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Anatomy, histology and histochemistry of accessory sex glands in male Persian squirrel (*Sciurus anomalus*)

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Abstract

Persian squirrel (*Sciurus anomalus*) is a species of squirrels in the Middle East. There is little information about the anatomy and histology of various organs of this species. Moreover, there are no practical data about the accessory male sex glands of this species of squirrel. In this study the anatomical, histological and histochemical properties of the male reproductive system accessory glands of Persian Squirrel was evaluated. Eight adult male squirrels were anesthetized and euthanized. The pelvic area was dissected and the male reproductive system was separated. The accessory sex glands were investigated for gross anatomical aspect. Samples were fixed in formaldehyde for histological and histochemical studies. The coagulating glands, prostate and bulbourethral glands were observed at gross anatomical level. A single heart-shape and compact prostate gland was situated on the dorsal side of pelvic urethra. Two small coagulating glands were observed on the cranio-dorsal side of prostate. Two large spiral shape bulbourethral glands were situated out of the pelvic cavity near the root of penis on both sides of anal area. Histologic studies revealed that all accessory sex glands were alveolar glands with cuboidal to columnar epithelium. The most positive reaction for PAS stain was observed in the trabeculae of glands. There was no positive reaction for lipids in glands stained with Oil red O and Sudan Black. Some anatomical differences were observed in the accessory sex glands of Persian Squirrel in comparison to other rodents, however the histology of glands was almost similar to other rodents.

Key words

Accessory sex glands, bulbourethral gland, coagulating gland, Persian squirrel, prostate, reproductive system.

Introduction

The prostate, vesicular glands, bulbourethral glands, coagulating glands, preputial glands and ampulla of the vas deferens are the male accessory genital glands which have an important role in the reproductive system function (Chughtai et al., 2005). There is a great variation in gross morphology and histology of these glands between different species of mammals (Thomson and Marker, 2006). The anatomical study of accessory genital glands could be effective for describing the structural differences of these glands among various species. It has been reported that in rabbit (Holtz, 1972), giant rat (Oke, 1988), Cape mole rat (Bennet and Jarvis, 1988) and the Pocket gopher (Vaughan, 1962) all of above mentioned glands except ampulla of the vas deferens

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were observed. In viscacha (Chaves et al., 2011) and agouti (Mollineau et al., 2006) the preputial glands were not observed.

The Persian squirrel (*Sciurus anomalus*) is a mammal belonging to the order of rodents. It is a wild species although some people keep it as a pet. The population of this species in wildlife is on decrease. Therefore, it is important to produce basic information on anatomy and histology of different systems, particularly the male reproductive system since it has considerable significance in breeding, in order to make a pathological diagnosis and provide clinical treatment of diseases in this species. In consequence, the aim of this study is to explore the male accessory sex glands as an important part of the male reproductive system.

The aim of this study was the evaluation of the anatomical and histological parameters of the accessory genital glands of Persian squirrel (*Sciurus anomalus*).

Material and methods

Macroscopic anatomy

Eight adult male Persian squirrels (*Sciurus anomalus*) were used in this study. The animals were collected from veterinary clinics with diseases other than genital diseases. The animals were weighed and then were euthanized by deep halothane inhalation followed by swift cervical dislocation.

All animal procedures used in this study were approved by the University of Tabriz standards for human care and use of laboratory animals, in accordance with the Research Ethical Committee of the Ministry of Health and Medical Education of Iran (adopted April 17, 2006) based on the Helsinki Protocol (Helsinki, Finland, 1975).

The dorsal part of body in the sacral region was dissected through a mid-dorsal lumbar incision. The sacral and caudal vertebrae and the rectum were completely removed to expose the reproductive organs. The accessory genital glands were exposed and were prepared for anatomical evaluation. Individual glands were separated, measured, weighed, photographed and characterized in terms of their location and direction in the body cavity, colour and structure.

