belong to the Caviidae family and are distributed throughout the semidry and arid caatinga vegetation of northeastern Brazil (OLIVEIRA et al., 2008).

Similar to other rodents such as *Mus musculus* (MENDONÇA et al., 2007), *G. spixii* females have a short pregnancy period, with an average duration of 48 days, and a 2-4 cubs per litter (LARCHER, 1981). The *G. spixii* reproduces in different seasons and females may sustain pregnancy even without the best nutritional conditions (LARCHER, 1981).

Females of *G. spixii* build nests among stones and vegetation for reproduction. They have crepuscular habits, live in herds and feed on leaves, branches and fruits of creeping plants, roots, tubercles and tree bark (LARCHER, 1981).

In Brazil, *G. spixii* is bred in captivity to provide alternative food (SANTOS et al., 2012), preserve the species and to better the development of a new model in animal research (OLIVEIRA et al., 2010; RODRIGUES et al., 2013). With regard to reproduction aspects, research has shown a type of inverted choriovitelline placenta (OLIVEIRA et al., 2008; 2012) and the start of puberty in the male occurs after 45 days of birth (SANTOS et al., 2012). The genital organs of the male *G. spixii* comprise testes, epididymis, accessory genital glands (prostate, vesicular and bulb urethral glands), penis and scrotum (RODRIGUES et al., 2013).

The variety of habitats occupied by different species of cavies provides a very wide diversity of social organizations, whilst different mating systems may be found. This diversity of social organization and mating systems may be based on morphophysiological adaptations of the reproductive organs of the family's members (ADRIAN; SACHSER, 2011).

The rodent's female reproductive apparatus comprises ovaries, uterine tubes and horns, uterus, vagina and vulva (TOUMA; PALME; SACHSER, 2001). A vaginal closure membrane, which develops according to oestral cycle phases, may be identified in species of the Caviidae family, such as *Cavia porcellus* (STOCKARD; PAPANICOLAOU, 1919), *Galea musteloides* and *Cavia aperea* (TOUMA; PALME; SACHSER, 2001) and in other rodent species, such as *Octodon degus* (MAHONEY et al., 2011) and *Thryanomys swinderians* (ADDO et al., 2007).

Since deeper knowledge on the morphophysiology of the reproductive apparatus organs of wild species is a must for the planning and development of more precise techniques for their reproduction in captivity and for species preservation, current investigation was developed to describe the morphology of the genital organs of the adult female of *G. spixii*, regardless of the stage of its estrous cycle, and provides other information for reproduction programs in captivity.

MATERIAL AND METHODS

Animals

Ten primiparous non-pregnant adult females of *G. spixii* were retrieved from the Center for Wild Animals Multiplication (CEMAS) (IBAMA 1478912/2011) of Federal Rural University of the Semiarid, Mossoró RN Brazil. This research was approved by the bioethics committee of the Faculty of Veterinary Medicine and Animal Science at São Paulo University under number 2400/2011.

Macroscopic and morphometric analysis

Monitoring of the development of the vaginal closure membrane *in vivo* starts at the rupture of the latter, which marks the estrus, as described by Stockard and Papanicolaou (1919) and Touma, Palme and Sachser (2001) to other caviidae. After the rupture of the membrane, the development of the vaginal closure membrane in all animals is monitored until the complete occlusion of the vaginal orifice and the next rupture, which lasts about 20 days. Photo documentation with digital camera Sony MAVICA 3.2mp has been undertaken at the moment of complete occlusion and complete rupture of vaginal closure membrane.

After the monitoring of the vaginal closure membrane *in vivo*, all animals were anesthetized with xylazine (4mg/kg/IM) and ketamine (60mg/kg/IM) and then euthanized with thiopental sodium (2.5% 60mg/Kg) by intracardiac cannulation.

The genital organs were dissected and removed together with the urinary organs which constitute the urogenital apparatus. The genital organs were measured with precision calipers (mm) and data grouped into a table and shown as mean with standard deviation before fixation (Table 1). Urogenital organs from all animals were fixed by formalin solution 10% by injection and submersion. Light microscopy process began after a 72-hour waiting period.

