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Ovariectomy increases the phenotypic plasticity of the female prostate epithelium in the Mongolian gerbil (*Meriones unguiculatus*)

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Abstract. The female prostate is a reproductive gland that typically presents a morphology similar to that of the male gland and is highly developed in female Mongolian gerbils. Two main cell populations compose the epithelium gland: basal and secretory luminal cells. However, during postnatal development, diverse secretory cell phenotypes are distributed among the typical ones. Prostate homeostasis is under the control of sexual hormones, such as oestrogen and progesterone. After hormonal deprivation the female gland undergoes several morphophysiological changes. The objective of this study was to identify and characterise, structurally and ultrastructurally, the cellular heterogeneity of the female prostate epithelium in normal conditions and after ovariectomy. Histological routine stains, such as haematoxylin–eosin, periodic acid–Schiff and silver impregnation, as well as immunocytochemical techniques were used to enable identification of the different cell types. Some secretory cells types were identified and characterised as mucinous, basophil, clear, ciliated, droplet, spumous and neuroendocrine cells. Population tally data showed that the hormonal suppression caused by ovariectomy resulted in a decrease in the proportions of basophil and clear cells and an increase in spumous cells. Thus, the secretory epithelial cells of the female gerbil prostate are not morphologically and functionally uniform, presenting a phenotypical plasticity according to the hormonal environment in which they operate.

Additional keywords: cell types, electron microscopy, hormonal deprivation, immunocytochemistry, secretory cells.

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Introduction

The existence of the female prostate has been established for both humans and rodents and their morphological similarities noted through histological methods (Shehata 1975, 1980; Gross and Didio 1987; Zaviačič *et al.* 2000*a*, 2000*b*; Santos and Taboga 2006), both histochemical and immunohistochemical (Tepper *et al.* 1984; Wernert *et al.* 1988, 1992; Zaviačič *et al.* 1997; Sloboda *et al.* 1998; Zaviačič and Ablin 2000; Custódio *et al.* 2004; Shinohara *et al.* 2013; Zanatelli *et al.* 2014).

The physiology of this organ and its functional role in females is the target of a growing number of studies (Zaviačič *et al.* 2000*a*, 2000*b*; Santos *et al.* 2003, 2006; Custodio *et al.* 2008, 2010; Da Silva *et al.* 2013). Research indicates similarities in the chemical constituents of the prostatic fluid released during female ejaculation with male prostatic fluid, and prostate-specific antigen (PSA) has been detected in the serum and urine of women (Zaviačič *et al.* 1993; Zaviačič 1999; Zaviačič and Ablin 2000; Schmidt *et al.* 2001). It is believed to have a function related to sexual behaviour (Zaviačič *et al.* 1993) with some studies associating it with the Gräfenberg spot (G-spot), since the stimulation of this point leads to female ejaculation (Schubach 2002), while other researchers argue that both are the same structure (Addiego *et al.* 1981; Hines 2001). In addition, the organ is susceptible to benign and malignant prostatic lesions (Uzoaru *et al.* 1992; Zaviačič *et al.* 1993, 2000*a*; Dodson *et al.* 1995; Sloboda *et al.* 1998; Zaviačič 1999; Custodio *et al.* 2008, 2010; Da Silva *et al.* 2013; Shinohara *et al.* 2013) similar to those developed by the male gland, especially during senescence.

The gerbil is a suitable experimental model to study the female gland, which has a para-urethral location in close contact with the wall of the median and distal urethra. Histologically it is

made up of the same components as the male prostate: duct–alveolar units within a muscle–fibre matrix. The duct portions that are inserted in the para-urethral muscle and that emerge in the urethra are lined by cuboidal epithelium and present a smaller diameter than the alveolar secreting portions. The latter are located more distantly to the urethra and may vary in shape and size, being lined by a cubic or columnar epithelium (Santos and Taboga 2006).

Like all organs of the female genital tract, prostate morpho-physiological maintenance throughout postnatal development is under the control of sexual hormones. Oestrogen (E2) and progesterone (P4) are steroid hormones produced mainly by the ovaries and both participate in the proliferation, differentiation and maintenance of the prostatic gland (Santos and Taboga 2006). Oestrogen and progesterone receptors are present in female gerbil prostate, as shown by immunocytochemical studies, proving the action of these hormones in the tissue (Shinohara *et al.* 2013; Zanatelli *et al.* 2014). Cyclic oscillations in E2 and P4 during the oestrous cycle are responsible for the morphological changes that occur in the female gerbil prostate. Suppression of these hormones, caused by ovariectomy, substantially compromises the gland's function; it typically becomes atrophic, which shows that it is highly sensitive to alterations in the serum levels of these hormones (Zanatelli *et al.* 2014).

Although the epithelial compartment is typically formed by secretory and basal cells (Santos *et al.* 2003), different cell types can be distinguished among them. The objective of this study was to characterise, structurally and ultrastructurally, this cellular heterogeneity of the female prostate secretory compartment, under normal conditions and after ovariectomy.

Materials and methods

Animals and sample preparation

Eighteen female gerbil (*Meriones unguiculatus*, Gerbillinae: Muridae) were used in this study, six for each inclusion material (historesin, paraffin and araldite resin). Two experimental groups were set as follows ($n = 9$ each): Co, adult females (90 days old) under no surgical interventions and killed between 110 and 120 days of age, in pro-oestrus and Ca, adult females ovariectomised at 90 days of age and killed at least 20 days after surgery, between 110 and 120 days of age.

The animals were kept under conventional conditions of temperature and humidity having free access to food and water. After being anesthetised by CO_2 inhalation, the animals were decapitated. The experimental protocol was approved by the Ethics Committee on Animal Use, IBLCE/UNESP, Univ. Estadual Paulista (Protocol 053/2011 CEUA). The experiments were performed in the Laboratory of Microscopy and Microanalysis at the Department of Biology of the Institute of Biosciences, Letters and Exact Sciences of UNESP, São José do Rio Preto.

Histological analysis

The prostatic complex was fixed by immersion in Karnovsky solution (4% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2) or in 4% paraformaldehyde in phosphate-buffered saline, for 24 h. After fixation, the tissues were washed, dehydrated in ethanol gradient, embedded in resin (Historesin; Leica) or in paraffin (Histosec; Merck) respectively and subjected to sectioning of 3–4 μm with a rotary microtome.

Histological sections embedded in historesin were stained with haematoxylin–eosin (HandE), periodic acid–Schiff (PAS) and silver impregnation (AgNOR; Howell and Black 1980). Those embedded in paraffin were stained with Gomori's Trichrome or submitted to immunocytochemistry for detection of basal and neuroendocrine cells.