Microscopic anatomy

Samples of the prostate gland, bulbourethral glands and coagulating glands were fixed in 10% buffered neutral formalin solution for 48 h. Tissue sections with a thickness of 6 microns were cut from paraffin embedded samples and stained with hematoxylin and eosin for routine histological studies.

For the histochemical evaluations of carbohydrate and lipid content of glands, sections from the paraffin embedded tissues were stained with periodic acid Schiff technique (PAS) and frozen sections were stained with oil red O and Sudan black B.

Results

Anatomical findings

The anatomical studies showed that, in *Sciurus anomalus*, the accessory genital glands consist of the prostate, bulbourethral glands and coagulating glands and the ampulla of vas deferens and vesicular glands were absent in this animal (Fig. 1).

Table 1 shows the gross measurements of the accessory sex glands of the Persian squirrels. Gross anatomy observations showed that the heart-shaped prostate was quite dense with smooth surface and was situated in dorsal part of pelvic urethra. A paired, small and horn-shaped coagulating gland was situated in the craniodorsal part of the prostate. A paired and large bulbourethral gland was located near the root of penis on either side of the anus (Fig. 1).

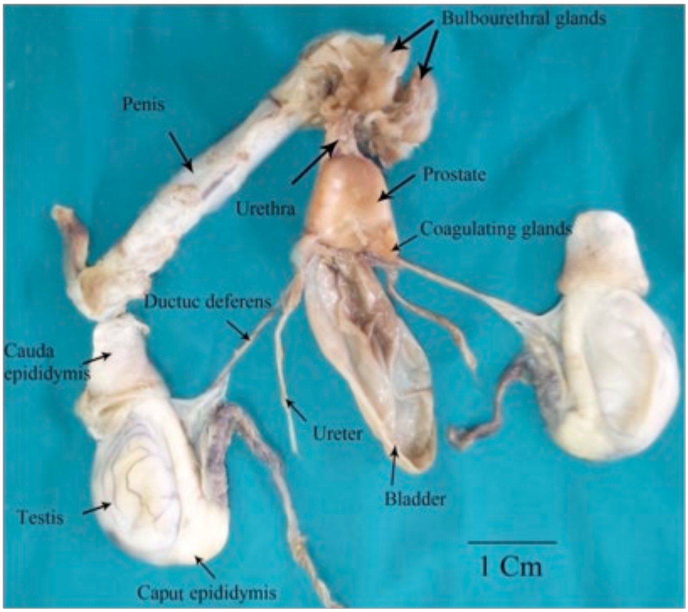

Figure 1 - Dorsal view of the male urogenital system of Persian squirrel.

Table 1 – Size of the accessory sex glands of the Persian squirrel (mean ± standard deviation; N = 8).

Glands	Length (cm)	Width (cm)	Weight (g)
Coagulating glands	0.42 ± 0.01	0.27 ± 0.01	0.23 ± 0.09
Prostate	1.29 ± 0.11	1.04 ± 0.03	1.05 ± 0.16
Bulbourethral glands	1.10 ± 0.08	0.22 ± 0.24	0.97 ± 0.12

Histological findings

A thin capsule of bulbourethral glands consisted of dense irregular connective tissue with bundles of skeletal muscle fibers (Fig. 2a). Capsular trabeculae of loose connective tissue divided the parenchyma of the gland into lobules that contained secretory units. The epithelium of the secretory ducts was simple squamous. This epithelium changed to stratified cuboidal epithelium in some areas (Fig. 2b). Mucosal invaginations (folds) were connected to each other in the central part of the gland. The longest folds gave rise to smaller secondary folds. In the connective tissue of secondary folds, the collagen fibers, few skeletal muscle cells and fibroblasts were observed. The epithelium of the secretory tubuloalveolar units was simple cuboidal. The epithelial cells had spherical nuclei with clear chromatin (Fig. 2b).

