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Actions of oestradiol and progesterone on the prostate in female gerbils: reversal of the histological effects of castration

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Abstract. The female prostate is a functionally active gland in several mammalian species, including humans and rodents. Investigations of prostate morphophysiology during the phases of the oestrous cycle have shown that the female prostate is influenced by fluctuations in serum concentrations of oestradiol (E2) and progesterone (P4). The aim of the present study was to evaluate the effect of combined prolonged administration of E2 and P4 on the prostate in ovariectomised female gerbils. Ovariectomy caused atrophy and decreased glandular secretory activity. Administration of E2 and P4 (0.1 mg kg^{-1} diluted in 0.1 mL of mineral oil, every 48 h over 30 days) resulted in a recovery of overall prostate structure, as evidenced by increased epithelial height, mass and prostatic secretory activity, without leading the appearance of significant lesions. Evaluation of androgen receptor (AR) expression revealed increased immunoreactivity in the E2+P4-treated group. Immunostaining for oestrogen receptor (ER) α was decreased in the castrated groups, but increased in the group subjected to hormone treatment. There were no significant differences in ERβ immunoreactivity among the groups. Assessment of cell proliferation revealed greater immunoreactivity in the treated group. Together, the results indicate that the interaction between E2 and P4 may be responsible for maintaining female prostate gland histophysiology.

Additional keyword: ovariectomy.

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Introduction

The female prostate surprises many people; it is probably the least known component of the female genital tract. Although it was initially interpreted as a vestigial gland, there have been many studies investigating its structure and ultrastructure (Santos *et al.* 2003, 2011), chemical constituents (Schmidt *et al.* 2001; Custódio *et al.* 2004) and morphological changes following hormone treatment (Santos *et al.* 2006, 2007; 2008; Perez *et al.* 2011; Rochel-Maia *et al.* 2011) and aging (Custódio *et al.* 2008, 2010; Oliveira *et al.* 2011). These studies demonstrate that the female prostate is a physiologically active gland.

The Mongolian gerbil *Meriones unguiculatus* (Criscetidae, Gerbilinae) has been used as a model for prostate studies owing to its similarities with the human prostate, particularly with regard to the compaction of the male prostate lobes (Price 1963; Pinheiro *et al.* 2003; Góes *et al.* 2007), the production of glycoprotein prostate-specific antigen (PSA)-positive fluid (Santos *et al.* 2006) and the presence of a voluminous prostate in approximately 90% of adult females (Santos and Taboga 2006). In female gerbils, the prostate consists of a set of ducts and acini in intimate contact with the wall of the urethra. The ducts are inserted into the muscles surrounding the urethra and open at many points into its lumen.

In adult female rodents, cyclical hormonal changes occur throughout the oestrous cycle. In gerbils, the oestrous cycle lasts 4–6 days, divided into pro-oestrus, oestrus, dioestrus I and dioestrus II (Nishino and Totsukawa 1996). Recent studies demonstrate major hormonal changes in the gerbil during the oestrus and dioestrus II, with peaks of E2 and P4, respectively (Santos *et al.* 2011). Thus, it is believed that cyclic oscillations in

E2 and P4 are responsible for the morphological changes that occur in the female prostate during the oestrous cycle in the gerbil rather than testosterone, the serum concentrations of which are relatively stable across all stages of the cycle.

Both E2 and P4 are steroid hormones produced mainly by the ovaries and both are largely related to female reproduction. Both hormones participate in the proliferation, differentiation and maintenance of the uterine epithelium and stroma and, during pregnancy, have a role in the development and differentiation of the mammary glands. Immunocytochemical studies have demonstrated that oestrogen receptors (ERα and ERβ) and progesterone receptors (PR) are present in the prostate tissue of female humans and rodents, indicating that E2 and P4 act to maintain the structure and function of this gland (Santos and Taboga 2006). Therefore, the aim of the present study was to evaluate the combined and prolonged effects of E2 and P4 on the maintenance of prostate histophysiology in ovariectomised female gerbils using a series of morphological and immunocytochemical analytical techniques.

Materials and methods

Experimental design

Thirty-two adult female gerbils (90 days old) were used in the present study. The gerbils were kept at the Biotherium of Institute of Biosciences, Letters and Exact Sciences of the São José do Rio Preto (SP) campus of São Paulo State University (UNESP) in polyethylene boxes with wood shavings as a base under conditions of adequate light and temperature, with food and water available *ad libitum*, in accordance with the ethical guidelines of UNESP and COBEA (Brazilian College for Animal Experimentation) (Protocol no. 16/07-CEEA).

With the exception of gerbils in the control group, all female gerbils were subjected to bilateral ovariectomy at 90 days of age after being anesthetised with 100 μL of ketamine (0.116 g mL^{-1}) and 30 μL xylazine (0.02 g mL^{-1}) (1 mL applied per kg bodyweight). After allowing 10 days for recovery after surgery, gerbils were assigned to one of the following experimental groups ($n = 8$ gerbils in each group): (1) castrated (Ca) females that underwent bilateral ovariectomy only; (2) a castrated + oil (CaO) group, in which ovariectomised female gerbils were given 0.1 mL, s.c., mineral oil (Nujol; Mantecorp, São Paulo, Brazil) every 48 h from Day 10 to Day 40 after surgery; (3) a castrated + E2 + P4 (CEP) group, in which the ovariectomised female gerbils were given subcutaneous injections of E2 (β-oestradiol 3-benzoate; Sigma, St Louis, MO, USA) and P4 (Sigma), both at a dose of 0.1 mg kg^{-1} (Santos *et al.* 2006; Scarano *et al.* 2008; Perez *et al.* 2012), diluted in 0.1 mL of mineral oil every 48 h from Day 10 to Day 40 after surgery; and (4) a control (Co) group, which consisted of intact female gerbils, in the pro-oestrus phase, which were killed at the same age as the females in the other groups.

All hormone and/or vehicle injections were made at 1000 hours. Gerbils in each group were killed 24 h after the last injection of the hormone or vehicle (131 days old). Females in the control group were cycled from 125 days of age and killed at the first pro-oestrus after this age (~135 days of age). All gerbils were killed by CO_2 inhalation and, after being weighed, were decapitated to enable blood collection for analysis of hormone concentrations. The prostate glands were removed together with the urethra (prostatic complex) and were weighed and fixed.