The scanning and quantitative analysis of histological sections were performed in Image-ProPlus analysing system Version 4.5 for Windows (Media Cybernetics) software coupled to an Olympus BX60 microscope.

Immunocytochemical analysis

Deparaffinised and rehydrated tissue sections were subjected to immunohistochemistry for the detection of the p63 protein and serotonin, as basal and neuroendocrine cell markers respectively. The sections were submitted to antigen retrieval in Tris–ethylene diamine tetraacetic acid (EDTA) buffer (10 mM Trizma base, 1 mM EDTA, pH 9.0) or citrate buffer (10 mM citrate buffer, pH 6.0) at 98°C for 20 min. To block endogenous peroxidase the slides were treated for 15 min with 10% hydrogen peroxide in methanol. Permeabilisation of cell membranes was performed when necessary by 10 min in 0.2% Triton and blocking of non-specific protein–protein interactions by incubating sections in 5% powdered skim-milk solution diluted in Tris base-buffered saline (TBS, 20 mm Tris pH 7.6, 140 mm NaCl) for 15 min. Mouse monoclonal p63 antibody (p63, sc-843; Santa Cruz Biotechnology) and rat monoclonal serotonin antibody (5-HT, sc 73025; Santa Cruz Biotechnology) were used as primary antibodies, diluted at 1 : 100 in Tris-buffered saline (TBS), pH 7.8, containing 1% bovine serum albumin (BSA), overnight at 4°C. Sections were then incubated with NovoLink Max Polymer detection system (Leica), revealed with diaminobenzidine (DAB; Sigma) and counterstained with Harris Haematoxylin.

Ultrastructural analysis

Prostatic fragments were fixed by immersion for 24 h in 3% glutaraldehyde plus 0.25% tannic acid solution in Milloning's buffer, pH 7.3, containing 0.54% glucose. After washing with the same buffer, they were post-fixed for 2 h with 1% osmium tetroxide, washed, dehydrated in a graded acetone series and embedded in Araldite resin. Ultrathin sections (50–75 nm) were contrasted with 2% uranyl acetate for 30 min, followed by 2% lead citrate in sodium hydroxide solution for 10 min. The samples were evaluated with a LEO-Zeiss 906 (Zeiss) transmission electron microscope operated at 80 kV.

Cell population analysis

To determine the frequency of each cell type, initially the area of each histological section was determined, considering 4× magnification, by the Image ProPlus analyser system Version 4.5 software for Windows. Basophil, clear, spumous, ciliated, droplets and basal cells were analysed in sections stained with HandE. Mucinous cells were counted in sections stained by PAS method and neuroendocrine cells were counted on histological

sections submitted to immunocytochemistry for serotonin. Each cell type was counted in all histological sections and divided by the respective section area. Six histological sections per animal were analysed. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software). Student's *t*-test was used for comparisons between groups. The level of significance adopted was 5% ($P \leq 0.05$).

Results

The female prostate gland has two main cell types, one more common, lining the gland lumen and known as secretory luminal cells, and the other more sparse, which rests on the basement membrane and known as basal cells. Both are integral cells distributed uniformly in the epithelial compartment of the gland (Fig. 1*a*), which varies, under normal conditions, from cuboidal

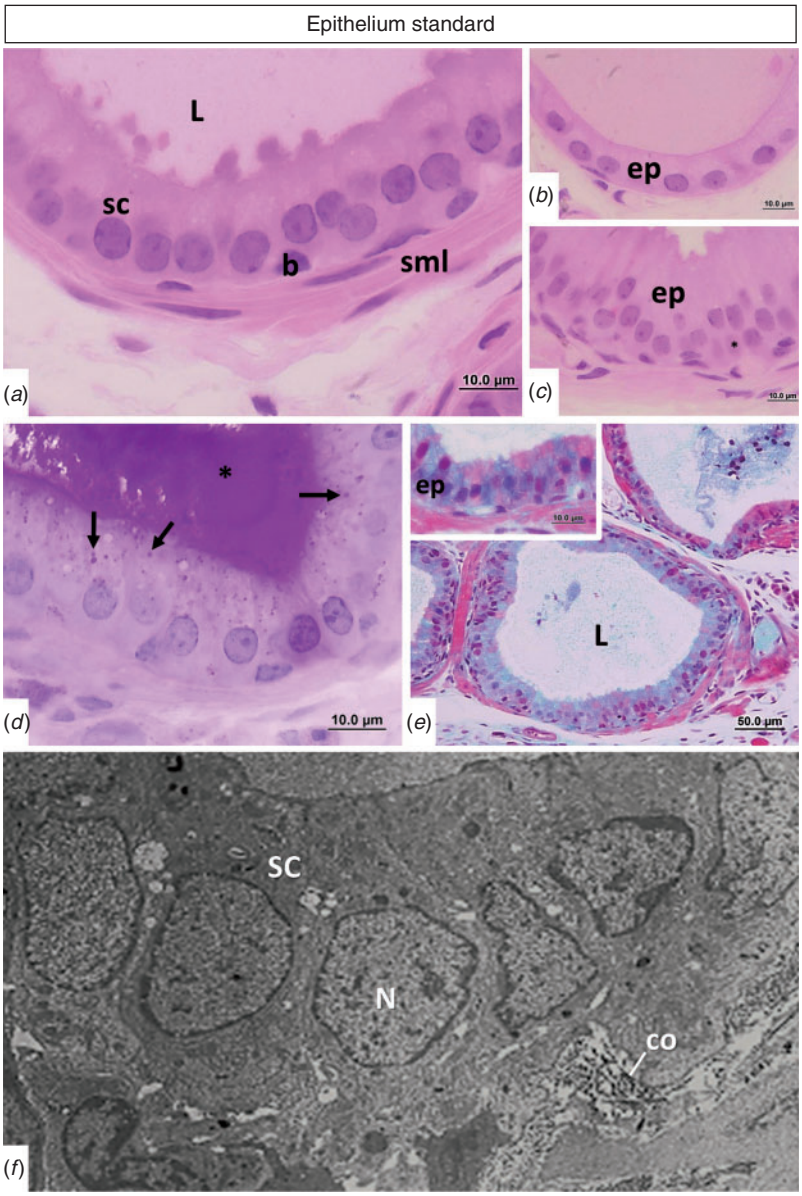

Fig. 1. Overview of typical epithelial compartment of the female prostate gland. (*a*) Morphology of acinar epithelium composed of luminal secretory cells (sc) and basal cells (b). L, lumen; sml, smooth-muscle layer involving the acini. (*b–c*) Epithelium (ep) varying from (*b*) the simple cubic to (*c*) columnar. HandE stain. (*d*) Secretory cells with PAS-positive granules (arrows) distributed and concentrated in their apical portion. *, glycoprotein secretion within the lumen. Periodic acid–Schiff (PAS) stain. (*e*) Typical acinar epithelium stained with Gomori's Trichrome, showing cells stained in green and red (in detail), reflecting the cytoplasmic composition diversity. (*f*) Ultrastructure of the epithelial compartment. co, collagen fibres; N, nucleus; sc, secretory cell. Magnification 2156×.

