Comparative characterization of the prostate gland in intact, and surgically and chemically neutered ferrets

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ABSTRACT

Surgical gonadectomy in ferrets was routinely performed in clinical practice in order to reduce their typical musky odor as well as inhibit aggressive behavior. Over recent years, however, early surgical neutering has been correlated with the occurrence of adrenal gland disease which causes various symptoms and prostatic hyperplasia. More recently, a synthetic long-acting GnRH analogue, deslorelin acetate, has been developed in order to overcome the above-mentioned complications. The aim of the present study was to compare the prostate gland in intact, and surgically and chemically neutered ferrets by means of deslorelin implants, from a morphological and morphometrical point of view. The macroscopic assessment of the prostate revealed a constant anatomical topography in all three categories. The prostatic tissue had similar organization, originating dorsally to the bladder

neck, extending laterally and then ventrally to the urethra, surrounding it entirely, dividing caudally into a dorsal and a ventral portion, and finally ending on the ventral surface of the urethra. In both the deslorelin-treated and the surgically-gonadectomized ferrets, a dramatic involution of the glandular prostatic tissue occurred, resulting in a decrease in acini size, more prominent in the chemically neutered ferrets, together with a hyperplasia of fibromuscular stroma. Epithelial acinar heights and acini cross-sectional areas measured from the prostatic tissues of the neutered ferrets showed significantly smaller values than those relating to the intact ferrets. Therefore, chemical castration by means of deslorelin implants, also being a non-surgical reversible procedure, could be considered an efficient method to prevent prostatic hyperplasia in ferrets.

Keywords: prostate, ferret, deslorelin, exotic small mammal, anatomy, histology.

INTRODUCTION

Despite the increasing popularity of ferrets as companion animals as well as the growing amount of scientific interest concerning this species [1,2], scant scientific studies dealing with the prostate gland of these mustelids exist, as opposed to what is the current state of art for other species, such as dogs [3,4,5,6], cats [7] and small exotic mammals, i.e. guinea pigs [8], chinchillas [9], rats [10], hamsters [11], gerbils [12,13], agoutis [14,15], squirrels [16], and rabbits [17]. In the past, this initially led to erroneous theories claiming the absence of the prostate gland in ferrets [18,19] and then to its controversial presence [20]. These mustelids, especially male ferrets, are characterized by a strong, musky smell; in order to reduce its intensity as well as inhibit aggressive behavior, surgical gonadectomy was routinely performed in clinical practice in the past [21-23]. Over recent years, however, early surgical neutering has been correlated with the occurrence of adrenal gland disease [24,25], a species-specific neoplastic condition. Chronic stimulation of the adrenal glands by a luteinizing hormone plays a key role in the pathogenesis of adrenocortical tumors in gonadectomized ferrets. Gonadotropin-releasing hormone (GnRH) is responsible for the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland. In intact ferrets, gonadotropins (FSH and LH) stimulate gonads to synthesize and secrete sexual hormones, which, by negative feedback, control the pulsatile release of GnRH and, consequently, that of LH and FSH. In neutered ferrets, however, the chronic stimulation of the adrenal receptors by uncontrolled gonadotropins (especially LH) leads to a hyperplastic response and eventually to the neoplastic evolution of the adrenal glands. Modified adrenal glands secrete abnormal quantities of the sexual hormones estradiol, 17-hydroxyprogesterone and androstenedione [24-26], which cause various symptoms including alopecia, pruritus, cutaneous atrophy, aggressive behavior, weight loss, lethargy, asthenia, vulvar swelling and prostatic hyperplasia in males [24,27-31]. Over the past few years, a synthetic long-acting GnRH analogue, deslorelin acetate, has been developed in order to overcome the above-mentioned complications. This molecule is a GnRH agonist having a seven-fold greater efficacy, a greater affinity for specific cellular receptors, and greater stability than its endogenous counterpart. It is commercialized in the form of a subcutaneous solid implant consisting of a cylindrical, biocompatible lipophilic matrix, containing the active substance opaque white in color and measuring 2.3 mm x 12.5 mm in diameter. It is applied subcutaneously and slowly absorbed; it is eliminated by the hepatic and the renal metabolism in the same way as naturally occurring GnRH. The continuous and prolonged release of low doses of the hydrosoluble active substance results in the inhibition of the hypothalamic-pituitary-gonadal (HPG) axis and, consequently, of the pituitary synthesis, and the secretion of gonadotropins (FSH and LH), thus leading to the suppression of

fertility [26,32-36] for a period of 10 to 40 months in treated ferrets [37]. To the authors' knowledge, scientific data concerning the morphological features of the prostate gland in ferrets are scant when it comes to intact ferrets [38], and absent with regard to surgically and chemically neutered ferrets. Therefore, the aim of the present study was to describe and compare the prostate in intact, and chemically and surgically neutered ferrets, from a morphological and morphometrical point of view, in order to reveal its properties and the differences in the study groups.

MATERIALS AND METHODS

The study population consisted of nine adult (aged 3-5 years) male ferrets: three intact, three surgically-gonadectomized and three chemically neutered by means of deslorelin implants (Suprelorin Virbac®) containing 4.7 mg of active substance, applied subcutaneously 6 to 12 months earlier. All ferrets died from diseases other than those affecting the urinary and reproductive tracts and tissues were collected following owners' permission. According to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, the Italian legislation (D. Lgs. n. 26/2014) does not require any approval by competent authorities or ethics committees, because this research did not influence any therapeutic decisions. The topography of the prostate gland was investigated in all ferrets by dissection, after placing the subjects in right lateral recumbency, removing the left lateral abdominal wall and resecting the homolateral posterior limb. The prostate gland was subsequently excised in its entirety and fixed in Bouin's solution for a minimum of 48 hours, dehydrated in alcohol, clarified in xylol, embedded in paraffin and finally serially transversely cut at 10µm by means of a rotary microtome. The sections were mounted on slides, stained with hematoxylin-eosin and Masson's trichrome, coverslipped with Entellan® and observed with a light microscope (Zeiss Axioplan®). Histological images were acquired using a Zeiss AxioCam ERc 5c® photo digital camera (Zeiss Group, Oberkochen, Germany) and were then processed with Adobe Photoshop CS®.