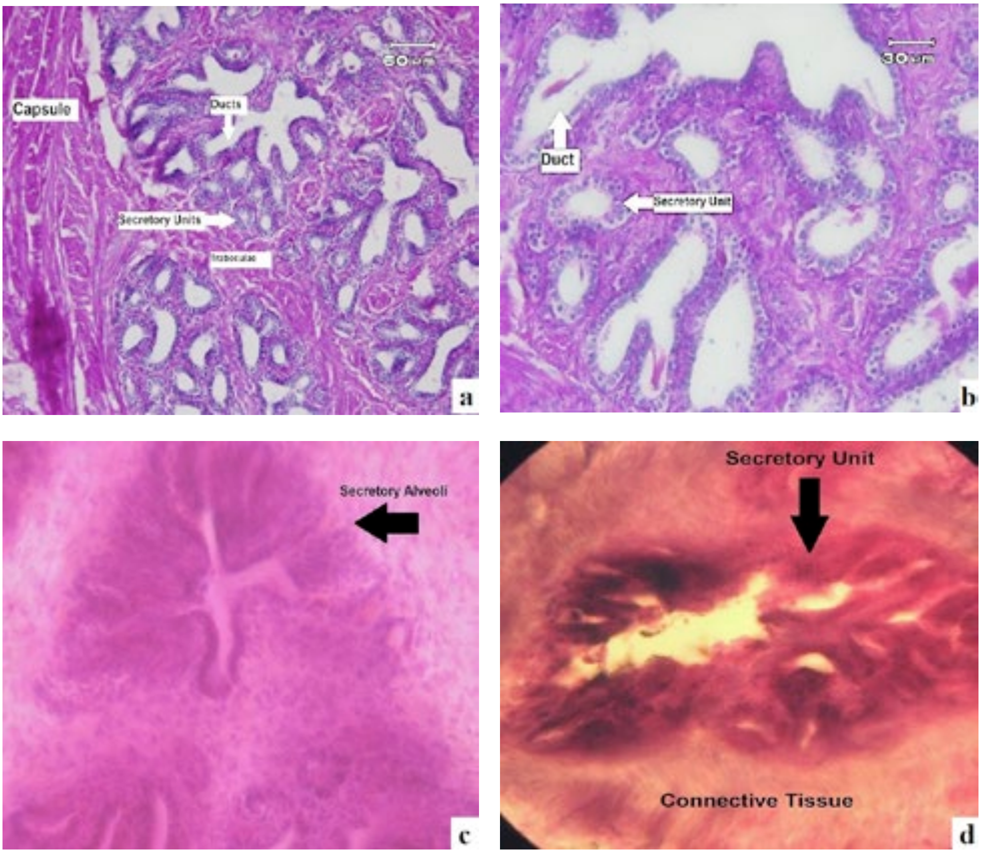

Figure 2 – Bulbourethral gland of male persian squirrel. **(a)** Transverse section. PAS-hematoxylin, 200×. **(b)** The epithelium of the secretory ducts was simple cuboidal. This epithelium changed to stratified cuboidal epithelium in some areas. PAS-hematoxylin, 400×. **(c)** No fat droplets were observed in secretory cells of alveoli. Oil red O, 100×. **(d)** No fat droplet was observed in secretory cells of alveoli. Sudan black B, 400×.

The prostate was surrounded by a dense collagenous connective tissue capsule without muscle fibers. Histologic studies revealed that the cranial part of this gland was divided in two portions by connective trabeculae (Fig. 3a). The caudal part of prostate was fully integrated between the two sides. Trabecular branches from the capsule divide the gland parenchyma into several secretory units. Trabeculae were connected to each other (Fig. 3b). Intertrabecular spaces were occupied by prostatic lobules. These lobules were composed of tubuloalveolar secretory glands. The glandular epithelial cells were simple columnar (Fig. 3c). The height of cells was varied. The prostate gland was completely attached to the dorsal wall of the urethra and the branching glandular ducts emptied directly into the colliculus seminalis. The main duct was found at the junction of the right and left lateral lobes. The epithelial tissue of the main duct changed to stratified columnar epithelium near the pelvic urethral opening (Fig. 4a).