Histochemistry

The prostatic complexes collected from five animals in each group were fixed by immersion for 24 h in Karnovsky's solution containing 5% paraformaldehyde and 2.5% glutaraldehyde. The material was then dehydrated in an ascending series of ethanol and embedded in historesin (Historesin embedding kit; Leica, Nussloch, Germany). Sections (3 μm) were produced on a rotary microtome (RM2155; Leica). To assess morphological differences in prostate glands from female gerbils in the different experimental groups, histological sections were stained with haematoxylin–eosin (HE) for general analysis of glandular structure and cellular quantification or with periodic acid-Schiff (PAS) for analysis of glycoprotein secretion. Sections were evaluated by light microscopy and histological images were digitised using an Olympus BX-60 microscope (Olympus, Hamburg, Germany) with a digital camera and Image-Pro Plus software version 4.5 for Windows (Media Cybernetics, Silver Spring, MA, USA).

Morphometric, karyometric, stereological and population analyses

Measurements were made on HE-stained histological sections of prostate glands. Stereological analysis was undertaken to determine the relative frequency of different prostatic compartments (epithelium, lumen, smooth muscle layer and non-muscular stroma) in each of the different groups. For this, six sections spaced uniformly throughout the block were used. Two fields were captured from each section, totalling 60 fields per group ($n = 5$). The measurements were then made in accordance with the M130 multipoint test system (Weibel 1963) applied to the prostate according to Huttunen *et al.* (1981). Thus, from the data obtained for each field examined, the relative frequency (%) of prostatic compartments was calculated by counting the points that coincided with each compartment and dividing them by the total number of points. Morphometric analysis was performed to determine epithelium height (μm) and muscle layer thickness (μm). Similarly, karyometric analysis determined the nuclear area (μm^2) and perimeter (μm) measures of secretory epithelial cells. All morphometric measurements were made using software (Image ProPlus, version 4.5 for Windows, Media Cybernetics, USA) calibrated with a micrometre ruler. For both analyses, 200 measurements were made for each experimental group. Determination of the population frequency per acini was made counting secretory, basal, clear and ciliated cells in 30 acini per experimental group, with these figures expressed as percentages.

Histopathological analysis

Histopathological analysis was performed using an Olympus BX-60 light microscope. Lesions were classified according to Shappell *et al.* (2004). Six histological sections stained with HE and spaced uniformly throughout the block were used for each animal ($n = 5$ animals per group). The incidence (percentage of

animals having a determined lesion) and the multiplicity of the lesions (number of foci in each section) were determined manually.

Serum hormone concentrations

Blood samples from eight animals per experimental group were collected in test tubes with 4 mL separation gel, centrifuged at 300*g* and serum concentrations of E2, P4 and testosterone determined. Measurements were made using an automatic Elisa Microplate Reader (DNM-9602; Perlong Medical Equipment Co. Ltd., Jiangsu, China) and a testosterone enzyme immunoassay kit (ADI-900-065; Enzo Life Sciences, New York, NY, USA), a 17β-oestradiol high-sensitivity ELISA kit (ADI-900-174, Enzo Life Sciences) and a P4 ELISA kit (ADI-900-011; Enzo Life Sciences). Test sensitivities were 7.81–2000 pg mL^{-1} testosterone, 15.6–3000 pg mL^{-1} E2 and 15.62–500 pg mL^{-1} P4.

Immunocytochemistry

The female prostatic complexes from three animals per group were fixed by immersion in 4% paraformaldehyde in phosphate buffer for 24 h. The tissues were then dehydrated in an ascending series of ethanol concentrations, cleared in xylene and embedded in paraffin (Histosec; MERCK, Darmstadt, Germany). Histological sections (4 µm) were produced on a rotary microtome (RM2155; Leica) and then subjected to immunocytochemistry to detect androgen receptors (AR), oestrogen receptors (ERα and ERβ) and the cell proliferation marker proliferating cell nuclear antigen (PCNA). Sections were dewaxed and then rehydrated in graded alcohol and distilled water. Antigenic recuperation was performed in citrate buffer (citric acid monohydrate, 0.21%, pH 6) at high temperature (100°C) for 45 min. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 45 min, followed by a rinse in phosphate-buffered saline (PBS). Sections were incubated with the following primary antibodies diluted 1 : 100 in 1% bovine serum albumin in PBS at 4°C overnight: anti-AR (rabbit polyclonal IgG; N-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-ERα (Clone 1D5; Code M7047; monoclonal mouse anti-human; Dako, Carpinteria, CA, USA), anti-ERβ (rabbit polyclonal IgG; SC-H-150; Santa Cruz Biotechnology) and anti-PCNA (M-2; Santa Cruz Biotechnology). The use of these antibodies was appropriate in gerbils because of the high level of sequence conservation of these proteins in mammals (Feldman and Feldman 2001; Prins and Korach 2008). Sections were then incubated with peroxidise-labelled secondary antibodies (Rabbit ABC Staining System SC-2018 (Santa Cruz Biotechnology) for AR, ERα, ERβ and PCNA) and revealed with diaminobenzidine (DAB; Sigma). Sections were counterstained with Harris haematoxylin. As a negative control, the primary antibodies were replaced with the corresponding normal isotype serum. Immunostaining was assessed using an Olympus BX-60 light microscope and counts were performed using Image-Pro Plus software, Version 4.5 for Windows (Media Cybernetics). The relative frequency (%) of AR, ERα, ERβ and PCNA nuclear demarcation was calculated as the number of positive prostatic cells per 1000 epithelial + 1000 stromal cells per experimental group ($n = 3$ animals per group). Results were graded from 0 to 3+ according to Corradi *et al.* (2009), where 1+ indicated <10% epithelial and stromal positive nuclear demarcation, 2+ indicated 10%–50% epithelial and stromal positive nuclear demarcation and 3+ indicated >50% epithelial and stromal positive nuclear demarcation.

Statistical analysis

Exploratory and statistical analyses of the results were performed using Microsoft Excel (Microsoft, Redmond, WA, USA) and GraphPad InStat 3.02 (GraphPad Software, San Diego, CA, USA), with data expressed as the mean ± s.d. All analyses were made using ANOVA and Tukey tests. The level of significance was set at 5% ($P \leq 0.05$).