Light microcopy

Fragments of ovaries. uterine tubes (isthmus, ampulla and infundibulum regions), uterine horns, uterus, medium third and ostium from vagina, with and without the vaginal closure membrane, were cut for analysis under light microscopy. The urethra was then severed from the clitoris adhered to the vaginal tube of the animals. All fragments described above were processed with routine technique of paraffin embedding and stained with hematoxylin-eosin (HE) (TOLOSA et al., 2003). Microscope slides were photomicrographed in Olympus BX, coupled to camera 60 HRc Axio CAM, using software Zeiss KS 400.

RESULTS

Macroscopic results

Two ovaries were found among adipose tissues, fixed by the mesovarium at the back of the abdominal cavity, tapering in the kidneys of *G. spixii*. The ovaries had the form of a flat almond in each antimere of the body.

The respective uterine tubes from the ovarian hilum (right and left) are suspended by the mesosalpinx and continue up to the respective right and left uterine horns. They communicate only when they reach the single uterine cervix positioned at the back-head of the urinary vesicle. The corpus of uterus is double. The uterine cervix ends at the fornix with an opening into the vaginal tube.

The vagina starts from the fornix and ends at the vaginal ostium which opens into the external vulva, although these animals lack the vestibule. The external vulva presents a clitoris trespassed by the urethra which opens into the urethral ostium at the clitoris apex, lacking a movable prepuce. Further, the anus lies on the back and in direct relationship with an external vulva in the shape of an inverted U. The vulva has slightly prominent rosy vulvar folds. The periodical development of a vaginal closure membrane is also extant. It covers the ostium of the vagina and ruptures naturally in a few days (Figure 1).

Table 1 shows morphometric data from current study. It should be emphasized that no morphometric data were found for comparison with other same-size species.



Figure 1: Photograph of female urogenital apparatus (*ex situ*) and external genitalia in ventral view (*in vivo*) of *G. spixii*. A: kidney (k); ureter (ur); ovary (o); uterine horn (uh), uterus (u); uterus large ligament (ull); bladder (b); vagina + urethra (v + urt). B: clitoris with urethral ostium (c); labia (l) without vaginal closure membrane; anus (a). C: clitoris with urethral ostium (c); labia (l) with the vaginal closure membrane; anus (a). Bar: 1cm.

Organ	Lenght (mm) Mean ± SD	Diameter (mm) Mean ± SD
Ovary	2.9 ± 0.2	2.8 ± 0.3
Vagina	35.6 ± 2.7	17.0 ± 4.0
Uterus	12.0 ± 2.0	17.8 ± 2.2
Uterus horns	29.0 ± 2.0	12.9 ± 2.8
Uterine tubes	32.6 ± 2.0	2.1 ± 0.9

SD= Standard deviation

Microscopic results

The surface of the ovaries of G. spixii is covered with a simple cubic epithelium. Microscopy reveals that it is divided into a cortical zone with follicles at different development stages and into a medullar zone with stromal tissues and vessels. Primary follicles are seen in great numbers, with nucleus and transparent cvtoplasm. central Development starts with a layer of cubic cells, which later form a multilaminar follicle with an antrum by cell proliferation. Secondary follicles have a granulose membrane and an internal and external theca. Tertiary follicles contain an oocyte surrounded by granulose membrane cells that form the cumulus oophorus and a corona radiata with a shiny zone separated from the oocyte. After oocytation and formation of the corpus luteum, the latter regresses and forms the *corpus albicans* (Figure 2).

The ovary-inserted uterine tubes in the female G. *spixii* have an epithelium with a prism-shaped internal lining. The lamina is made up of a loose connective tissue, a smooth muscular layer and a serous layer. The smooth muscular layer in the isthmus section is thicker and less folded than the ampulla and the infundibulum which has the least thick muscular layer and the greatest folds. Uterine glands and loose conjunctive tissue occur in the uterine horns, whereas the septum of non-modeled dense connective tissue may be found in the double uterus (Figure 3).



Figure 2: Photomicrograph of the ovary of *G. spixii* in HE. A: lining epithelium of the ovary (thick arrow); initial primary follicle (arrow); and group of primordial follicles (circle). B: primordial follicles (circle); developed primary follicle (arrow). C: tertiary follicles (arrow). D: *corpus albicans* (arrow).