simple (Fig. 1*b*) to columnar (Fig. 1*c*). When stained by HandE, typical luminal and basal cells present a homogeneous acidophilic cytoplasm (Fig. 1*a–c*). Cells stained by PAS show a few PAS-positive granules distributed throughout the cytoplasm, with a higher concentration in the apical portion of the cell (Fig. 1*d*). When stained by Gomori's Trichrome, the epithelium of the female gland features secretory cells stained in green and red interspersed, showing differences in the cytoplasmic composition of these cells, reflected by the affinity of each to the Fast Green and Cromotrope pigments used in the technique (Fig. 1*e*). Ultrastructurally, luminal cells present nuclei with the same cell shape and have few condensed chromatin clusters (Fig. 1*f*).

Basal cells are less abundant and form a discontinuous layer in the epithelial compartment. They are smaller than the secretory cells and present a pyramidal shape (Fig. 2*a*, *b*). Their most important ultrastructural characteristics are the presence of minimal endoplasmic reticulum, Golgi vesicles, mitochondria and the absence of secretory vesicles. The cell nucleus contains a considerable amount of condensed chromatin associated with the nuclear envelope (Fig. 2*c*, *d*).

Eventually some cells present changes in morphological or cytochemical characteristics, which distinguished them from other typical cells in the gland epithelium. Some of the luminal cells, despite showing a similar morphology to other luminal cells, presented a more basophilic tone in HandE staining (Fig. 3*a*), besides a granular aspect. Generally these cells were displayed in groups or next to each other. When stained by the PAS method, these cells showed positive cytoplasmic granules for the technique, in variable quantities and stain levels (Fig. 3*b–d*). Granules indicating the precipitation of silver with the product synthesised and stored by the cell were also observed using the AgNOR technique (Fig. 3*e*). It was found that prostatic mucinous cells present the same chemical nature as vaginal epithelium cells stained by PAS (Fig. 3*f*) and by AgNOR (Fig. 3*g*).

Tall columnar cells with an intense basophily, but no granular aspect, observed in sections stained by HandE (Fig. 4*a*) were also differentiated when compared with the other epithelial cells. The ultrastructural analysis of these basophil cells demonstrated that they present electron-dense cytoplasm, with poorly developed organelles and some secretory vesicles in the apical portion (Fig. 4*b*).

Moreover, some cells showed a pale cytoplasm when stained by HandE and were named clear cells (Fig. 5*a*). Ultrastructurally, these cells also presented less electron-dense cytoplasm (Fig. 5*b*).

Other cells showed a process of gradual vacuolisation in the cytoplasm, giving them a droplet aspect by HandE staining (Fig. 6*a*). These cells almost always appear in groups arranged in the prostatic acini. The ultrastructural analysis showed the presence of large secretory vesicles dispersed throughout the cytoplasm with electron-dense product inside them (Fig. 6*b*).

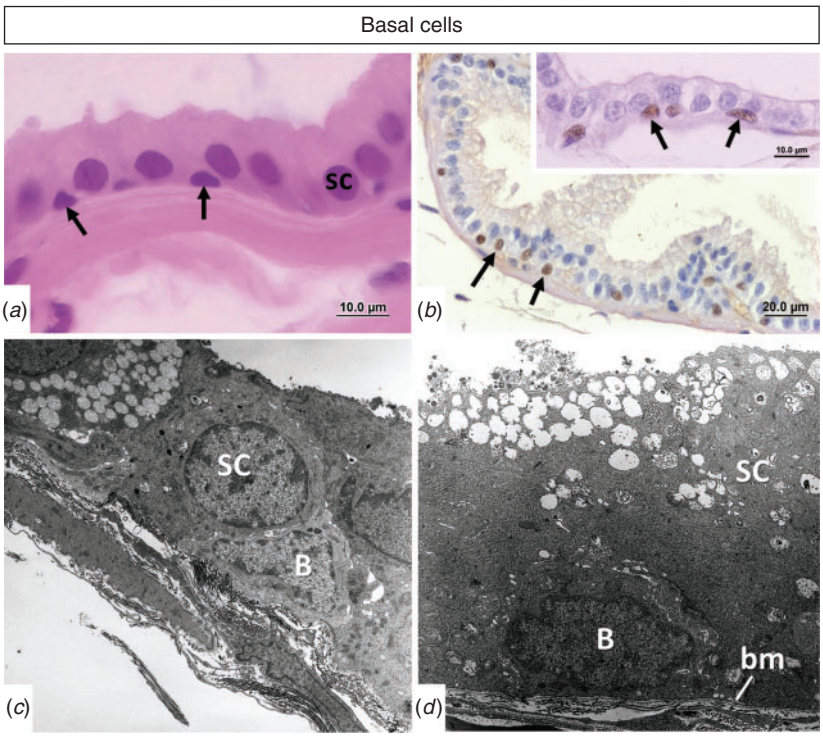

Fig. 2. Basal cells (arrows) visualised by (*a*) HandE staining and (*b*) immunocytochemistry for p63, a common marker of basal cells. (*c–d*) Ultrastructure of basal cell (B) in triangle shape, amidst the secretory cells (SC). Note the location connected to the basement membrane (bm). Magnification (*c*) 4646× and (*d*) 7750×.

Voluminous polymorphic cells with a 'bubbly' cytoplasm, filled with cytoplasmic vesicles, with nuclei often displaced to the periphery of the cell were also distinguished by HandE staining (Fig. 7*a*, *b*), appearing in an isolated manner in the acinar epithelium. The tenuous cytoplasmic network presented by these cells was PAS positive (Fig. 7*c*). Eventually, inside the vesicles, were observed some corpuscles indicative of cellular secretion (Fig. 7*c*), which could also be seen in the ultrastructural analysis. The organelles of spumous cells were found in limited spaces between the secretory vesicles (Fig. 7*d*, *e*).