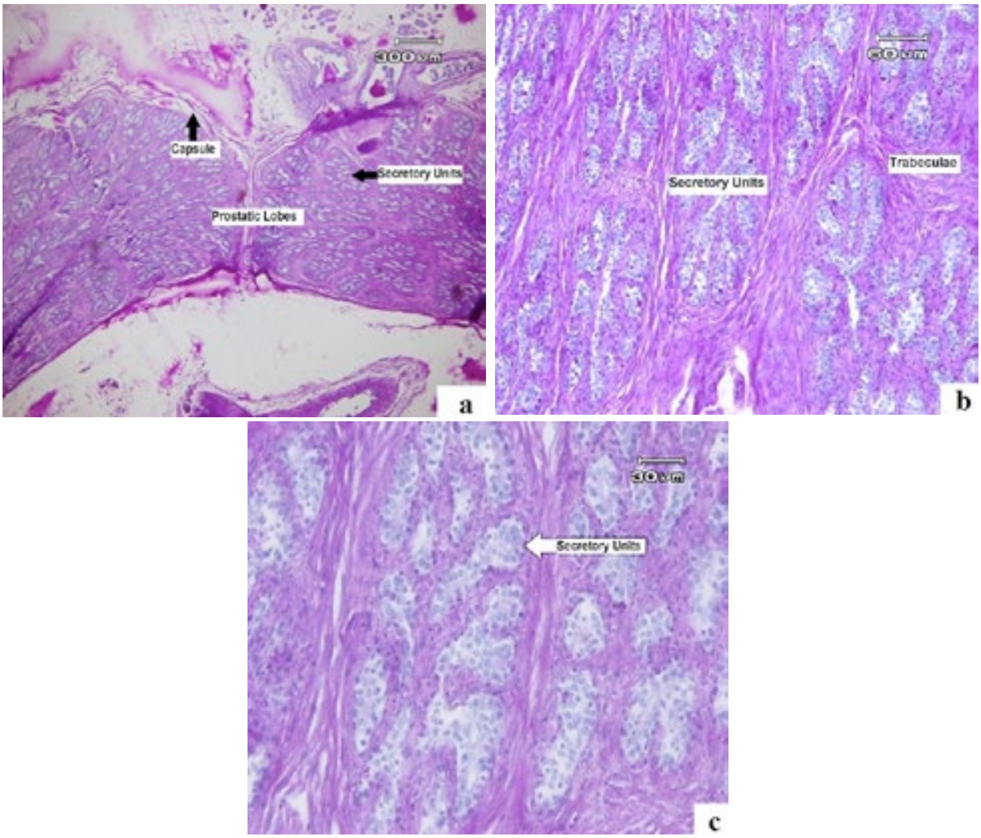

Figure 3 – Prostate gland of male Persian squirrel. **(a)** Overview of the prostate gland. The cranial part of this gland was divided in two portions through connective tissue trabeculae. Hematoxylin and eosin, 40×. **(b)** Cross section of the prostate gland. Trabeculae and glandular lobules with secretory units are shown. PAS-hematoxylin, 200×. **(c)** Prostate gland parenchyme. Secretory units with simple columnar epithelium with spherical nucleus. PAS-hematoxylin, 400×.