Figure 3: Photomicrograph of the cervix, double uterus and uterine tube of *G. spixii* in HE. A: folds of the fornix with cornified epithelium in the dorsal region (D) and ventral region of the cervix (V). B: cervix with uterine glands (arrow). C: double uterus (arrows) separated by a septum of connective tissue. D: endometrium with columnar epithelium (e) and uterine glands (arrow). E: Isthmus slightly folded from uterine tube (arrow) and its lumen. F: infundibulum of uterine tube (arrow) with larger folds oriented to the lumen (l).

The uterus is divided into the following layers: the serous external layer; the intermediate layer, or myometer, made up of muscles; the internal layer, or endometer, lined with the cubic columnar epithelium and uterine glands in the lamina's connective tissue.

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The uterine cervix has a horn-like epithelium at its ceiling within the transitional region leading towards the fornix. A stratified pavementous-type epithelium with many uterine glands and a cubic epithelium may be found in the basal region (Figure 3).

A horn-like epithelium lies in the fornix region and a non-modeled dense connective tissue occurs in the lamina (Figure 3).

The vaginal microscopy morphology of the female *G. spixii* reveals an organ made up of

mucous, submucous and adventitious layers. The mucous layer is formed by a keratinized stratified epithelium. The latter undergoes several morphological variations according to the different oestral cycle phase in which the material was randomly collected. An epithelium with horn-like cells was detected, whereas other animals exhibited only a poorly stratified epithelium without any keratinization. The vaginal tissue has several longitudinal folds throughout the extension of the vagina (Figure 4).



Figure 4: Photomicrograph of the vagina, clitoris, fornix and vaginal closure membrane of *G. spixii* in HE. A: vaginal epithelium (e); vaginal muscular layer (m); ischiocavernous muscles of the clitoris (isqm); urethral glands (u); greater caption showing the longitudinal folds of vagina (lf). B: cornified stratified epithelium of the middle portion of the vagina (e); connective tissue of submucous layer (ct). C: epithelium of clitoris with glands (e) in the basal region; venous sinuses (vs); muscular layer (m); adventitial layer (a). D: fornix folds with cornified epithelium. E: vagina (circle) with the completely formed closure membrane (arrow) and base of clitoris (c) with urethra (U). F: vaginal closure membrane (vcm) with nucleated cells (arrow) and intercellular vacuoles (dotted arrow).

A non-modeled connective tissue with blood vessels in the vaginal submucous layer was found. The muscular layer features an internal longitudinal layer and an external transversal one. The adventitious layer is contiguous to the muscular layer in which lie both the unilocular adipose tissue and the blood vessels

The vulva of female *G. spixii* lacks a vestibule. In fact, the vagina's ostium is cyclically obstructed by the vaginal closure membrane.

Several gland ducts in the mucous layer occur within the region of the vagina's ostium.

The vulva's internal epithelium consists of a non-keratinized pavement. The vulva is lined on the outside with a keratinized pavementous-like epithelium with pilous follicles and sebaceous and acinary glands.

The vaginal closure membrane consists of an agglomeration of nucleated and enucleated cells and of connective tissue, with intercellular vacuoles (Figure 4).

The vaginal tissue at its most tapering section is continuous with the clitoris tissue which is trespassed by the urethra. The latter is surrounded by several urethral glands internally lined by the epithelium, which ranges from prismatic to columnar. The clitoris still shows an ischiocavernous muscle (Figure 4).

DISCUSSION

The female genital organs of *G. spixii* have characteristics that go beyond the common features of genital organs of other domestic animals such as the dog, horse, cows and pigs (DYCE; SACK; WENSING, 2010). When compared to other rodents, several similarities may be found which seem to be evolution strategies that warrant the reproduction of this order of mammals within the most diverse habitats (ADRIAN; SACHSER, 2011).