Acinar epithelial heights and cross-sectional areas of 300 acini were measured in the three study groups. The acini sizes were divided into seven groups: $\leq$ 2,000 µm^2, 2,001 to 4,000 µm^2, 4,001 to 6,000 µm^2, 6,001 to 8,000 µm^2, 8,001 to 10,000 µm^2, 10,001 to 12,000 µm^2 and $\geq$ 12,001 µm^2. DeltaPix InSight® measurement software was used for this analysis.

Statistical analysis

Statistical analysis was carried out in order to compare epithelial acinar heights and cross-sectional areas in the three groups, by menas of commercially available software (R 2.13.1). The normality of

the variables was assessed using the Shapiro-Wilk test. Since the data did not meet the assumption of Gaussian distribution, a non parametric test was employed. All data were expressed as mean ± standard deviation (SD) and median (interquartile range, IQR) and differences between the groups were evaluated using the Kruskal-Wallis test, with a significance level at $p < 0.01$. Microsoft Excel 2016 was used for tables and descriptive statistics.

RESULTS

GROSS ANATOMY OF THE PROSTATE GLAND

The prostate gland in ferrets is topographically located on the boundary between the abdominal and pelvic cavities, and it completely surrounds the urethra (Fig. 1a). It makes dorsal contact with the rectum and the deferent ducts which pass through the dorsal part of prostate and open into the prostatic part of the urethra; it makes ventral contact with the caudal part of the abdominal cavity floor and with the cranial part of the pelvic floor at the level of the pubis. In one obese ferret, interposition of abundant adipose tissue between the prostate and the above-mentioned structures was observed. From macroscopic investigation (Figs. 1b,c), it emerged that the prostate in ferrets extended for 15 mm from the caudal part of the bladder neck. In the intact ferrets, the prostate appeared round in shape, measuring up to 8 mm in thickness and 6 mm in width. On the other hand, in the neutered ferrets, it was more fusiform, reaching a maximum of 4 mm in thickness and width. Its surface was uniformly smooth and shiny as it was entirely covered by the peritoneum.

MICROSCOPIC FEATURES OF THE PROSTATE IN THE THREE STUDY GROUPS

Under the light microscope, observation of the prostate gland of the intact ferrets revealed its entirely compact architecture, with the disseminate part completely missing. The prostate was located on the dorsal (Fig. 2a), lateral (Fig. 2b) and ventral (Fig. 2c) surfaces of the bladder neck and urethra, forming a complete ring around these structures. The two deferent ducts passed through the dorsal and broadest part of the prostate and opened into the urethra (Fig. 2d). Connective tissue and muscular bands located laterally to the urethra divided the caudal part of the prostate into a dorsal and a ventral portion (Fig. 2e). The most caudal segment of the prostatic parenchyma was located exclusively on the ventral surface of the urethra, as its dorsal part, moving caudally, progressively decreased in size and eventually regressed (Fig. 2f). The prostatic gland was surrounded by a fibromuscular capsule from which variably-sized septa originated and divided the glandular parenchyma into irregular lobes and lobules. It was a compound tubuloalveolar gland, with a secretory epithelium consisting of

polyhedral cells of various heights based on their functional state, with basal rounded nuclei (Fig. 3a). The cytoplasm of the tallest cells was light in color and had a granular appearance. No lipid droplets were observed. The lumen of the adenomeres was filled with a secretion containing structurally homogeneous granules of different sizes. Smaller granules tended to aggregate in clusters (Fig. 3b).

In the surgically neutered ferrets, the prostate gland was found to have the same topography as in the intact ferrets. The glandular tissue, however, was quantitatively inferior, consisting of few tubuloalveolar adenomeres disseminated inside the abundant fibromuscular stroma (Fig. 4a). Most adenomeres showed signs of regression, cuboid epithelium (Fig. 3c), a reduced lumen and scarce secretion within which structurally homogeneous granules were found (Fig. 3d). At the level of the mediocaudal portion of the gland, excretory ducts opened individually into the ventral part of the urethra (Fig. 3d).

The prostate in the chemically neutered ferrets presented an analogous topography, with a significant prevalence of fibromuscular stroma (Fig. 4b). Several islands of glandular tissue consisting of small alveoli with a reduced or even inappreciable lumen were surrounded by well-developed fibromuscular stromal tissue (Figg. 3e,f).

Lastly, the present study revealed that ampullary glands were prominent in intact ferrets but were totally absent and entirely replaced by abundant connective tissue in both deslorelin-treated and surgically-gonadectomized ferrets (Fig. 5).

DISCUSSION

The existence of the prostate gland in ferrets has been under debate for a long time [20]. In fact, some authors have claimed its absence in this species [18,19], while others have described it on both a macroscopic and a microscopic level [29,38-41]. Identification difficulties are due to the indistinct appearance of the prostatic tissue in young, neutered [23], and deslorelin-treated ferrets [29,30,41,42]. On the other hand, in intact adult ferrets, the prostate appears as a small spindle-shaped mass surrounding the proximal part of the urethra, measuring 15 mm in length and 6 mm in width [29,38-40]. Among the three intact ferrets examined, the dimensions of the prostate were completely overlapping, maybe because the animals died in the same period (February-July), when the spermatogenic activity in the hob occurs [40]. The prostate and the ampullary glands were found to be the only accessory sexual glands present in ferrets therefore lacking bulbourethral glands and seminal vesicles; this finding was consistent with previous studies by Jacob and Poddar [28], and Orcutt [29]. Based on these anatomical data, ferrets resemble dogs [3], but differ from cats which have bulbourethral glands but lack ampullary glands [7], and from rodents [13] which possess seminal

vesicles and coagulation glands. Similarly to dogs and chinchillas, the prostate in ferrets completely surrounds the distal part of the bladder neck and the proximal urethra, and is located on the boundary between the abdominal and the pelvic cavities [3,9,29,30,39,40,42]. Unlike other carnivores and humans, the prostate does not have a bilobar organization in ferrets [3,43-46]. In addition, similarly to rodents, rabbits and men, it also lacks a visible external lobulation [38]. In agreement with research findings provided by O'Malley [30], Lewington [42], and Powers and Brown [40], the deferent ducts extend across the caudal half of the prostate gland and open into the urethra. Comparable to other carnivores [3,7], glandular secretions are released into the urethra through many small tubules unlike other species, such as hairy-nosed wombats (*Lasiorhinus latifrons*) [29,38,47] and rodents [13,48,49], which possess long prostatic ducts.