Results

Biometric analysis

The biometric analysis shown in Fig. 1*a–c* revealed no significant difference in the bodyweight of animals in the different experimental groups. Conversely, the weight of the female prostate gland decreased with castration and increased above that in the Co group weight after E2 + P4v treatment.

Relative weight was calculated as the ratio of the prostatic complex/bodyweight. After ovariectomy, there was a decrease in the relative weight of the prostatic complex in the Ca and CaO groups. After hormone treatment, the relative weight of the prostatic complex increased to values above those in the control (Co) group.

Morphometric, karyometric, stereological and population analyses

Morphometric analysis (Fig. 1*d*) revealed a significant reduction in epithelial height and muscle layer thickness in the Ca and CaO groups. In the CEP group, muscle layer thickness recovered to near normal values, whereas the height of the epithelial cells increased beyond that in the Co group. Karyometric analysis (Fig. 1*e*) revealed a reduction in the nuclear area and perimeter of secretory epithelial cells in the Ca and CaO groups compared with the Co group, whereas in the CEP group there was a clear recovery of these nuclear morphological characteristics.

The stereological data presented in Fig. 1*f* show that there was no significant change in the luminal and epithelial compartments among groups. The smooth muscle layer increased in the CaO group, whereas there was a decrease in the volume of the non-muscular stroma in this group. There were no significant changes in these parameters in the other groups.

The frequency of different cell types in the epithelium is shown in Fig. 1*g*. The proportion of secretory cells differed significant between the Ca (lower) and CEP (higher) groups, whereas there were no significant differences in the proportion of secretory cells in the other groups. The population of basal and ciliated cells did not change among experimental groups. The frequency of clear cells increased in the Ca group.

Serological analysis

Serum concentrations of testosterone, E2 and P4 in the experimental groups are given in Table 1. Testosterone concentrations

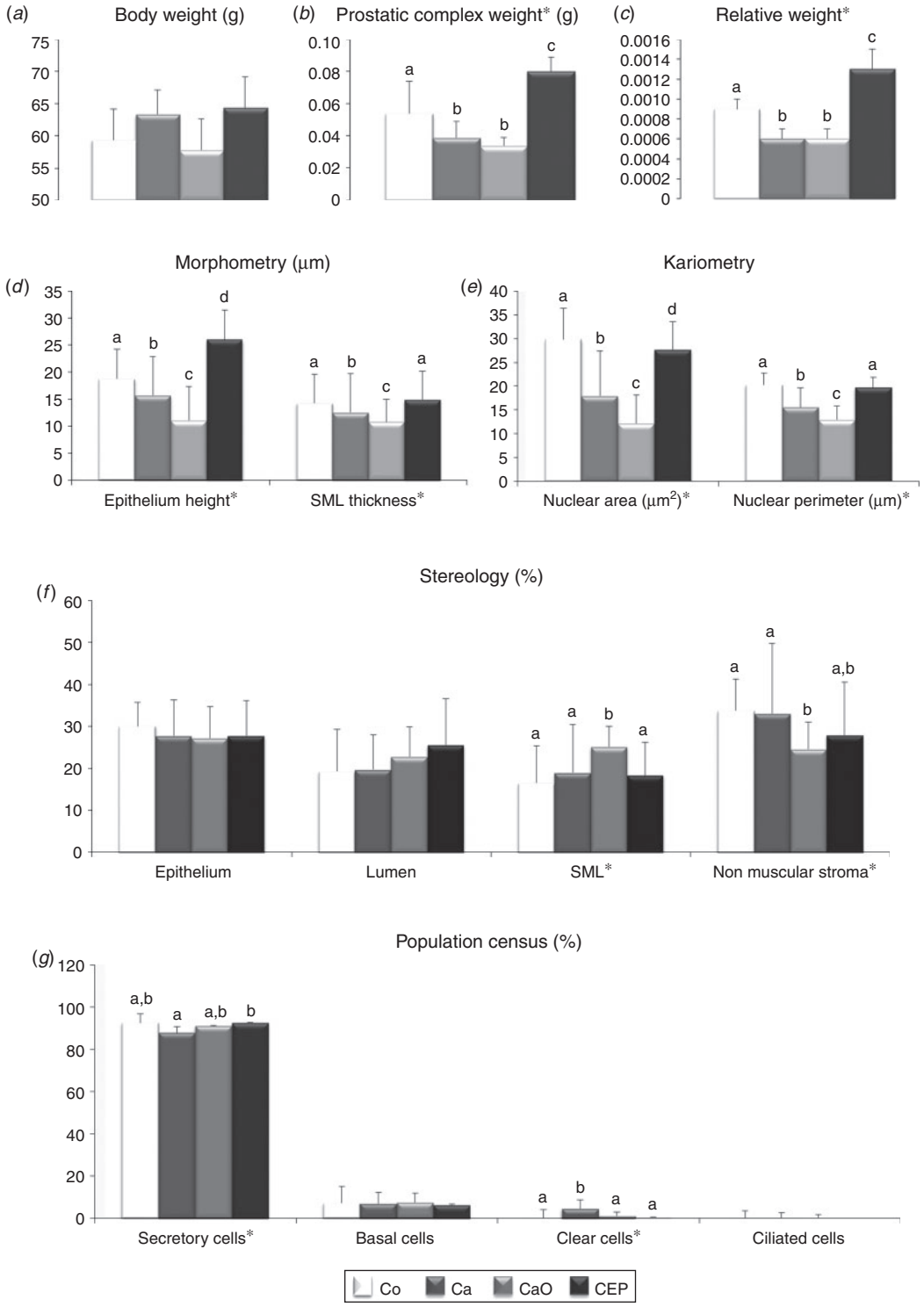

Fig. 1. (*a–c*) Biometric analysis, (*d*) morphometric analysis, (*e*) kariometric analysis of secretory cells, (*f*) stereological results and (*g*) population census of prostate glands from female gerbils in the control (Co), castrated (Ca), castrated plus vehicle (mineral oil)-treated (CaO) and castrated plus oestrogen- and progesterone-treated (CEP) groups. Data are the mean ± s.d. Asterisks indicate parameters for which significant changes were noted. Within graphs, columns with different superscript letters differ significantly ($P \leq 0.05$).