The epithelium stained by HandE also revealed the presence of isolated ciliated cells (Fig. 8*a–c*), which showed cilia in the apical region, towards the lumen of prostatic acini. Ultrastructurally, it can be observed the organisation of basal bodies and the concentration of mitochondria at the apical region of these cells (Fig. 8*d*).

The last cell type observed, the neuroendocrine cells, cannot be distinguished by HandE staining, but was studied by immunocytochemistry to serotonin, a known marker for this cell type. Neuroendocrine cells are distributed throughout the acinar

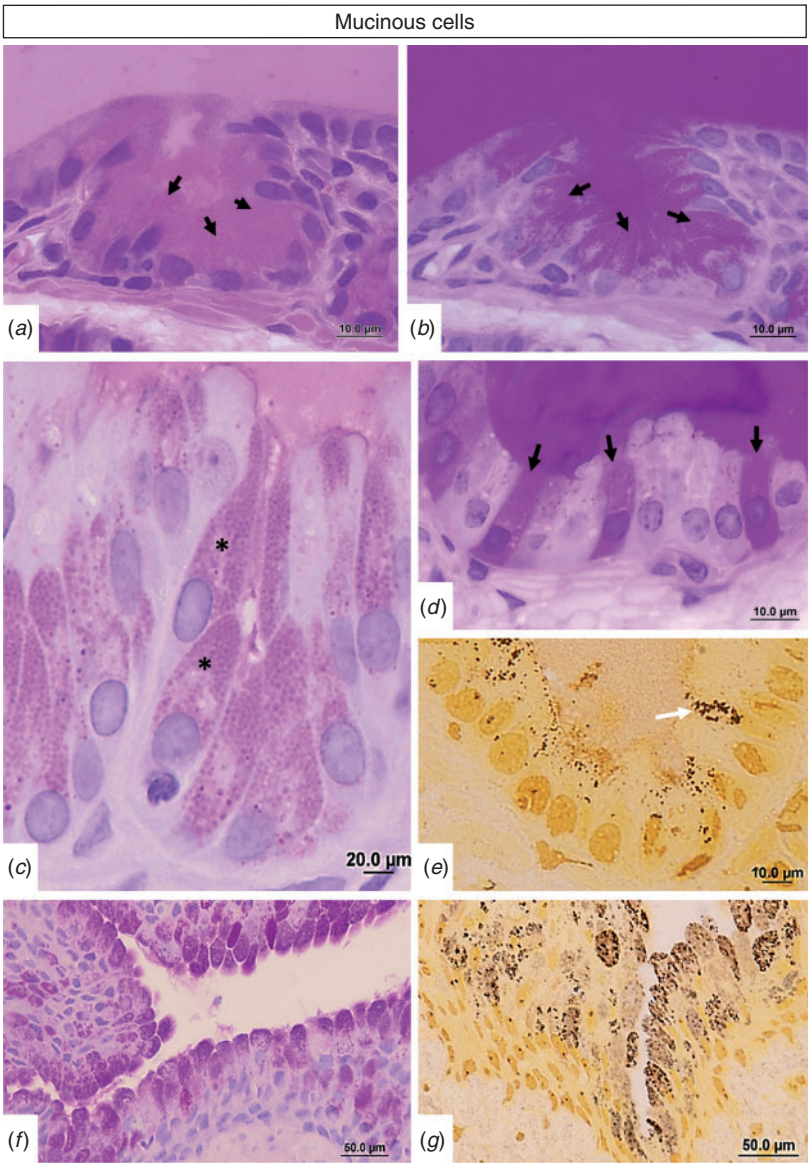

Fig. 3. Mucinous cells of the female prostate epithelial compartment. Phenotype of mucinous cells viewed by (*a*) HandE and (*b*) PAS staining. Arrows indicate the presence of mucin secretory granules. (*c*) Detailed view of the PAS-positive cytoplasmic granules (*). (*d*) Mucinous cells (arrows) among the typical secretory cells of the epithelium. PAS stain. (*e*) Argyrophil cells (arrow) detected by granules labelled by silver precipitation. AgNOR stain. (*f–g*) Presence of mucus-producing cells in the vaginal epithelium, stained by (*f*) PAS and (*g*) AgNOR.

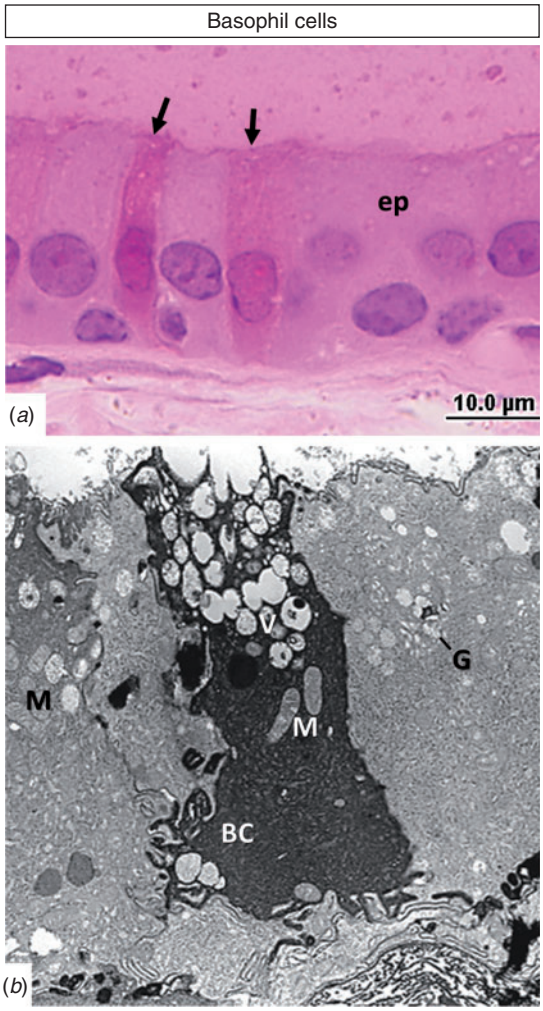

Fig. 4. (*a*) Basophil cells (arrows) of the epithelial compartment (ep) stained by HandE. (*b*) Ultrastructure of basophil cell (BC) showing typically electron-dense cytoplasm. Some secretory vesicles (V) are concentrated in the apical portion. G, Golgi complex; M, mitochondria. Magnification 7750×.