The topography of the ovaries of G. spixii follows the patterns featured in Cuniculus paca (REIS et al., 2011), Dasyprocta agouti (ALMEIDA et al., 2003) and Myocastor coypus (FELIPE; CABODEVILA; CALLEJAS, 1999). The results found in the Dasyprocta agouti (ALMEIDA et al., 2003) present particularities because in these animals, the ovaries are not immersed in adipose tissue as that found in the G. spixii and Dasyprocta prymnolopha (GIMARÃES; MATOS; VALE, 1994). Ovarian follicles at different maturation stages reveal that female G. spixii were sexually mature according to data from *Myocastor covpus* (FELIPE; CABODEVILA; CALLEJAS, 1999). Ovaries of G. spixii by present study and that found in Myocastor coypus (FELIPE; CABODEVILA; CALLEJAS, 1999) consist of simple cubic epithelial lining, peripheral cortex and central medulla formed by connective tissue interspersed with blood vessels.

The same uterine tubes in the ovaries of *G. spixii* have also been described in *Dasyprocta aguti* (FORTES et al., 2005) and *Cuniculus paca* (REIS et al., 2011). The uterine tubes in this species above are divided into mucous, submucous and serous

layers. Moreover, the characteristics of the genital organs of *G. spixii* are similar even to the capybara (*Hydrochoerus hidrocaeris*) (COSTA et al., 2002) and the paca (*Cuniculus paca*) (REIS et al., 2011). Similarity among the *G. spixii*, capybara (COSTA et al., 2002) and the paca (REIS et al., 2011) lies in the double uterus with its uterine horns, which form a double uterus. Then, the double uterus opens into the single cervix and communicates with the single vagina.

With regard to the double uterus from *G. spixii*, common to other rodents described above, a different result is found in the *Lagostamus maximus* females where the uterine horns do not merge into a single cavity of the cervix, but remain independent (WEIR, 1971). In fact, they form a double cervix as in marsupials (GONÇALVES et al., 2009).

Variations in the microscopy morphology of the vaginal epithelium of *G. spixii*, with differences between different animals, have been described in other studies on the differences of oestral cycle phases. In the guinea pig *Cavia porcelus* (SELLE, 1922) and the viscacha *Lagostamus maximus* (WEIR, 1971), an epithelium with intense proliferation and cornification in the proestrus phase is extant. However, current study does not intend to compare the morphology during the phases of the estrous cycle.

Similar to the capybara (POCOCK, 1922) and paca (REIS et al., 2011), *G. spixii* features a clitoris in the vulva with a vaginal closure membrane, but lacks the vaginal vestibule.

Other rodents, such as *Thryonomys swinderianus* found in Africa (ADDO et al., 2007), *Lagostamus maximus* (WEIR, 1971), *Galea musteloides, Cavia aperea* (TOUMA; PALME; SACHSER, 2001), *Myocastor coypus* (FELIPE et al., 2000; FELIPE; CABODEVILA; CALLEJAS, 2001) and *Octodon degus* (MAHONEY et al., 2011) found in South America, the domestic guinea pig (*Cavia porcelus*) (KELLY; PAPANICOLAOU, 1927) and the *Rattus norvegicus* (LEPHART et al., 1989) also have a vaginal closure membrane up till its rupture in puberty. In this species above, the membrane develops at each oestral cycle and ruptures only during estrus phase.

The vaginal closure membrane in *G. spixii* develops and ruptures constantly, although results in this study do not take into account the phases of the oestral cycle as in the above-described studies since the aim of current study is different. Microscopy shows that the vaginal closure membrane of *G. spixii* merges from vaginal epithelium and displays layers of nucleated cells and intercellular vacuoles filled with the connective tissue.

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Similar microscopic results on the vaginal closure membrane from present research are not found in other research. The authors believe that further studies would demonstrate the importance of this phenomenon which was described at the beginning of the last century by Stockard and Papanicolaou (1919) and Kelly and Papanicolaou (1927) in *Cavia porcellus*, but has not yet been properly detailed to highlight its importance for the reproduction of the species that exhibit this characteristic.