Table 1. Serum testosterone, oestradiol and progesterone concentrations in female gerbils in the different experimental groups

Data are the mean ± s.d. ($n = 8$ animals per group). Within rows, values with different superscript letters differ significantly ($P \leq 0.05$). Co, control group; Ca, castrated; CaO, castrated + vehicle (mineral oil)-treated group; CEP, castrated and oestradiol + progesterone-treated group

	Co	Ca	CaO	CEP
Testosterone (ng mL^{-1})	0.14 ± 0.09^{a}	0.05 ± 0.05^{ab}	0.04 ± 0.02^{b}	0.10 ± 0.01^{ab}
Oestradiol (pg mL^{-1})	39.5 ± 56.9^{ab}	20.3 ± 5.3^{b}	20.7 ± 8.3^{b}	79.3 ± 43.8^{a}
Progesterone (ng mL^{-1})	11.0 ± 3.5	6.7 ± 6.1	7.2 ± 7.1	11.3 ± 3.4

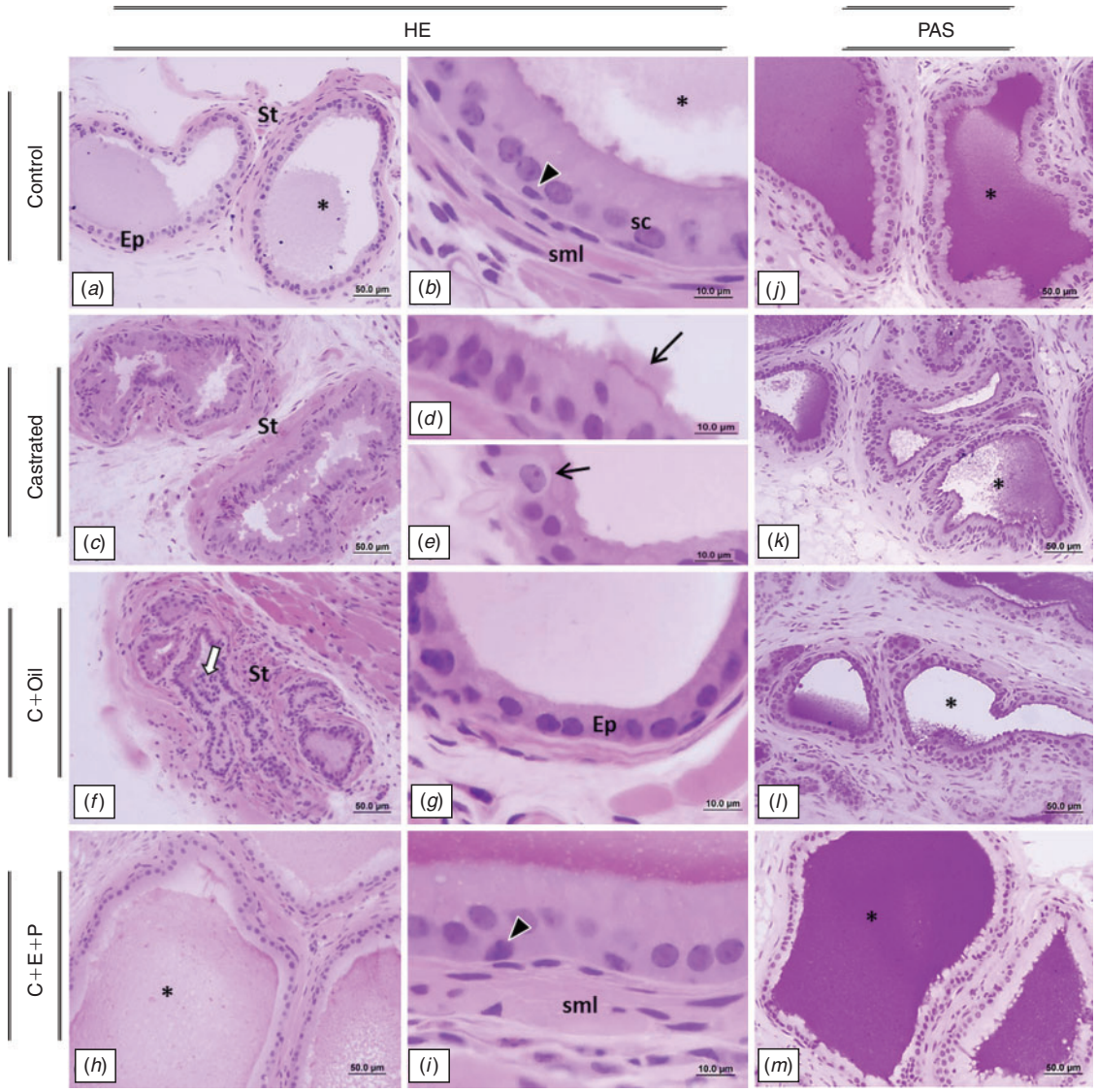

Fig. 2. Structural and histochemical aspects of the female prostate in the control, castrated, castrated plus vehicle (mineral oil)-treated (C+Oil) and castrated plus oestrogen- and progesterone-treated (C+E+P) groups. Tissues were stained by haematoxylin and eosin or periodic acid–Schiff (PAS), as indicated. (*a*) Histological section of a normal adult female prostate in the pro-oestrous phase, showing the fibromuscular stroma (St), epithelium (Ep) and lumen (*). (*b*) Detail of the secretory epithelium showing secretory cells (sc), basal cells (arrowhead) and the adjacent smooth muscle layer (sml). (*c*) Overview of the female prostate after castration. (*d*) Ciliated cells (arrow) among the secretory cells. (*e*) A clear cell (arrow) among the secretory cells in the acinar epithelium. (*f*) Overview of the female prostate after castration and vehicle (mineral oil) administration showing the presence of cellular debris (white arrow) inside the lumen. (*g*) Detail of the prostatic secretory epithelium (Ep) of the C+Oil group. (*h*) Overview of the prostate in the C+E+P group. (*i*) Detail of the secretory epithelium showing prismatic secretory cells, basal cells (arrowhead) and the adjacent smooth muscle layer (sml). (*j*) Lumens in a normal prostate gland with large amounts of secreted glycoprotein (*). (*k*, *l*) Regressed lumens with almost no glycoprotein secretion (*). (*m*) Higher magnification view of increased lumens containing large amounts of secreted glycoprotein (*).