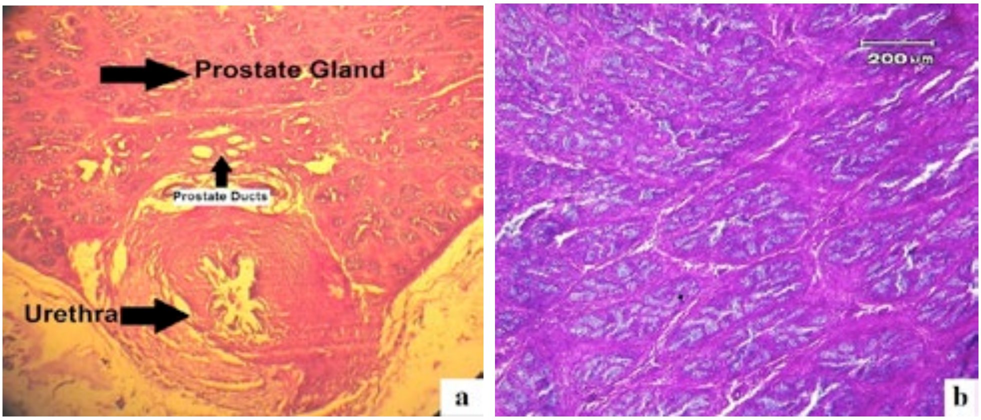

Figure 4 – Prostate gland of male Persian squirrel. **(a)** Cross section of prostate gland. The main ducts appear near the pelvic urethra. PAS-hematoxylin, 40×. **(b)** Cross section of secretory unit. Most PAS reaction is observed in interalveolar trabeculae. PAS-hematoxylin, 100×.

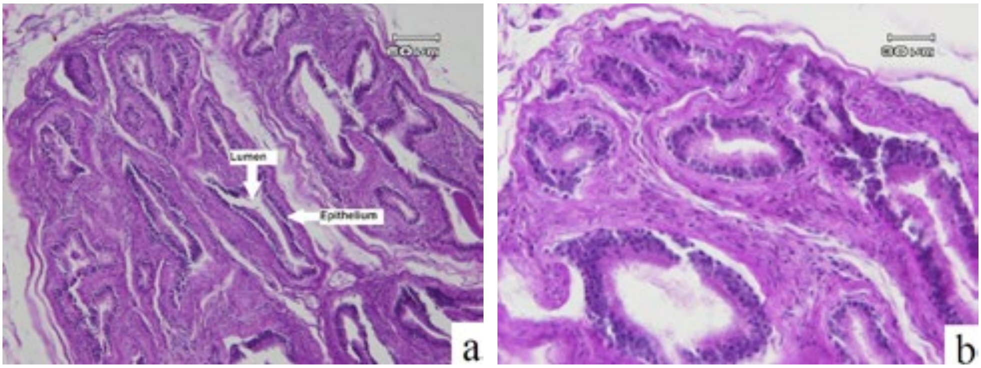

Figure 5 – Coagulating gland of male Persian squirrel. **(a)** Cross section. Alveolar secretory units with large lumen are observed. Pseudostratified columnar cells are seen and cells with long nucleus resembling smooth muscle cells are located around the secretory alveoles. Hematoxylin and eosin, 200×. **(b)** Cross section. PAS reactivity was limited to the basement membrane and connective tissue around the secretory alveoli. PAS-hematoxylin, 400×.

Histological studies of coagulating glands revealed that the secretory units of this alveolar gland had a large central lumen lined with pseudostratified columnar cells (Fig. 5a). The nuclei of the epithelial cells were spherical and clear. A collagenous capsule with connective tissue cells with long nucleus which resembled smooth muscle cells were situated around the secretory alveoles (fig. 5a). The ducts of coagulating glands were lined with stratified columnar epithelium and were surrounded by connective tissue completely.

Histochemical findings

Histochemical study of bulbourethral glands by PAS reaction showed that the highest positive reaction was observed in trabeculae depending on large amounts of connective tissue fibers in trabecules. The PAS reaction decreased in secondary mucosal folds due to decrement of connective tissue fibers (Fig. 2c). Moreover, PAS reaction was observed in the basal part of secretory alveoles, in the basement membrane. Histochemical evaluations by Oil Red O and Sudan Black B showed that there were no fat droplets in secretory cells of alveoles (Fig. 2b and d).

In prostate, most PAS reaction was observed in interalveolar trabecules. There was no PAS reaction on the luminal surface of secretory alveoles (Fig. 4b). The histochemical observations of prostatic alveoles upon staining for fat were similar to bulbourethral glands.

Histochemical studies of coagulating glands showed that the PAS reaction was limited to the basement membrane and connective tissue around the secretory alveoles (Fig. 5b). Similar to above mentioned glands, there was no positive reaction for lipids.