The prostate in ferrets, similarly to other domestic species [3,5,17,43], consists of tubuloalveolar glands surrounded by a fibromuscular capsule from which variably-sized fibrovascular septa depart and divide the glandular parenchyma into irregular lobules, in contrast to the distinct prostatic lobules of dogs [7,50]. Similarly to dogs [3,6,43,51] and rabbits [17], the glandular epithelial cells are cuboidal or columnar with basal rounded nuclei of different heights depending on the age and the functional state of the gland [3], and with a light-colored and granular-looking cytoplasm [29]. Unlike dogs [5], however, lipid droplets were not detected. The adenomeres were found to be filled with a glandular secretion containing, as in rabbits [17], several structurally-homogeneous granules; no lamellar concretions, normally found in the prostate glands of dogs [3,43,51] were noted. Microscopic investigation has revealed the absence of the disseminate part of the prostate gland, which, instead, is present in domestic carnivores in the form of scattered lobules in the wall of the urethra [7,50,51].

The present study provided the first macroscopic and microscopic comparative description of the prostate gland in intact, surgically neutered and chemically neutered (deslorelin-treated) ferrets. From a macroscopic point of view, the prostate gland in surgically and chemically neutered ferrets has been found to be smaller than that belonging to intact ferrets; however, as opposed to previous reports by Orcutt [29] and O'Malley [30], it was always macroscopically visible to the naked eye. More specifically, while its length in surgically and chemically neutered ferrets was comparable to that in intact ferrets, its width and height were slightly lower in the former groups, measuring, on average, 4x4 mm and 4x3 mm, respectively. On the microscopic level, all prostatic samples had the same organization: at its cranial origin, few adenomeres were present dorsally to the bladder neck; the prostatic tissue then extended laterally and then ventrally to the urethra, surrounding it entirely; it caudally divided into a dorsal and a ventral portion, and finally ended, with a small cluster of

adenomeres, on the ventral surface of the urethra. To the authors' knowledge, such a detailed description of the microscopic organization of the prostate gland in ferrets has not previously been provided in the literature. Consistent with Orcutt [29], O'Malley [30] and Lewington [42], both surgical castration and chemical neutering by means of deslorelin implants resulted in a dramatic decrease in glandular prostatic tissue, as opposed to an increase in fibromuscular stroma. In neutered mice, hyperplasia of the fibromuscular stroma (of moderate degree 8 days after surgery, increasing in degree with the increase in time after castration) was identified, and epithelial cells lining the acini proliferated into several layers, without however showing squamous metaplasia [52]. Castration in dogs leads to atrophy of the prostate gland, characterized primarily by atrophy of the acini [6]. Epithelial acinar heights and acini cross-sectional areas measured from the prostatic tissues of the surgically and chemically neutered ferrets showed significantly smaller values than those relating to the intact ferrets (6.09 μm (5.65-6.69) and 1,974 μm^2 (1,027-6,399) in surgically neutered ferrets, *versus* 19.58 μm (17.21-22.47) and 8,835 μm^2 (2,690-26,671) in intact ferrets, respectively; $p < 0.00000$. 3.74 μm (3.21-4.42) and 1,055 μm^2 (576-1,982) in chemically neutered ferrets, *versus* 19.58 μm (17.21-22.47) and 8,835 μm^2 (2,690-26,671) in intact ferrets, respectively; $p < 0.00000$) (Tables 1, 2). Table 1, illustrating the percentage distribution of acini cross-sectional areas in the three study groups, shows how adenomeres having a cross-sectional area > 12,000 μm^2 accounted for 47% of all adenomeres in intact ferrets while showing a progressively decreasing trend in surgically neutered ferrets (15%) and deslorelin-treated ferrets (5%). Acini having a cross-sectional area of 0-2,000 μm^2 showed an opposite trend, making up 21% of all adenomeres in intact ferrets, accounting for 50% in surgically neutered ferrets, and increasing all the way up to 74% in chemically neutered ferrets. Adenomeres having an area of 2,001-12,000 μm^2 were evenly distributed throughout the three study groups. In addition, in comparing surgical and chemical castration, epithelial acinar heights and cross-sectional areas of prostatic acini of deslorelin-treated ferrets were significantly smaller than those relating to surgically neutered ferrets (3.74 μm (3.21-4.42) in chemically neutered ferrets, versus 6.09 μm (5.65-6.69) in surgically neutered ferrets and 1,055 μm^2 (576-1,982) in chemically neutered ferrets, versus 1,974 μm^2 (1,027-6,399) in surgically neutered ferrets, respectively; $p < 0.00000$) (Tables 1, 2). Treatment with deslorelin implants seemed, therefore, to induce more pronounced prostatic glandular atrophy when compared to a surgical gonadectomy. It is possible that, on the macroscopic level, the limited size reduction of the prostate gland after neutering is due to a partial replacement of glandular tissue by connective tissue. Furthermore, it should be noted that the maximum height of the acini in neutered ferrets was inferior to the minimum height of acini in intact ferrets (Table 2).

In conclusion, the findings of the present study revealed that the prostate gland was both macroscopically and microscopically visible in all three groups of ferrets. In both the chemically and the surgically neutered ferrets, involution of the prostatic glandular tissue occurred, resulting in a decrease in acini size, together with hyperplastic proliferation of the connective tissue. In addition, regression of prostatic tissue was found to be more prominent in the deslorelin-treated ferrets than in the surgically castrated ferrets. Therefore, chemical castration by means of deslorelin implants, also being a non-surgical reversible procedure, could be considered an efficient method to prevent prostatic hyperplasia in ferrets.

REFERENCES

[1] Beeber NL. Abdominal surgery in ferrets. Vet Clin North Am Exot Anim Pract 2000;3:647-62.

[2] Fox JG, Marini RP. Taxonomy, history, and use. In: Fox JG, Marini RP, editors. Biology and diseases of the ferret, 3rd ed. Ames: Wiley Blackwell; 2014, p. 5-22.