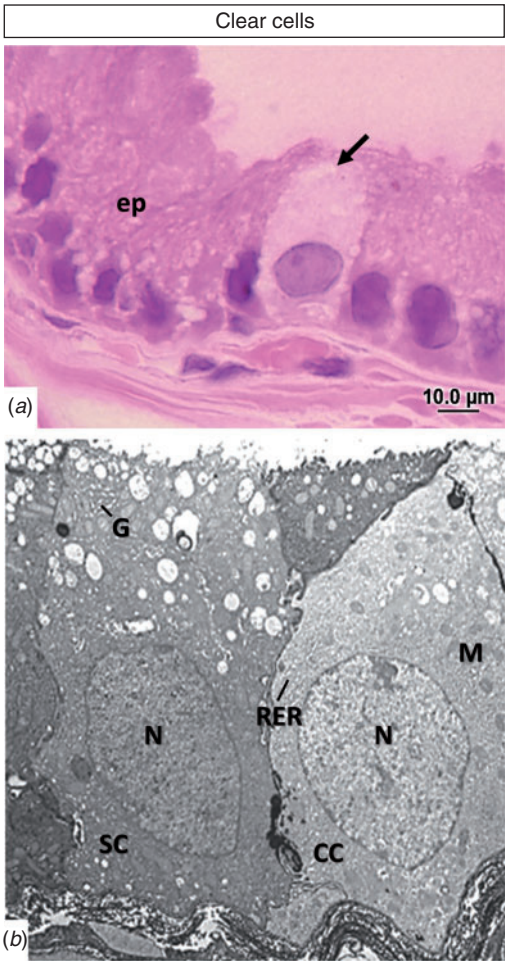

Fig. 5. (*a*) Clear cell (arrow) of the female prostate epithelial compartment (ep) stained by HandE. (*b*) Ultrastructure of clear cell (CC) showing low electron density contrasting to the typical secretory cell (SC). G, Golgi complex; M, mitochondria; N, nucleus; RER, endoplasmic reticulum. Magnification 3597×.

epithelium, interspersed or adjacent to the luminal cells, and are rarely detected in the female gerbil prostate (Fig. 9*a*). Histological sections of female gerbil urethra were used as a positive control for immunocytochemistry, since the presence of these cells in this tissue is very abundant (Fig. 9*b*). Ultrastructurally, these cells were characterised by heterogeneously sized electron-dense granules dispersed throughout the cytoplasm (Fig. 9*c*, *d*).

The results of counting the cell types identified revealed that basal cells increased significantly in the prostate gland after ovariectomy, compared with the Co group (Fig. 10*a*). Basophil and clear cells suffered a significant decrease in Ca (Fig. 10*c*, *d*) while spumous cells increase in this group (Fig. 10*f*). The mucinous, droplet and ciliated cells showed no changes in frequency between the Co and Ca groups (Fig. 10*b*, *e*, *g*). Neuroendocrine cells were counted, but showed extremely low frequencies and were unchanged between the two groups.

Discussion

It has already been established that the female gerbil prostate mature epithelium is composed of 90–92% secretory cells and 8–10% basal cells (Santos *et al.* 2007; Zanatelli *et al.* 2014). The typical secretory cells have a cytoplasm with a well-developed endoplasmic reticulum, Golgi apparatus and a great number of secretory vesicles, which continuously carry the secretory products. The basal cells, on the other hand, are not responsible for producing and secreting prostatic fluid, but are stem cells that rest on the basement membrane and renew the epithelial compartment through their proliferation and differentiation (Santos *et al.* 2003).

After ovariectomy the prostate gland suffers an atrophy process characterised mainly by a decrease in the epithelial and luminal compartments and reduced secretory activity. The proportion of the cell populations is not changed (Zanatelli *et al.* 2014); however, this study demonstrated that some different phenotypes of secreting cells become more or less frequent due

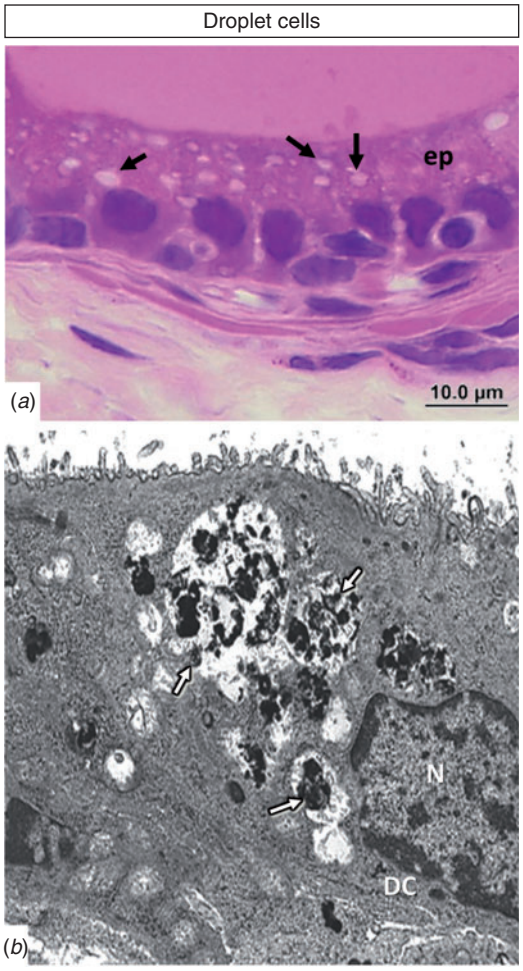

Fig. 6. (*a*) Droplet cells of the female prostate epithelial compartment (ep) stained by HandE. Droplets (arrows) identified in the cell type. (*b*) Ultrastructure of droplet cell (DC) showing heterogeneous secretory vesicles (white arrows) with secretion accumulation. N, nucleus. Magnification 10 000×.

to the hormonal deprivation. This change also occurred with the population of basal cells, which increased after ovariectomy. In this study, the population tally was determined in relation to the prostate tissue area. Thus, as the prostate suffers a size reduction after ovariectomy, the quantity of basal cells appeared higher in the Ca group.

The heterogeneous phenotypes among the predominant group of secretory cells were differentiated in the female gerbil prostate by their morphological characteristics and by the staining properties of the dyes and markers used.