Discussion

The morphology of the accessory sex glands in the Persian squirrel is different from that in other rodents. For instance, there are no ampullary and vesicular glands in this creature. In fact, unlike other rodents, it possesses only three types of glands, namely prostate, bulbourethral glands, and coagulating glands. It has also been reported that ampullary glands are absent in the tomcat (Ellenport, 1975), blind mole rat (Gottreich et al., 2001), hedgehog (Mann, 1964), and boar (Getty, 1975). Moreover, it has been indicated that there are no vesicular glands in the tomcat (Ellenport, 1975), blind mole rat (Gottreich et al., 2001), and European mole (Brown et al., 1994; Racey, 1978).

The bulbourethral gland is located on the ischiocavernosus muscle at the base of the penis in the rat (Rowett, 1965) and greater cane rat (Adebayo et al., 2009). This gland in the Persian squirrel is located outside the pelvis in the perineal region. According to our observations, the gross morphology of bulbourethral gland of Persian squirrel was tortuous and labyrinthine, whereas it is pea-shaped, small and spheroid, and gourd-shaped respectively in the agouti (Mollineau et al., 2009), viscacha (Chaves et al., 2011), and greater can rat (Adebayo et al., 2009). The gland was covered with a capsule containing smooth and striated muscle in the greater cane rat (Adebayo et al., 2009) and the lining was simple columnar. The bulbourethral gland in the Persian squirrel is of the alveolar-duct type and its lining is simple cuboidal.

Studies carried out on the prostate of the agouti (Menezes et al., 2010), indicate that the gland is divided into two parts: dorsal and ventral. In the capybara (Menezes et al., 2010) and chinchilla (Cepeda et al., 2006), the prostate is divided into three equal lobules—two lateral and one medial. The morphology of the prostate in the Persian squirrel, as in the viscacha (Chaves et al., 2011) and dog (Miller et al., 1965), was macroscopically dense and homogenous. Nevertheless, microscopic studies revealed that a wall divided the cranial part of the gland into two separate parts, left and right.

It is reported that the prostate epithelial lining in the agouti is pseudostratified columnar (Mollineau et al., 2009). But it is simple columnar in the guinea pig (Cordeiro et al., 2004). The prostate lining in the Persian squirrel is simple cuboidal.

In different mammalian species, the presence and number of the bulbourethral glands vary. They are not present in the dog (Miller et al., 1965), while marsupials possess three pairs (Barbour, 1981). It is a non-paired gland in the rabbit (Dimitrov and Stamatova, 2011). As in the viscacha (Chaves et al., 2011), the bulbourethral gland in the Persian squirrel is paired and situated outside the pelvis in the perineal region.

Our investigations showed that the coagulating glands of the Persian squirrel are located in the craniolateral and dorsal part of the prostate and have a horn-like shape. These glands are located along the concave base of the vesicular glands in the agouti (Menezes et al., 2010), and have a prolonged and flat shape and reside medially to the vesicular glands in the lesser bandicoot rat (Hikim and Maiti, 1981).

Our observations revealed that connective tissue cells with long nucleus which resemble smooth muscle cells were situated around the secretory alveoli in the Persian squirrel. Similar cells have been described in the lesser bandicoot rat (Hikim and Maiti, 1981) and agouti (Menezes et al., 2010).

It has been indicated that the PAS reaction is negative for the prostate portion of the prostatic complex in the rabbit (Dimitrov and Stamatova, 2011) and for the prostate central zone in the chinchilla (Cepeda et al., 2006). Chaves et al. (2011) investigated the prostate gland in the viscacha and demonstrated that the two regions, namely central zone and peripheral zone, were PAS-positive.

In this study, the histochemical search for fat in the reproductive accessory glands in the Persian squirrel did not reveal a positive reaction. The highest PAS-positive reaction in these glands was in the connective trabeculae.

This study was the first step in the anatomical, histological, and histochemical examination of the accessory glands in the reproductive system of the male Persian squirrel. It is expected that the results achieved in this research will form the basis of further studies on the reproductive systems of this rodent.

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