[3] Berg OA. The normal prostate gland of the dog. Acta Endocrinol 1958;27:129-39.

[4] Gordon N. Surgical anatomy of the bladder, prostate gland, and urethra in the male dog. J Am Vet Med Assoc 1960;136:215-21.

[5] Gouvelis A, Baker JR, Rosenkrantz H. Histochemistry of the canine prostate. Inves Urol 1971;8:426-41.

[6] Lai CL, van den Ham R, van Leenders G, van der Lugt J, Teske E. Comparative characterization of the canine normal prostate in intact and castrated animals. Prostate. 2008;68:498-507. https://doi.org/10.1002/pros.20721.

[7] Dyce KM, Sack WO, Wensing CJG. Textbook of Veterinary Anatomy. St Louis: Elsevier Saunders; 2010.

[8] Neuhaus J, Dorschner W, Mondry J, Stolzenburg JU. Comparative anatomy of the male guinea-pig and human lower urinary tract: histomorphology and three-dimensional reconstruction. Anatom Histol Embryol 2001;30:185-92. https://doi.org/10.1046/j.1439-0264.2001.00323.x|

[9] Cepeda RC, Adaro LA, Peñailillo PG. Morphometric variations of chinchilla laniger prostate and plasmatic testosterone concentration during its annual reproductive cycle. Int J Morphol 2006;24:89. https://doi.org/10.4067/S0717-95022006000100017.

[10] Jesik CJ, Holland JM, Lee C. An anatomic and histologic study of the rat prostate. Prostate 1982;3:81-97. https://doi.org/10.1002/pros.2990030111.

[11] Toma JG, Buzzell GR. Fine structure of the ventral and dorsal lobes of the prostate in the young adult Syrian hamster, *Mesocricetus auratus*. Am J Anat 1988;181:132-40. https://doi.org/10.1002/aja.1001810203.

[12] Góes RM, Zanetoni C, Tomiosso TC, Ribeiro DL, Taboga SR. Surgical and chemical castration induce differential histological response in prostate lobes of Mongolian gerbil. Micron 2007;38:231-36. https://doi.org/10.1016/j.micron.2006.06.016.

[13] Rochel SS, Bruni CA, Taboga SR, Vilamaior PS, Góes RM. Lobe identity in the Mongolian gerbil prostatic complex: a new rodent model for prostate study. Anat Rec 2007;290:1233-47. https://doi.org/10.1002/ar.2058.

[14] Mollineau WM, Adogwa AO, Garcia GW. The gross and micro anatomy of the accessory sex glands of the male agouti (*Dasyprocta leporina*). Anat Histol Embryol 2009;38:204-7. https://doi.org/10.1111/j.1439-0264.2008.00922.x.

[15] Borges EM, Branco E, de Lima AR, Leal LM, Martins LL, Reis AC, et al. Morphology of accessory genital glands of spotted paca (*Agouti paca Linnaeus*, 1766). Anim Reprod Sci 2014;145:75-80. https://doi.org/10.1016/j.anireprosci.2013.12.014.

[16] Çakir M, Karatas A. Histo-anatomical studies on the accessory reproductive glands of the anatolian souslik (*Spermophilus xanthoprymnus*) (*Mammalia: Sciuridae*). Anat Histol Embryol 2004;33:146-50. https://doi.org/10.1111/j.1439-0264.2004.00517.x.

[17] Vásquez B, Del Sol M. Complejo prostático en el conejo (*Oryctolagus cuniculus*). Rev Chil Anat 2002;20:175-80. https://doi.org/10.4067/S0716-98682002000200010.

[18] Thornton PC, Wright PA, Sacra PJ, Goodier TE. The ferret, *Mustela putorius furo*, as a new species in toxicology. Lab Anim 1979;13:119-24.

[19] Capello V. Approccio clinico e chirurgia preventiva del furetto domestico. Veterinaria 1995;1:7-19.

[20] Rosenthal KL. Adrenal gland disease in ferrets. Vet Clin North Am Small Anim Pract 1997;57:401-18. https://doi.org/10.1016/S0195-5616(97)50039-8.

[21] Bennett RA, Pye GW. General surgery. In: Lewington JH, editor. Ferret husbandry, medicine and surgery. 2nd ed. Philadelphia: Saunders; 2007, p. 430-39.

[22] Lightfoot T, Rubinstein J, Aiken S, Ludwig L. Soft tissue surgery. In: Quesenberry KE, Carpenter JW, editors. Ferrets, rabbits and rodents: Clinical medicine and surgery, 3rd ed. Philadelphia: Saunders; 2012, p. 154.

[23] Mehler SJ. Surgery. In: Fox JG, Marini RP, editors. Biology and diseases of the ferret, 3rd ed. Ames: Wiley Blackwell; 2014, p. 285-310.

[24] Schoemaker NJ, Schuurmans M, Moorman H, Lumeij JT. Correlation between age at neutering and age at onset of hyperadrenocorticism in ferrets. J Am Vet Med Assoc 2000;216:195-97. https://doi.org/10.2460/javma.2000.216.195.

[25] Schoemaker NJ, Teerdsb KJ, Molc JA, Lumeij JT, Thijssen JH, Rijnberk A. The role of luteinizing hormone in the pathogenesis of hyperadrenocorticism in neutered ferrets. Mol Cell Endocrinol 2002;197:117-25. https://doi.org/10.1016/S0303-7207(02)00285-X.

[26] Wagner RA, Finkler MR, Fecteau KA, Trigg TE. The treatment of adrenal cortical disease in ferrets with 4.7 mg Deslorelin acetate implants. J Exot Pet Med 2009;18:146-52. https://doi.org/10.1053/j.jepm.2008.11.003.

[27] Rosenthal KL, Peterson ME, Quesenberry KE, Hillyer EV, Beeber NL, Moroff SD, et al. Hyperadrenocorticism associated with adrenocortical tumor or nodular hyperplasia of the adrenal gland in ferrets: 50 cases (1987-1991). J Am Vet Med Assoc 1993;203:271-75.