The mucinous cells, containing by PAS-positive granules, showed no phenotypic change compared with other secretory cells stained by HandE and were distinguished only by the nature of their cytoplasmic content. According to Bancroft and Gamble (2002), PAS-positive granules indicate sulfated mucins, denoting the predominance of secretion rich in carbohydrates. These mucin-producing cells, or high molecular-weight glycoproteins, lubricate and protect the epithelial compartment. According to their function, mucins are divided into two groups: those that bind to membranes, such as MUC 1, MUC 3 and MUC 4, which contribute to the cell–cell epithelium interaction, and those that are secreted, such as MUC 2, MUC 5AC, MUC 5B and MUC 6, which are macromolecular components of mucus (Legrier *et al.* 2004; Cozzi *et al.* 2005). Thus, variation in the intensity and distribution of PAS-positive granules found in cells may indicate the presence of different types of mucins or may also reflect the different levels of synthetic activity of each cell. The silver impregnation technique (AgNOR) can confirm the chemical nature of mucinous cells, since the carbohydrate precipitated by the silver allows identification of dark granules dispersed throughout the cytoplasm, thereby characterising the argyrophil property of this cell type. This cell phenotype has been previously observed in work by our research group involving male gerbil prostates (Campos *et al.* 2010; Perez *et al.* 2011). A more tenuous basophilia, featuring clear cells, was previously described by Santos *et al.* (2003) in the epithelial compartment of this gland. According to the descriptions of the group, these cells are a subtype of merocrine secretory cells, which differ from the typical merocrine by presenting pale cytoplasm and less electron-dense cytoplasmic contents with low-density and variable sizes of secretory vesicles, as evaluated ultrastructurally. These cells, which accumulate neutral carbohydrate in their cytoplasm, are rare in normal epithelium, but after the administration of testosterone (Santos *et al.* 2006), letrozol (Santos *et al.* 2008) and in senile female gerbils (Custodio *et al.* 2008) gradually become more frequent.

In this study, the number of these cells relative to the prostate area decreased in the castrated group (Ca) compared with the control group. The suppression of sexual hormones caused by ovariectomy may have been the cause of reducing the population of these cells in the female prostate. Data from Zanatelli *et al.* (2014) showed that, after castration, the number of clear cells increased in the prostate epithelial compartment. These data conflict with those obtained in the present study. This divergence of results may be explained by the more detailed analysis of this cell phenotype performed in the present study, considering the morphological and ultrastructural differentiation and characterisation for counting clear cells, making the data presented here more reliable than those from the previous study.

Another phenotype identified in the epithelium was the basophil cell, also a merocrine cell type with markedly eosinophilic cytoplasm. Most of the time these cells were high columnar with restricted cytoplasmic volume, tending to coalescence. Ultrastructurally, they showed electron-dense cytoplasm with reduced organelles. It is believed that these cells are a pre-death stage of the typical secretory luminal cells of the epithelial compartment. The experimental ovariectomy performed in this study led to a significant reduction in the population of these cells. The hormonal fall caused by castration may have accelerated the discard process of these cells from the epithelial compartment.

The droplet cells were characterised by the presence of droplets of varying sizes dispersed throughout the cytoplasm in HandE staining. Custodio *et al.* (2008) have described these cells as fragmented in appearance due to an intense accumulation of heterogeneous multivesicular bodies, seen ultrastructurally.

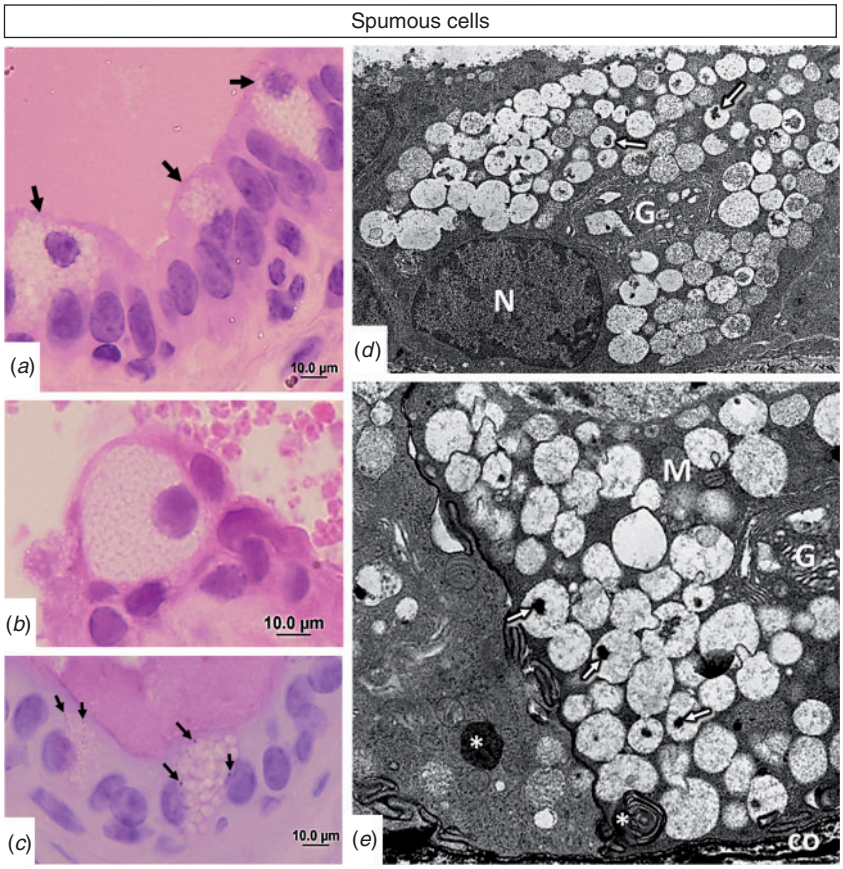

Fig. 7. (*a–b*) Spumous cells (arrows) of the female prostate epithelial compartment. HandE stain. (*c*) PAS-positive stain of the tenuous cytoplasmic network distributed through the cell. Arrows, secretion corpuscles. (*d–e*) Ultrastructure of the cell type showing cytoplasm entirely covered by large secretory vesicles. Note the distribution of biosynthetic organelles, such as mitochondria (M) and Golgi complex (G) in the intra-cytoplasmic vesicular spaces. N, nucleus; *, ceramide deposits; white arrows, secretion corpuscles. Magnification (*d*) 4646× and (*e*) 12930×.

Despite this apparent fragmentation, the metabolism of these cells remains active, since merocrine and apocrine secretions appear to occur and an endomembrane system is developed and functional. In female gerbils this cell phenotype is normally found in adult animals but appears more frequently with aging and starts to present deposits of lipofuscin. This accumulation of non-degraded material constrains the cell functions and promotes the development of age-related diseases (Custodio *et al.* 2008).

Phenotypically abnormal cells occupied entirely by a cytoplasmic network and presenting a bubbly aspect were identified in the epithelial compartment as spumous cells. This fragile cytoplasm was PAS positive, suggesting the presence of carbohydrates in its composition. The ultrastructural analysis showed a cytoplasm filled with juxtaposed secretory vesicles. In restricted cytoplasmic spaces located between the vesicles a developed endomembrane system can be identified with enlarged Golgi cisterns. Some secretory vesicles were occupied by polymorphic corpuscles. These ultrastructural characteristics suggest that these spumous cells have a more differentiated metabolism compared with typical luminal cells.