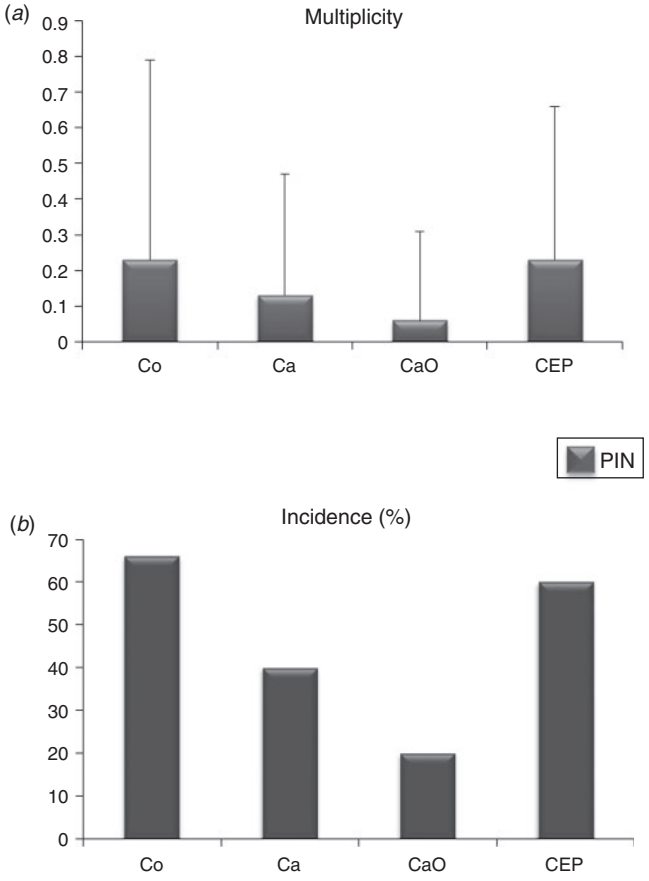

Fig. 3. (*a*) Multiplicity (number of foci in each section) and (*b*) incidence of premalignant prostatic intraepithelial neoplasia (PIN) in prostate glands from female gerbils in the control (Co), castrated (Ca), castrated plus vehicle (mineral oil)-treated (CaO) and castrated plus oestrogen- and progesterone-treated (CEP) groups.

declined in the CaO group compared with the other groups. There were no significant differences in E2 concentrations between the Co, Ca and CaO groups, but E2 concentrations were higher in the CEP compared with Ca group. Finally, P4 concentrations were variable in all groups, with large standard deviations; consequently, there were no significant differences among the treatment groups. However, there was a trend for a decrease in P4 concentrations in the Ca group, which was reversed by P4 administration.

Morphological aspects

Anatomically, the female gerbil prostate in the pro-oestrous phase consists of a set of ducts and acini that are embedded in a fibromuscular stroma. Under normal conditions, as observed in the Co group in the present study, the acini are lined by a simple epithelium that varies from cuboidal to columnar, with large lumens that store secretory products (Fig. 2*a*). The epithelium contains two main cell types: basal and secretory cells (Fig. 2*b*).

The general morphology of the gland in the Ca and CaO groups showed marked structural regression. The prostatic acini that had exhibited an essentially circular configuration in the control group had become wavy and deformed, reflecting regression of the gland (Fig. 2*c*, *f*). Similarly, a decrease in the height of the epithelial cells indicated atrophy of the gland (Fig. 2*g*). In addition, acinar regression was reflected by a reduction in the secretory activity of the prostate compared with the Co group (Fig. 2*j*, *k*, *l*). Among the secretory cells, ciliated and clear cells were observed (Fig. 2*d*, *e*). The clear cells are characterised by a chromophobic cytoplasm and an elliptical nucleus and are so named because of their pallid aspect after staining with HE. Cellular debris in the acinar lumen were also frequently found in the Ca and CaO groups (Fig. 2*f*).

In the CEP group, treated with E2 and P4, major restructuring in the arrangement of the female prostate tissue was observed. The glands showed a reversal of the regression process, as evidenced by the morphology of the acini, which were circular again, an increase in epithelial height and recovery of secretory activity, as evidenced by the large amount of glycoprotein secretion within the acini (Fig. 2*h*, *i*, *m*).

Histopathologically, it was possible to determine the occurrence of premalignant prostatic intraepithelial neoplasia (PIN) as the main morphological lesion in the prostates of gerbils in all experimental groups. However, as shown in Fig. 3*a*, *b*, hormone treatment had no effect on either the multiplicity or the incidence of PIN. There were no significant differences in either parameter among the different groups.

Immunoreactivity for AR

Immunoreactivity for the AR in prostates from gerbils in the different experimental groups is shown in Fig. 4*a–h*; immunostaining nuclear quantification is given in Table 2. Similar immunomarkings were seen in the Co, Ca and CaO groups, including epithelial and stromal cells (Fig. 4*a–f*). Intense AR immunostaining was seen in the CEP group that was present in virtually all acinar epithelial cells and most stromal cells (Fig. 4*g*, *h*).

Immunoreactivity for ERα and ERβ

Immunostaining for the oestrogen receptors is shown in Fig. 4*i–x*. In prostates from the control (Co) group, ERα immunostaining was found mainly in stromal cells (Fig. 4*i*, *j*). This immunomarcation decreased in the Ca and CaO groups (Fig. 4*k–n*; Table 1), but approaching normal levels in the CEP group (Fig. 4*o*, *p*). Immunoreactivity for ERβ was similar in all experimental groups, and was found mainly in the nuclei of epithelial cells (Fig. 4*q–x*; Table 2).

Marker of cell proliferation: PCNA

The results of immunohistochemical analysis for PCNA are shown in Fig. 5*a–l* and Table 2. There were significant decreases in the number of proliferative epithelial and stromal cells in the Ca and CaO groups compared with the Co (control) group. There was a significant increase in the number of immunostained epithelial and stromal cells in the CEP group compared with the other groups.