[28] Coleman GD, Chavez MA, Williams BH. Cystic prostatic disease associated with adrenocortical lesions in the ferret (*Mustela putorius furo*). Vet Path 1998;35:547-49. https://doi.org/10.1177/0300985898035006121O.

[29] Orcutt CJ. Ferret urogenital diseases. Vet Clin North Am Exot Anim Pract 2003;6:113-38. https://doi.org/10.1016/S1094-9194(02)00023-3.

[30] O'Malley B. Clinical anatomy and physiology of exotic species. Philadelphia: Saunders; 2005.

[31] Jekl V, Hauptman K. Reproductive medicine in ferrets. Vet Clin North Am Exot Anim Pract 2017;20:629-63. https://doi: 10.1016/j.cvex.2016.11.016.

[32] Wagner RA, Piche CA, Jöchle W, Oliver JW. Clinical and endocrine responses to treatment with deslorelin acetate implants in ferrets with adrenocortical disease. Am J Vet Res 2005;66:910-14. https://doi.org/10.2460/ajvr.2005.66.910.

[33] Schoemaker NJ, van Deijk R, Muijlaert B, Kik MJ, Kuijten AM, de Jong FH, et al. Use of a gonadotropin releasing hormone agonist implant as an alternative for surgical castration in male ferrets (*Mustela putorius furo*). Theriogenology 2008;70:161-7. https://doi.org/10.1016/j.theriogenology.2008.03.006.

[34] Neal LB. Surgical management of adrenal tumors and insulinomas in ferrets. J Exot Pet Med 2011;20:206-16. https://doi.org/10.1016/j.cvex.2016.07.004.

[35] Lennox AM, Wagner R. Comparison of 4,7 mg deslorelin implants and surgery for the treatment of adrenocortical disease in ferrets. J Exot Pet Med 2012;21:332-35. https://doi.org/10.1053/j.jepm.2012.09.001.

[36] Schoemaker NJ. Gonadotrophin-releasing hormone agonists and other contraceptive medications in exotic companion animals. Vet Clin North Am Exot Anim Pract 2018;21:443-64. https://doi.org/10.1016/j.cvex.2018.01.011.

[37] Risi E. Control of reproduction in ferrets, rabbits and rodents. Reprod Domest Anim 2014;49Suppl:81-86. https://doi.org/10.1111/rda.12300.

[38] S. Jacob, S. Poddar, Morphology and histochemistry of the ferret prostate, Acta Anat. 1986;125:268-73. https://doi.org/10.1159/000146175.

[39] Pollock CG. Disorders of the Urinary and Reproductive Systems. In: Quesenberry KE, Carpenter JW, editors. Ferrets, rabbits and rodents: Clinical medicine and surgery, 3rd ed, Philadelphia: Saunders; 2012, p. 46-61.

[40] Powers LV, Brown SA. Basic anatomy, physiology and husbandry. In: Quesenberry KE, Carpenter JW, editors. Ferrets, rabbits and rodents: Clinical medicine and surgery, 3rd ed, Philadelphia: Saunders; 2012, p. 1-12.

[41] Evans HE, An NQ. Anatomy of the ferret. In: Fox JG, Marini RP, editors. Biology and diseases of the ferret, 3rd ed. Ames: Wiley Blackwell; 2014, p. 64-5.

[42] Lewington JH. Ferret husbandry, medicine and surgery. 2nd ed. Philadelphia: Saunders; 2007.

[43] Barone R. Anatomie comparée des mammifères domestiques. Splancnologie II. Paris: Vigot; 2001.

[44] Clemente CD. Anatomy, a regional atlas of the human body. Baltimore, Philadelphia: Lippincott Williams & Wilkins; 2011.

[45] Evans HE, De Lahunta A. Miller's anatomy of the dog. St Louis: Elsevier Saunders; 2013.

[46] ICVGAN, International Committee on Veterinary Gross Anatomical Nomenclature. Nomina Anatomica Veterinaria. 6th ed. Hannover, Columbia, Gent, Sapporo; 2017.

[47] Barbour RA., Histology and histochemistry of the accessory reproductive glands in the male hairy nosed wombat. Histochemistry 1981;72:133-48. https://doi.org/10.1007/BF00496788.

[48] Pinheiro PF, Almeida CC, Segatelli TM, Martinez M, Padovani CR, Martinez FE. Structure of the pelvic and penile urethra-relationship with the ducts of the sex accessory glands of the Mongolian gerbil (*Meriones unguiculatus*). J Anat 2003;202:431-44. https://doi.org/10.1046/j.1469-7580.2003.00181.x|.

[49] Adebayo AO, Akinloye AK, Olukole SG, Ihunwo AO, Oke BO. Anatomical and immunohistochemical characteristics of the prostate gland in the greater cane rat (*Thryonomys swinderianus*). Anat Histol Embryol 2015;44:138-45. https://doi.org/10.1111/ahe.12122.

[50] Eurell JA, Frappier BL. Dellmann's textbook of veterinary histology. Ames: Blackwell; 2006.

[51] König HE, Liebich HG. Veterinary anatomy of domestic mammals. Berlin: Schattauer Verlag; 2007.

[52] Brandes D, Bourne G. The histochemistry of the prostate in normal and in castrated and hormone-treated mice and of prostatic homografts exposed to 20-methyl-cholanthrene. Br J Exp Pathol 1954;35:577-88.

Table 1 - % distribution of prostate acini in intact, and surgically and chemically neutered ferrets by range of cross-sectional area, mean ± standard deviation (sd) values and median (interquartile range, IQR).

Cross- sectional area	Intact ferrets*	Surgically neutered ferrets*	Chemically neutered ferrets*
0-2,000 μm^2	21	50	74
2,001-4,000 μm^2	13	16	9
4,001-6,000 μm^2	9	7	5
6,001-8,000 μm^2	5	5	2
8,001-10,000 μm^2	3	3	2
10,001-12,000 μm^2	2	4	3
> 12,000 μm^2	47	15	5
Mean ± sd (μm^2)	21,770±31,524	6,197±10,409	2,580±5,536
Median (IQR)	8,835 (2,690-26,671)	1,974 (1,027-6,399)	1,055 (576-1,982)
Range (min-max)	102-231,370	46-73,536	20-73,418

This morphometrical analysis was carried out on 300 acini for each ferret.
* refers to not normally distributed data.