Comparing the characteristics reported for droplet and spumous cells, we may suggest that both are a different physiological stage of the same secretory cell type. By ultrastructural analysis it was possible to verify that the secretory vesicles gradually increased in number from one cell to another, reaching its maximum and resulting in a cytoplasm entirely covered by them, which featured the spumous phenotype. A secretory overactivation was possibly the start of this phenotypic and physiological change. The data from the population tally showed that the peak frequency of spumous cells occurred after ovariectomy. The hormone suppression may have been the stimulus for the phenotypic and functional changes of these cells. Interestingly, after ovariectomy, the population of droplet cells tended to decrease, while the frequency of spumous cells increased, which also suggests that these are the same cell type changing from one stage to another. Other work by our research group has observed cells with a spumous phenotype in the male gerbil prostate after hormone ablation by treatment with anti-oestrogen Tamoxifen (Campos *et al.* 2010) and in the female gerbil prostate after castration and treatment with progesterone (Fochi *et al.* 2013).

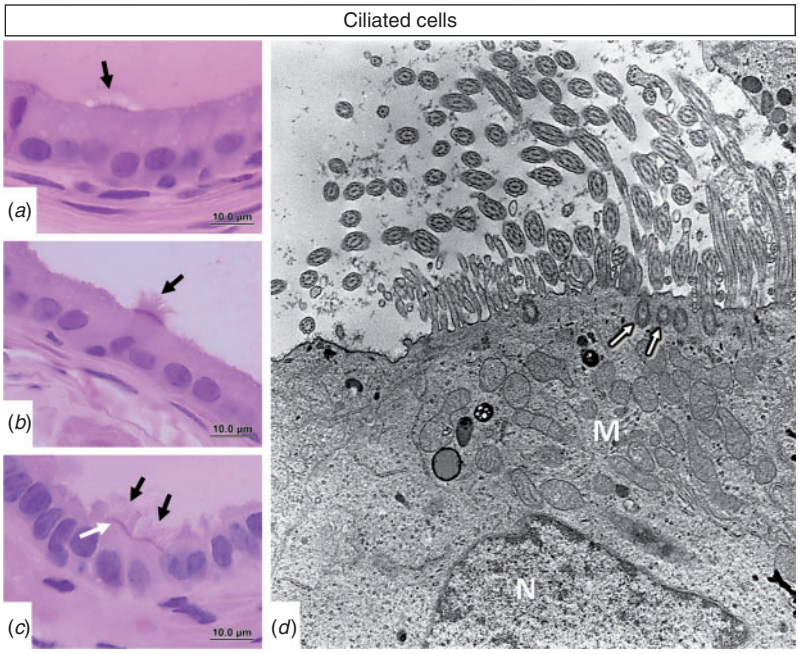

Fig. 8. (*a–c*) Ciliated cells (arrows) of the female prostate epithelial compartment, stained with HandE. White arrows, basal bodies. (*d*) Ultrastructure of the ciliated cells highlighting the concentration of mitochondria (M) near the basal bodies (white arrows). N, nucleus. Magnification 10 000×.

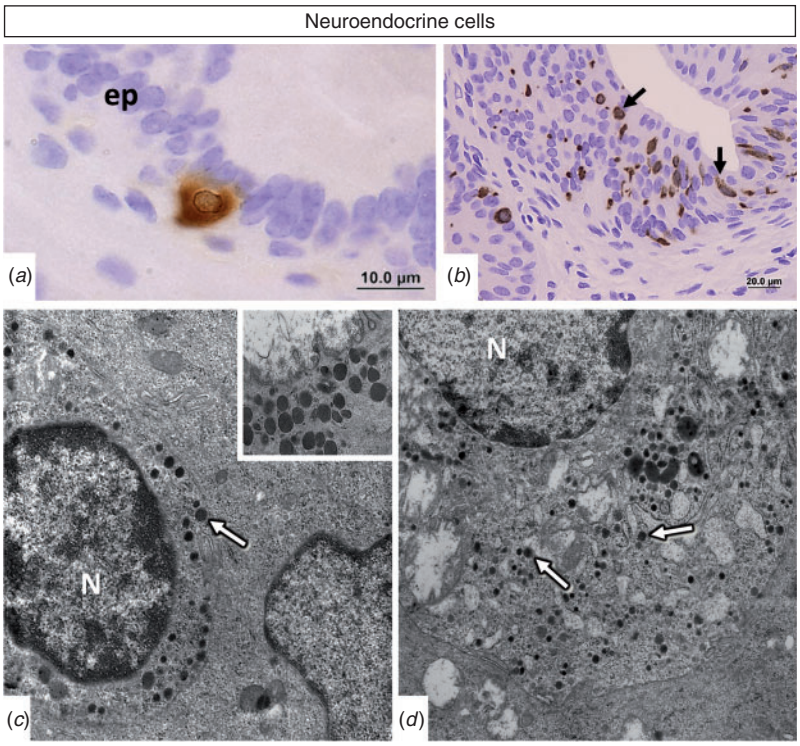

Fig. 9. (*a*) Neuroendocrine cell, open type, immunostained for serotonin, among the secretory cells of the epithelial compartment (ep). (*b*) Histological section of female urethra used as positive control for the technique. Presence of large numbers of neuroendocrine cells (arrows). (*c–d*) Ultrastructure of the cell type highlighting the characteristic electron-dense granules (white arrows). N, nucleus. Magnification (*c*) 16 700×, (*c*, inset) 35 970× and (*d*) 12 930×.