Discussion

Previous studies have shown that the female prostate is a functionally active organ and that its performance in reproduction is

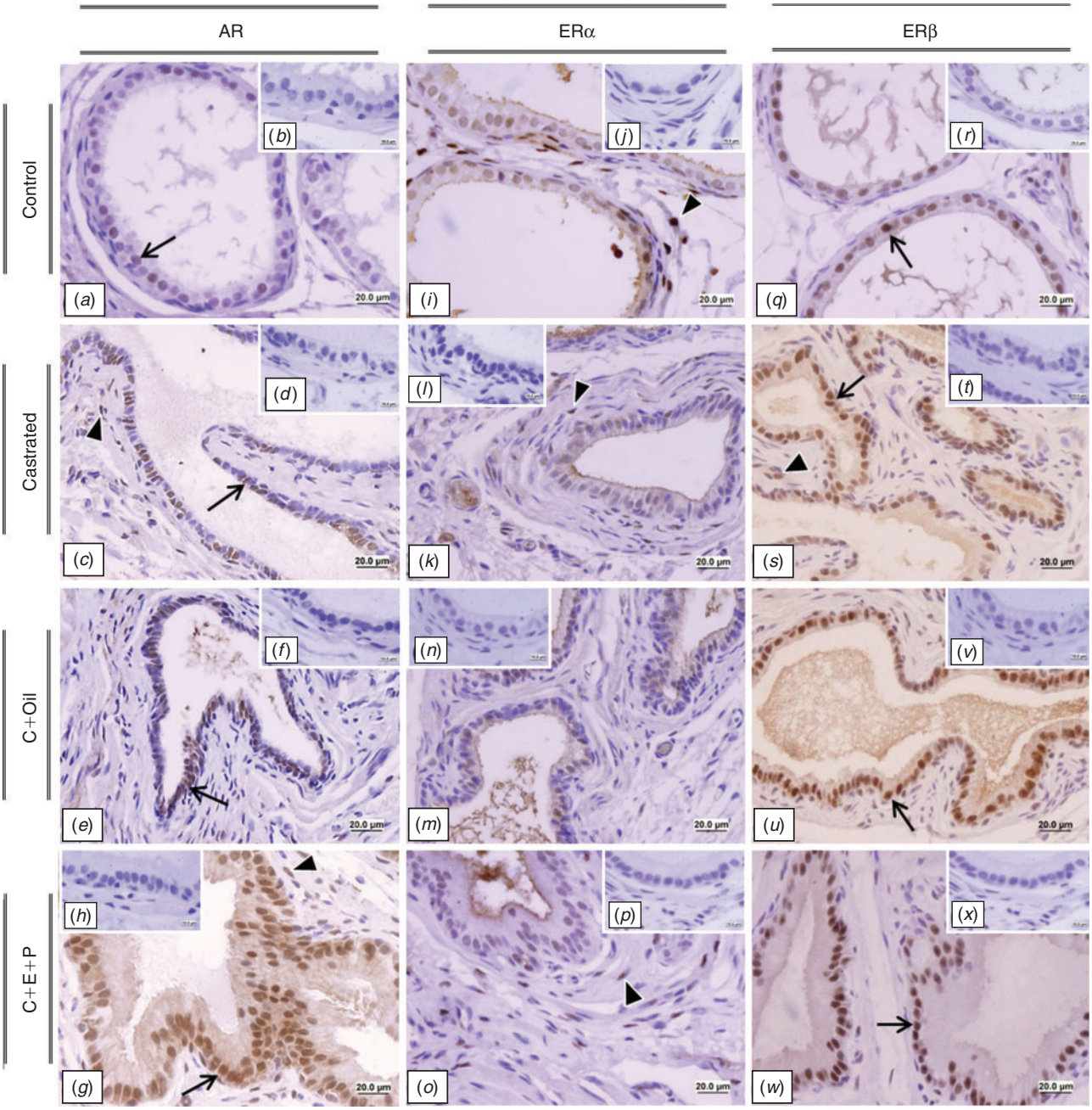

Fig. 4. Immunocytochemical analysis of androgen receptor (AR) and oestrogen receptors ERα and ERβ, counterstained with Harris haematoxylin. (*a*, *c*, *e*) AR immunoreactivity was seen in epithelial (arrows) and stromal (arrowheads) cells of gerbils in the control, castrated and castrated plus vehicle (mineral oil)-treated (C+Oil) groups. (*g*) There was evidence of high AR immunoreactivity in epithelial cells from the female prostate of the castrated plus oestrogen- and progesterone-treated (C+E+P) group. (*b*, *d*, *f*, *h*) Control reactions. (*i*) ERα immunomarking (arrowhead) in stromal cells from female gerbils in the control group. (*k*, *m*) Low ERα immunostaining (arrowhead) in stromal cells from female gerbils in the castrated and C+Oil groups. (*o*) Evidence of increased ERα expression (arrowhead) in stromal cells from female gerbils in the C+E+P group. (*j*, *l*, *n*, *p*) Control reactions. (*q*) ERβ immunostaining (arrow) in the prostate gland from female gerbils in the control group. (*s*, *u*, *w*) ERβ immunostaining was seen in epithelial (arrows) and stromal (arrowhead) cells of prostate glands from female gerbils in the castrated, C+Oil and C+E+P groups. (*r*, *t*, *v*, *x*) Control reactions.

dependent on the action of steroid hormones (Santos *et al.* 2008). Natural fluctuations in these hormones during the oestrous cycle in female gerbils are accompanied by major changes in the structure and function of the prostate gland.