Table 2 - % distribution of prostate acini in intact, and surgically and chemically neutered ferrets by range of epithelial acinar height, mean ± standard deviation (sd) values and median (interquartile range, IQR).

Epithelial acinar height	Intact ferrets*	Surgically neutered ferrets	Chemically neutered ferrets*
< 3 µm	0	0	17
3-8 µm	0	98	82
9-13 µm	9	2	1
14-18 µm	26	0	0
19-23 µm	43	0	0
> 23 µm	22	0	0
Mean ± sd (µm)	19.99±4.80	6.11±0.87	3.89±1.05
Median (IQR)	19.58 (17.21-22.47)	6.09 (5.65-6.69)	3.74 (3.21-4.42)
Range (min-max)	12.00-35.25	4.26-7.79	2.44-6.82

This morphometrical analysis was carried out on 100 epithelial cells for each ferret.
* refers to not normally distributed data.

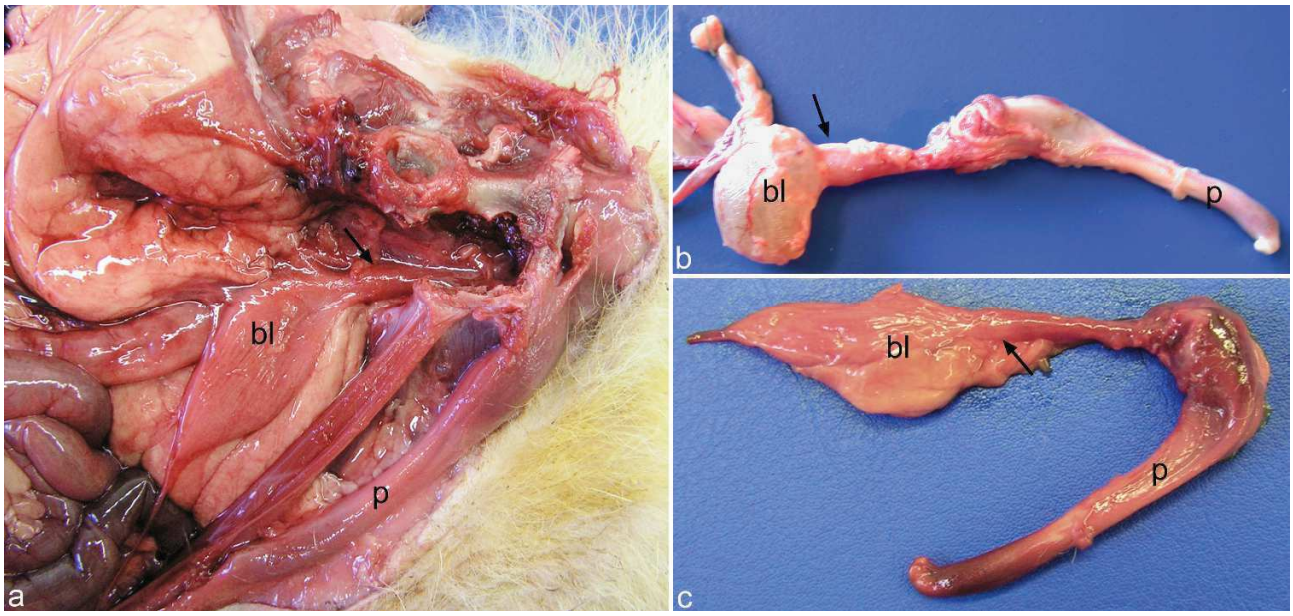

Fig. 1 Gross anatomy of the prostate in the intact (a, b) and the surgically neutered (c) ferrets. The prostate gland (arrow) is located between the abdominal and pelvic cavities, between the distal part of the bladder (bl) and the origin of the urethra. p: penis.