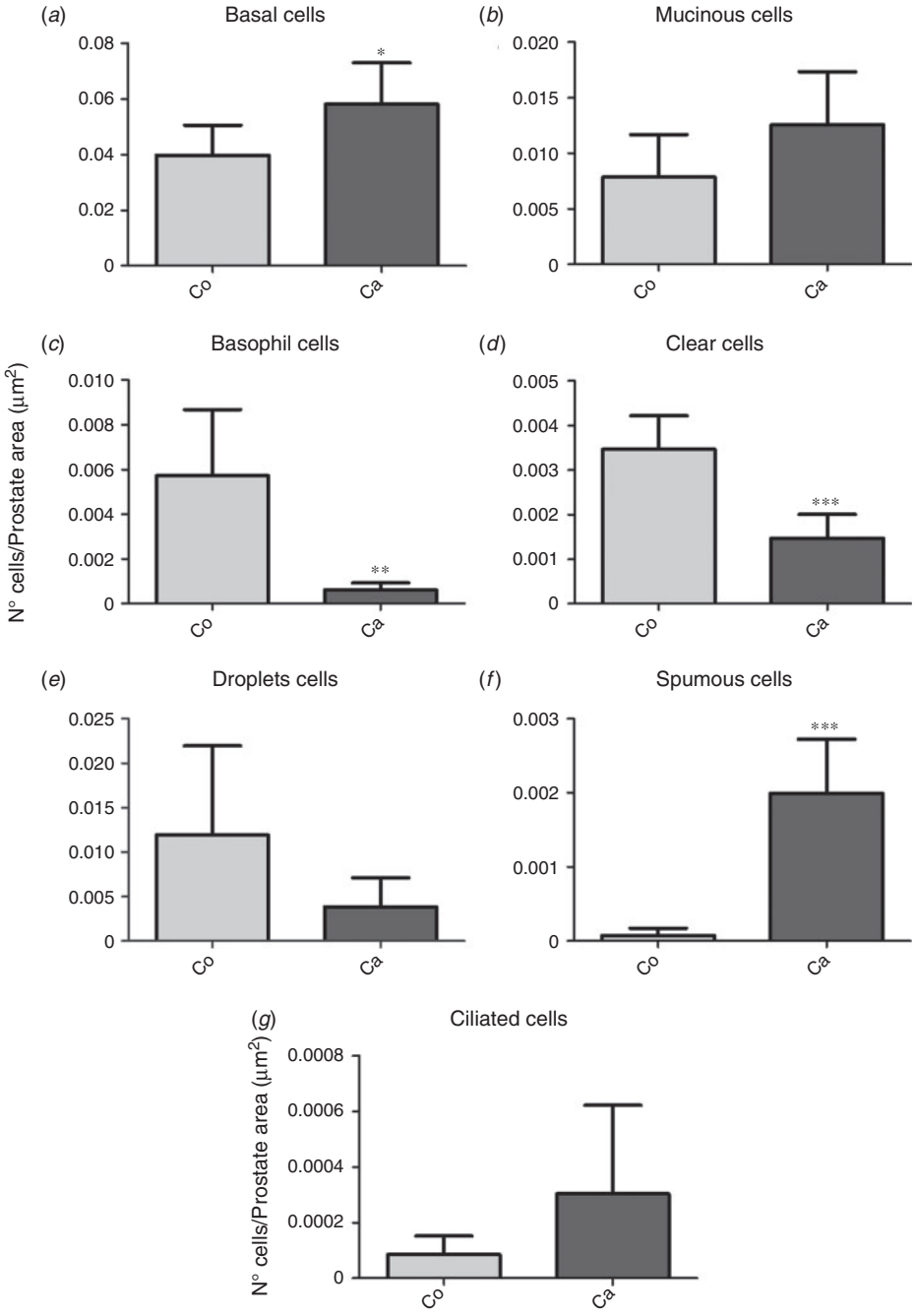

Fig. 10. Graphics of cell population tally relative to the prostatic area. (*a*) Basal cells, (*b*) mucinous cells, (*c*) basophil cells, (*d*) clear cells, (*e*) droplet cells, (*f*) spumous cells and (*g*) ciliated cells. The data represent the mean ± s.e.m. of the number of cells divided by the prostate area of the histological section. *Indicates significant differences between the experimental groups ($P \leq 0{,}05$). Co, control; Ca, castrated.

Santos *et al.* (2007) previously described the existence of ciliated cells in the prostatic compartment of female gerbils. Several other studies have also observed this cell type (Fochi *et al.* 2008; Oliveira *et al.* 2011; Shinohara *et al.* 2013; Zanatelli *et al.* 2014). The appearance of these cells is probably related to an anomalous differentiation of prostatic basal cells under the influence of a hormonal imbalance, according to the researchers, but in the present study ovariectomy was not sufficient to significantly change the frequency of this cell type, causing only a tendency to increase.

Neuroendocrine cells were identified in the female gland in this study by ultrastructural studies and immunocytochemistry

with a specific marker. Zaviačič (1999) confirmed the presence of this cell type in the gland of women. As with the male prostate gland, it is believed that these cells are involved in growth, differentiation and regulation of the secretory process in the mature organ (Di Sant'Agnese 1992). Morphologically, two types of neuroendocrine cells are distinguished: open, balloon-shaped, with thin extensions reaching the prostate lumen and closed, lacking in luminal extensions. Both types have complex morphology with irregular dendritic processes extending underneath and between the adjacent epithelial cells (Abrahamsson 1999*a*, 1999*b*). Ultrastructurally, neuroendocrine cells are characterised by the presence of dense cytoplasmic granules, with a marked heterogeneity of size and shape. These dense granules are characteristic of endocrine cells and are involved in the storage and secretion of a variety of endogenously active substances: chromogranin, serotonin, calcitonin and somatostatin, among others (Di Sant'Agnese 1992; Abrahamsson 1999*a*, 1999*b*).

Serotonin is a good marker to visualise neuroendocrine cells from the prostate of rodents (Rodríguez *et al.* 2003). This study demonstrated the presence of neuroendocrine cells in the female prostate gland of Mongolian gerbils. No one knows for sure the role of these cells in the prostate, but they are believed to be involved in the growth, differentiation and regulation of tissue (Ingelmo *et al.* 2007). It is likely that they secrete products into the stroma and present receptors to stromal factors, which provide all the necessary interaction for growth and normal prostate development (Montuenga *et al.* 2003). Furthermore, neuroendocrine cells are found in prostatic tumours and their incidence is considered a promising prognostic indicator for the development of androgen-independent disease. They secrete paracrine factors that stimulate the growth, survival and metastasis of prostatic carcinoma cells (Amorino and Parsons 2004).

Although cellular heterogeneity has been described for the female gerbil prostate epithelium, there is an extensive morphological similarity with the typical secretory cells of male gerbil prostates (Santos *et al.* 2003, 2006; Santos and Taboga 2006; Rochel *et al.* 2007). The morphological evaluation performed in this study described the structural and ultrastructural profile of different secretory cell types found in the female prostate gland. The experimental ovariectomy showed that the prostate responds to hormonal imbalance by changing the proportions of its cell populations and activating phenotypes and specific functions. Some of these populations are also observed in the prostate of male gerbils, as previously mentioned. Further studies should investigate new peculiarities of each cell type, seeking to understand more clearly how they act for the homeostasis of the female gland.

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