In the present study, the general appearance of the gland during the pro-oestrous phase in gerbils in the Co (control) group was similar to that described by Santos *et al.* (2003, 2006). A similar structure was described by Scarano *et al.* (2008) for the

Table 2. Immunostaining nuclear quantification of androgen receptors, oestrogen receptors ERα and ERβ and proliferating cell nuclear antigen in prostatic epithelium and stroma in female gerbils in the different experimental groups

Data show the values of the counts expressed as a percentage. Nuclear immunostaining was quantified according to Corradi *et al.* (2009) as follows: 1+, <10% positive epithelial and stromal cell nuclei; 2+, 10%–50% positive epithelial and stromal cell nuclei; 3+, >50% positive epithelial and stromal cell nuclei. Co, control group; Ca, castrated; CaO, castrated + vehicle (mineral oil)-treated group; CEP, castrated and oestradiol + progesterone-treated group; PCNA, proliferating cell nuclear antigen

	Co	Ca	CaO	CEP
Androgen receptors				
Epithelial cells	28.11 (+2)	27.7 (+2)	48.88 (+2)	86.66 (+3)
Stromal cells	4.34 (+1)	5.41 (+1)	8.67 (+1)	25.96 (+2)
ERα				
Epithelial cells	19.47 (+2)	12.27 (+2)	46.7 (+2)	13.15 (+2)
Stromal cells	34.06 (+2)	5.49 (+1)	8.48 (+1)	20.32 (+2)
ERβ				
Epithelial cells	91.58 (+3)	91.65 (+3)	94.07 (+3)	94.47 (+3)
Stromal cells	24.88 (+2)	9.25 (+1)	21.3 (+2)	16.4 (+2)
PCNA				
Epithelial cells	20.43 (+2)	9.88 (+1)	14.52 (+2)	55.12 (+3)
Stromal cells	16.14 (+2)	5.8 (+1)	1.29 (+1)	28.68 (+2)

ventral prostate male gerbils of the same species as used in the present study (*Meriones unguiculatus*).

The gerbils that underwent ovariectomy in the Ca and CaO groups had similar histophysiological results, which implies that administration of the vehicle was not responsible for the changes observed in the E2 + P4-treated (CEP) group.

The hormonal suppression caused by ovariectomy resulted in atrophy of the female prostate gland, as evidenced by numerous morphological features. The reduction in the height of the epithelium of acini in the Ca and CaO groups represents a similar response to that seen in prostate glands from male gerbils after castration, in which the acinar epithelium often appears cuboidal or takes on a short cylindrical form (Scarano *et al.* 2008). The decrease in nuclear area and perimeter of prostate epithelial cells revealed by karyometric analysis can be ascribed directly to the reduction in the synthetic activity of these cells in the Ca and CaO groups. The reduction in synthetic activity was also evident as the almost complete absence of glycoprotein secretion (PAS staining) within the prostate acini. The large decrease in relative prostate weight in castrated female gerbils is another indicator of strong acinar regression caused by hormonal suppression. Cellular debris were commonly found in the acinar lumen of the prostate glands in the Ca and CaO groups. These debris are formed in response to the hormonal restriction: in this way, the gland discards large numbers of epithelial cells that are no longer stimulated to secrete substances. Circulating levels of all three hormones (E2, P4 and testosterone) tended to fall following castration, but increased again after hormone replacement, although the effects were not consistently significant. The considerable variation among animals, particularly in terms of P4 concentrations, may reflect background production of these hormones by the adrenal glands and other non-ovarian sources (Davis and Trans 2001; Ghayee and Auchus 2007).

The thinning of the smooth muscle cell layer observed in the prostate glands of gerbils in the Ca and CaO groups disagrees with previous observations of prostates from castrated male gerbils, in which an increase in the fibromuscular stromal compartment has been suggested as a result of a rearrangement of these cell types (Góes *et al.* 2007). However, because of differences in male and female hormone profiles, it is likely that the responses of the smooth muscle layer surrounding the acini also differ between the sexes. Santos *et al.* (2011) reported that the largest stromal changes in the female prostate coincide with hormonal peaks of E2 and P4 during the oestrous cycle, which demonstrates the importance of these hormones in the regulation of stromal remodelling.

Female gerbils in the Ca group exhibited a significant increase in the frequency of clear cells. When adult female gerbils were subjected to treatment with exogenous testosterone (Santos *et al.* 2006) and letrozole, an aromatase inhibitor (Santos *et al.* 2008), the frequency of clear cells also increased. Similarly, Custódio *et al.* (2008) reported an increase in the clear cell population in the senile female gerbil prostate, identifying a greater proliferation of this cell type in dysplastic and neoplastic lesions. This may imply that the occurrence of clear cells is related to a hormonal imbalance in the gland. However, the factors involved in this response need be clarified in future studies.

The reversal of glandular atrophy observed in the CEP group was reflected by a general morphological restructuring of the organ, which again comprised acini with large lumens and a circular aspect, as well as increases in epithelium height and smooth muscle layer thickness. In addition, there was evidence of recovery of synthetic and metabolic activity of the prostate from gerbils in the CEP group: there were considerable increases in the nuclear area and perimeter of epithelial secretory cells and a large volume of secretion was found within the lumen. These observations demonstrate the involvement of E2 and P4 in the function of the gland. Moreover, it can be said that double hormonal treatment restored the overall structure of the gland without causing large foci of lesions, with no significant differences in histopathological derangements of the prostate observed in females among all experimental groups.

The increased expression of AR and ERα in prostate tissue from the CEP group is believed to be due to hormone treatment of this group. It is known that secretory epithelial cells and fibroblasts in the female prostate are positive for AR expression (Santos and Taboga 2006). The ERα, which is often expressed in prostate stromal cells in humans and rodents (Prins *et al.* 2001; Royuela *et al.* 2001; Omoto *et al.* 2005), was also detected in secretory epithelial cells of the female gland, but without significant changes in expression among experimental groups.

There was a significant increase in AR immunostaining in the CEP group compared with the other groups. The expression of this receptor in virtually all secretory epithelial cells and most stromal cells is consistent with increased circulating androgen levels in gerbils treated with P4.