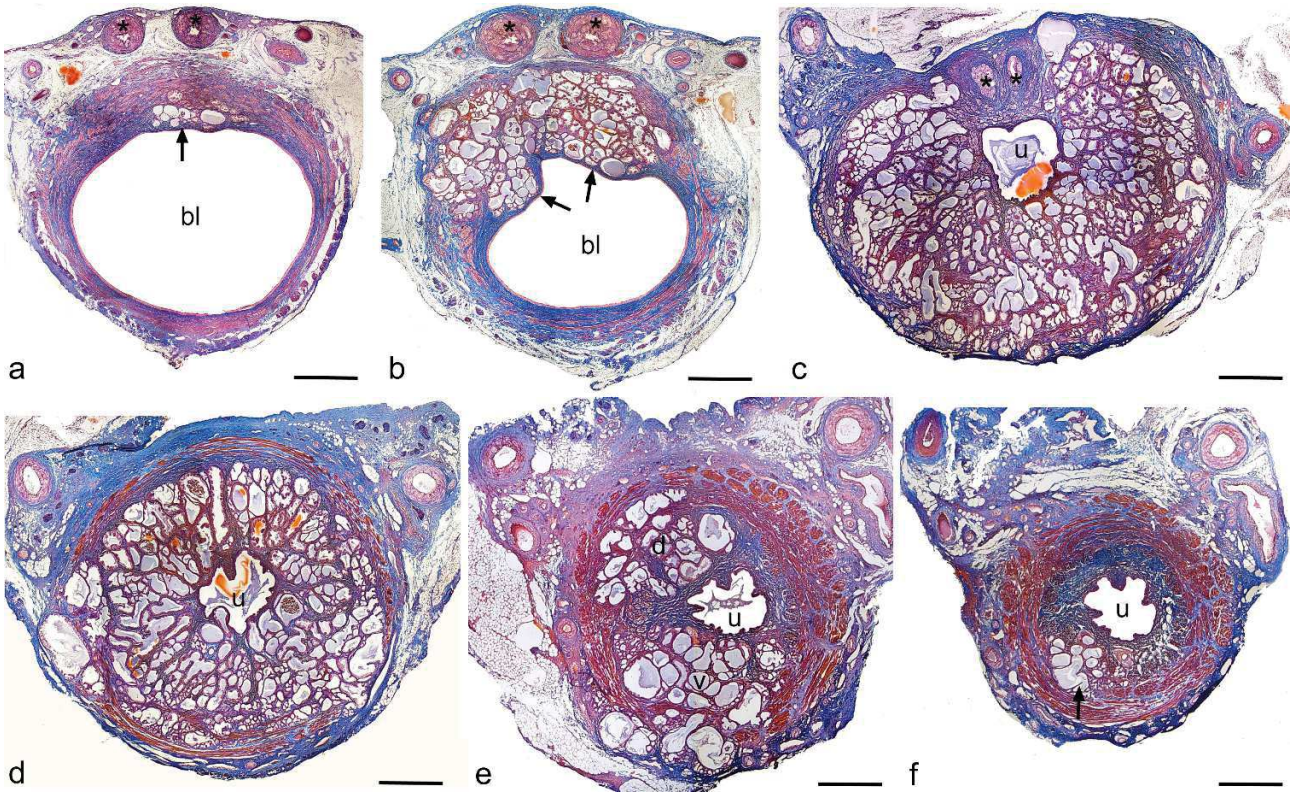

Fig. 2 Trichrome Masson's staining of representative transverse sections illustrating the distribution, from rostral to caudal, of the prostate in intact ferrets. a: The prostate gland (arrow) originates dorsally to the bladder neck; b: The prostate gland (arrow) extends laterally to the bladder (bl) and makes dorsal contact with deferent ducts (asterisks); c: Point of maximum extension of the prostate gland. Note that the deferent ducts (asterisks) pass through the gland before opening into the urethra (u); d: The prostate completely surrounds the urethra (u) at the level of the opening of the deferent ducts into the urethra; e: Connective tissue and smooth muscular bands divide the prostate into a dorsal (d) and a ventral (v) portions, and f: The caudal extremity of the prostate gland (arrow) is located ventrally to the urethra (u). Bar = 1 mm.

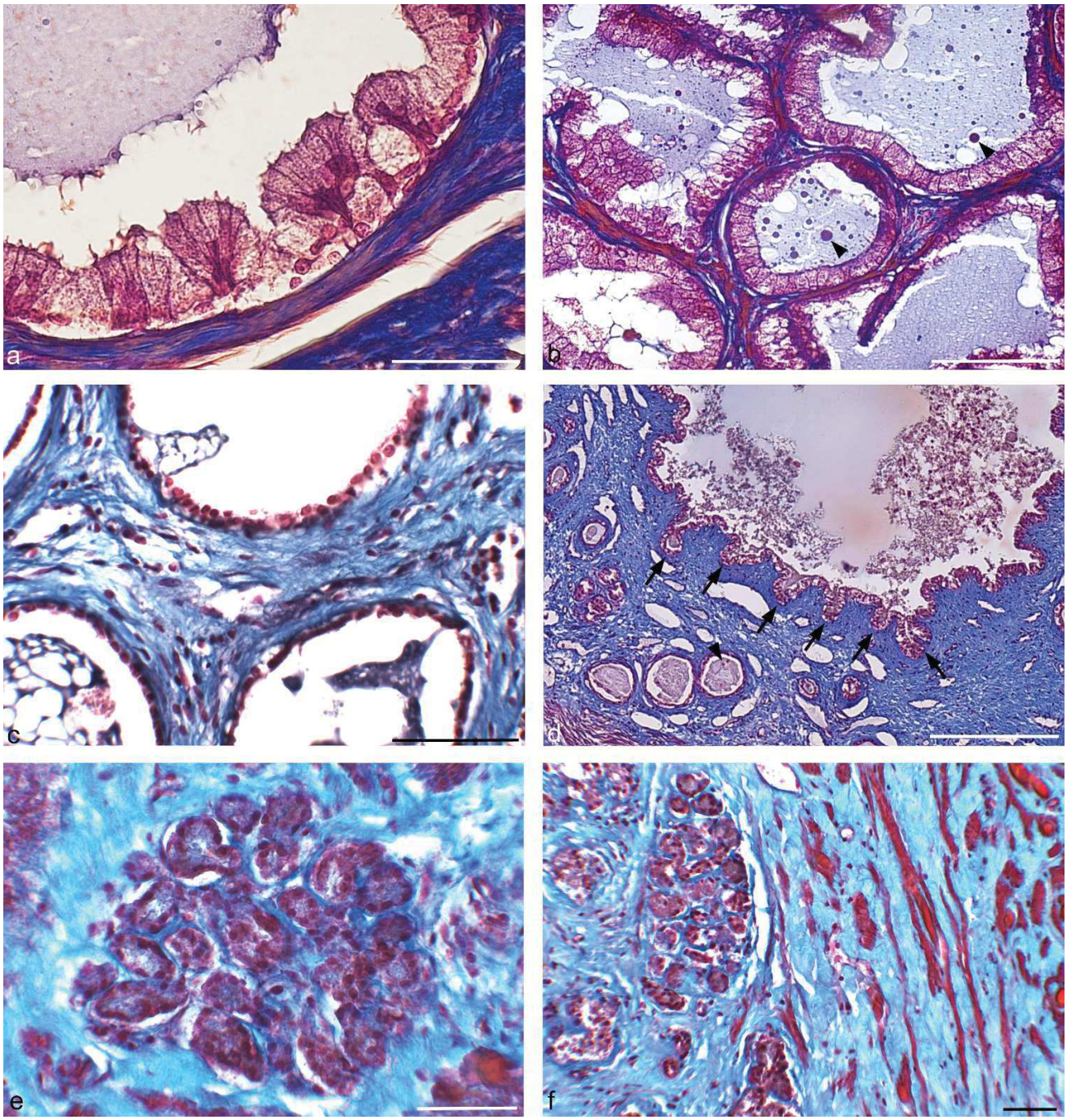

Fig. 3 Microscopic structure of the prostate gland in intact (a, b), surgically neutered (c, d) and chemically neutered (e, f) ferrets (Masson's Trichrome). a: The secretory epithelium is composed of a single layer of columnar cells with elongated or rounded basal nuclei. Bar = 50 µm; b: The acinar lumen of the gland contains some granules of different sizes with a homogeneous structure (arrow head). Bar = 100 µm; c: In the surgically neutered ferrets, the acinar epithelium appears cuboidal. Bar = 100 µm; d: Numerous short prostatic ducts open into the urethral lumen (arrows). Note the decrease in the acinar lumen and the presence of small granules (arrowhead). Bar = 200 µm; e: In the neutered ferrets, the acini are small in size with a reduced or inconspicuous lumen. Bar = 50 µm, and f: Abundant fibromuscular stroma replaces the acini of the gland. Bar = 50 µm.