Another interesting feature in G. spixii, to Cavia porcellus (STOCKARD; similar PAPANICOLAOU. 1919: **KELLY:** PAPANICOLAOU, 1927) is the clitoris trespassed by the urethra in the vulva and not in the vaginal vestibule as normally found in domestic four-footed bovines. equines and swine-related animals (HAFEZ; HAFEZ, 2004) and in two-footed animals of the genus Leontopithecus (PISSINATTI et al., 2008). In other caviomorphs species, as in Cuniculus paca (REIS et al., 2011) and capybara (POCOCK, 1922), the clitoris trespassed by the urethra is not described. The clitoris trespassed by urethra is related to the equilibrium in the production of androgens and estrogen hormones during intrauterine sexual differentiation shown in mice (YANG et al., 2010).

Many authors call the hormonal variations towards different anatomic configurations of female genital organs as masculinization or androgenization (OSTNER; HEISTERMANN; KAPPELER, 2003; GLICKMAN et al., 2006; CONLEY et al., 2007; DREA et al., 2007). The masculinization of female mammals can ranges from behavior aspects up to severely masculinized external genitalia, as in the case of the hyena *Crocruta crocruta*, with a penile clitoris trespassed by the urethra and the lack of vaginal aperture (GLICKMAN et al., 2006). On the other hand, the lemurs *Eulemur fulvus rufus* (OSTNER; HEISTERMANN; KAPPELER, 2003) and *Lemur catta* (DREA, 2007) and the common squirrel monkey *Saimiri sciureus* (BRANCO et al., 2010) have a hypertrophied clitoris and a vagina.

The European mole *Talpa europea* and the star-nose mole *Candilura cristata* have a penile clitoris, which is not trespassed by the urethra, and an ovotestis (RUBENSTEIN et al., 2003), which are bipolar gonads with the characteristics of testicles and ovaries (BARRIONUEVO et al., 2004).

Intra-uterine hormonal factors are involved in all the above-mentioned masculinization of the female (PLACE et al., 2002; GLICKMAN et al., 2006), especially those related to the androgenproducing steroidogenic enzymes and/or to the low aromatization of androgen hormones in estrogens produced by aromatase cytochrome P450 enzyme or the production of androgen hormones at similar or higher levels than the males (WHITWORTH et al., 1999) and the production of anti-Müllerian hormone by the ovary of the female fetus (BROWNE et al., 2006).

Studies on the social behavior of *G. spixii* in its natural environments should be enhanced since they describe the species's females as highly aggressive when the oestral period approaches. In fact, the females select the males to be mated with (ADRIAN; SACHSER, 2011).

In fact, the morphology of the Galea spixii genitalia has several interesting female characteristics, such as the vaginal closure membrane and a clitoris trespassed by urethra that further studies. In addition. needs further investigations in developmental biology would demonstrate a possible intrauterine masculinization in the Galea spixii female.

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RESUMO: O preá silvestre do semiárido (*Galea spixii*) é um roedor histricomorfo da família Caviidae. No Brasil, *G. spixii* são criados em cativeiro com intuito de gerar uma fonte alternativa de proteína e preservar a espécie. Embora, existam poucos dados sobre os órgãos genitais femininos destes animais. A presente pesquisa descreve a morfologia dos órgãos genitais femininos de *G. spixii* adultas, sem considerar as fases do ciclo estral e permite conhecimento básico sobre a anatomia que poderá ajudar futuros projetos de reprodução em cativeiro. Os órgãos genitais femininos de *G. spixii* adultas, cujo epitélio e camada muscular se modificam ao longo das regiões do istmo, ampola e infundíbulo; útero duplo e dois cornos uterinos com glândulas, os quais se abrem em uma única cérvix, a qual se conecta com a vagina pelo fórnix; uma vagina com epitélio variado entre diferentes animais; uma vulva com clitóris transpassado pela uretra; ausência de vestíbulo vaginal e a presença de uma membrana de oclusão vaginal. A morfologia da genitália feminina de *G. spixii* possui interessantes características como a membrana de oclusão vaginal e um clitóris transpassado pela uretra, as quais

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necessitam de maiores estudos. Outras pesquisas sobre biologia do desenvolvimento poderiam demonstrar uma possível masculinização intrauterina das fêmeas de *G. spixii*.

PALAVRAS-CHAVE: Membrana de Oclusão Vaginal. Preás. Reprodução. Roedores.

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