In groups of ovariectomised females that did not receive hormone treatment, ERα expression in stromal cells of the

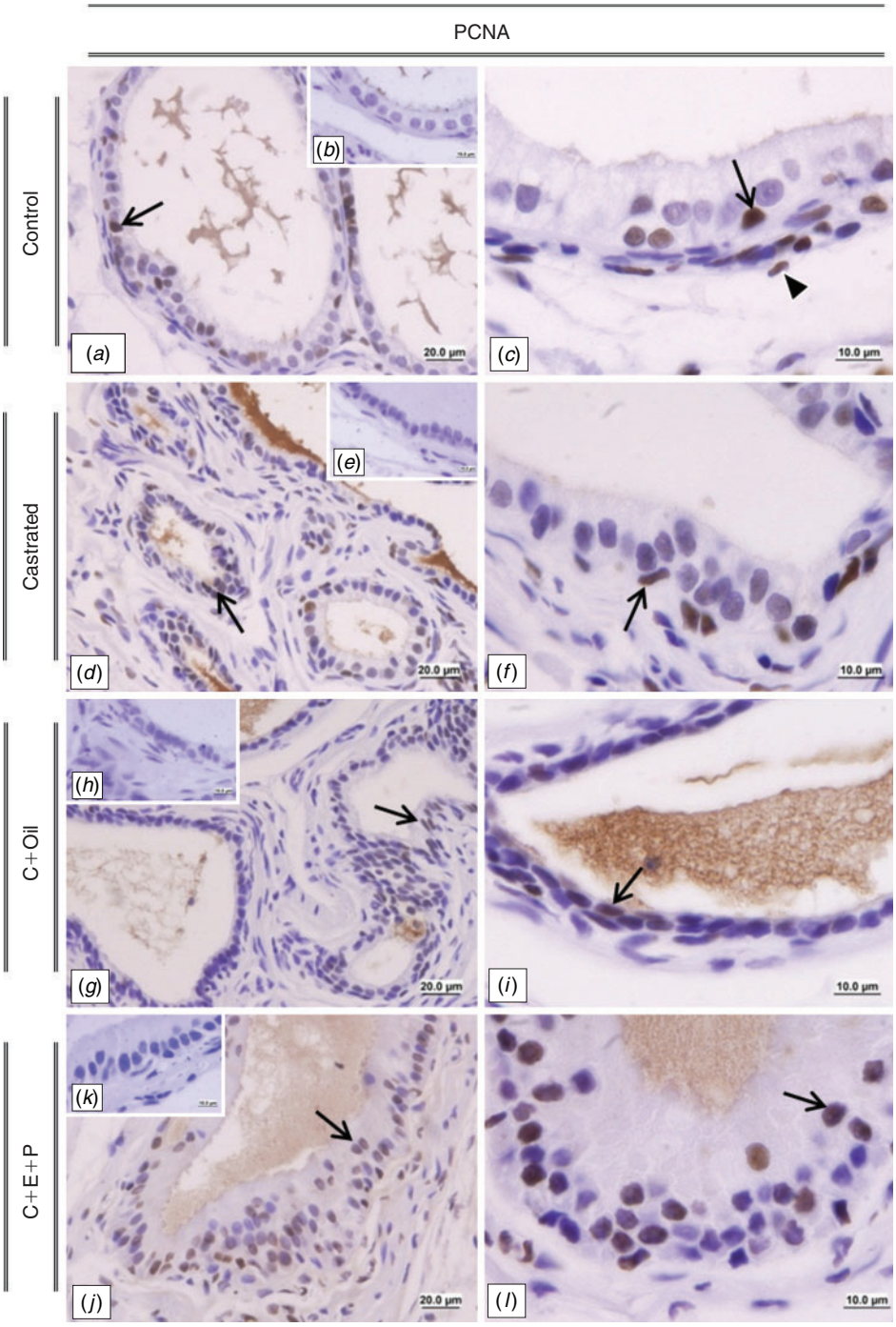

Fig. 5. Histological sections subjected to immunocytochemistry for the detection of the cell proliferation marker proliferating cell nuclear antigen (PCNA). Slides were counterstained with Harris haematoxylin. (*a*, *c*) PCNA immunostaining of epithelial (arrow) and stromal (arrowhead) cells in prostate glands of female gerbils in the control group. (*d*, *f*, *g*, *i*) Immunostaining of prostate epithelial (arrow) and stromal cells in prostate glands of female gerbils in the castrated and castrated plus vehicle (mineral oil)-treated (C+Oil) groups. (*j*, *l*) Increased PCNA expression (arrows) in prostate glands from castrated female gerbils treated with oestradiol and progesterone (C+E+P). (*b*, *e*, *h*, *k*) Control reactions.

prostate decreased compared with that in the control group. Wada-Hiraike *et al.* (2006) investigated the expression of oestrogen receptors in the uterine luminal epithelium of ovariectomised rats and found similar results for ERα expression, which was reduced after hormonal suppression. In the present study, ERα expression in the stromal cells of the prostate recovered in gerbils in the CEP group. These data are consistent with a study that evaluated the combined effect of synthetic E2 and P4 on the uterus of female gerbils and found that this treatment increased ERα expression in this tissue (Lv and Shi 2012). Thus, it is believed that the expression of ERα is under the influence of a synergistic action between E2 and P4 in the female gerbil prostate.

In contrast, ERβ immunostaining decreased in stromal cells in the Ca group. In adult male rats, castration leads to an accentuated decline in ERβ synthesis and activity in prostate tissue, which is reversed by testosterone replacement (Prins *et al.* 1998). These findings could not be elucidated using the immunocytochemistry technique in female gerbils in the present study. ERβ was highly expressed in the epithelium in the control group, and more sensitive methods are required to detect changes in ERβ expression this cell type.

The results of PCNA immunostaining demonstrated a reduction in the number of epithelial and stromal cells in mitosis in the Ca and CaO groups. Similar data have been reported by Campos *et al.* (2010) in a study of senescent male gerbils that were also subjected to hormonal suppression. After combined treatment with E2 and P4, there was a significant increase in the number of proliferating epithelial cells and recovery of the number of stromal cells in proliferation, indicating again that the hormones strongly influenced the morphological and functional recovery of the gland, reversing the atrophy process and stimulating cell division for maintenance and tissue repair.

The data presented herein lead to the conclusion that ovarian hormones are important for the maintenance of the female prostate physiology, whereas ovariectomy causes glandular regression and a decrease in secretory activity. We also show that E2 plays an important role in combination with P4 in regulating the female prostate. It is known that the integrity of the gland is related not only to levels of E2 and P4, but also to the cyclic balance between these hormones. However, studies of the combined action of E2 and P4 in the prostate may lead to the development of treatments for important prostatic diseases, which, according to other studies, affect both males and females (Zaviačič *et al.* 1993; Dodson *et al.* 1994, 1995; Ali *et al.* 1995; Ebisuno *et al.* 1995; Sloboda *et al.* 1998; Islam *et al.* 2001; Sharifi-Aghdas and Ghaderian 2004; Kato *et al.* 2005; McCluggage *et al.* 2006).

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