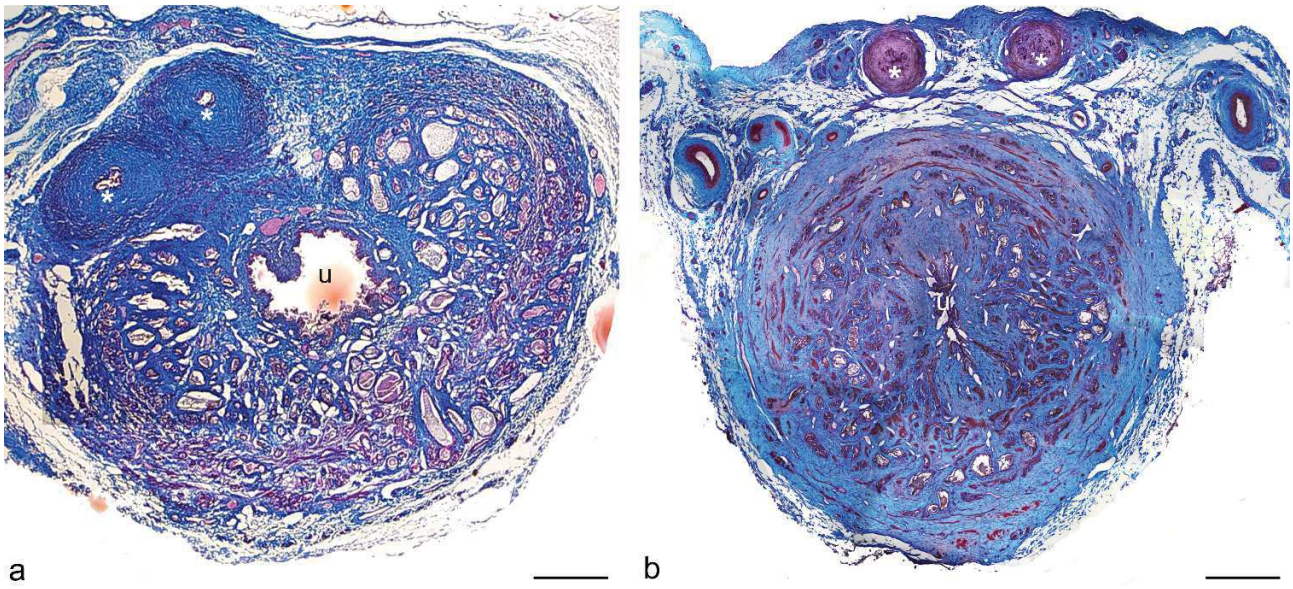

Fig. 4 Cross section of a prostate gland in surgically (a) and chemically (b) neutered ferrets (Masson's Trichrome). Note that the prostate acini of the chemically neutered ferrets are smaller than those of the surgically neutered ferrets, and the loose connective tissue is particularly abundant in the glands of the latter. The asterisks indicate the deferent ducts. Bar = 500 μm.

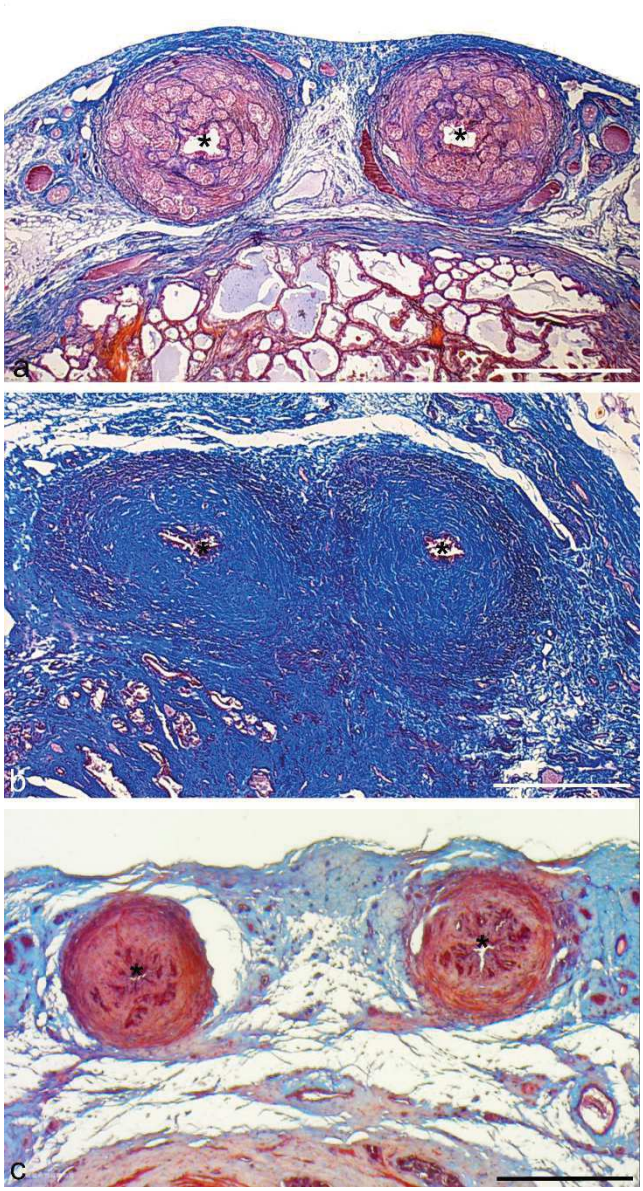

Fig. 5 Deferent ducts (asterisks) in intact (a), and surgically (b) and chemically (c) neutered ferrets (Masson's Trichrome). Notice how the ampullary glands, well developed in the intact ferrets (a), are absent in the surgically neutered ferrets (b), and atrophic in the chemically neutered ferrets (c). The asterisks indicate the deferent ducts. Bar = 350 µm.

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