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MMP-2 and TIMP-2 in the prostates of male and female mongolian gerbils: effects of hormonal manipulation

Sabrina S. Rochel-Maia[1*], Fernanda C. A. Santos[2,5*], Patrícia S. L. Vilamaior[5], Luis A. Justulin Jr[3], Sérgio L. Felisbino[4], Rejane M. Góes[5] and Sebastião R. Taboga[5]

[1]Department of Cell Biology, Institute of Biology, State University of Campinas (UNICAMP), São Paulo, Brazil, [2]Department of Morphology, Institute of Biology Sciences, Federal University of Goiás (UFG), Goiás, Brazil, [3]Department of Morphology, Federal University of Triângulo Mineiro – UFTM, Uberaba, Minas Gerais, Brazil, [4]Department of Morphology – Institute of Biosciences (IBB) – São Paulo State University (UNESP), São Paulo, Brazil and [5]Department of Biology, Institute of Biosciences, Humanities, and Exact Sciences (IBILCE), São Paulo State University (UNESP), São Paulo, Brazil

*The first two authors contributed equally to this paper

Summary. TIMPs in the prostates of male and female gerbils and evaluated the effects of testosterone on the expression of these enzymes. Ventral prostates from male gerbils that were either intact or had been castrated for 7 or 21 days, along with prostates from female gerbils that were either intact or had been treated with testosterone for 7 or 21 days, were submitted to histological, stereological and immunohistochemical analyses. Stereology of prostatic components showed significant alterations of tissue compartments in the ventral male prostate after castration, especially after 21 days, with a significant increase in stroma. Administration of testosterone led to disorganization in the female prostate, with a significant increase in collagen fibers and smooth muscle cells after 21 days, along with the development of epithelial lesions such as PINs. MMP-2 increased after 21 days of castration in males; however, the TIMP-2 immunoreaction for this group was weak or absent. In females, the expression of MMP-2 appeared to decrease after 7 days of treatment with testosterone, but after 21 days, both epithelium and stroma showed a stronger reaction for MMP-2 than the controls. The expression of TIMP-2 in the treated females was similar to its expression in the castrated males. We conclude that the distribution of MMPs and TIMPs in both male and female prostates is altered by androgen manipulation, but the mechanism of stromal regulation appears to be distinct between genders because both the lack of T in castrated males and the excess levels of T in treated females lead to the same effect.

Key words: MMP, TIMP, Female prostate, Ventral prostate, Testosterone

Offprint requests to: Prof. Dr. Sebastião Roberto Taboga, Cristóvão Colombo 2265. CEP 15054–000. São José do Rio Preto, SP, Brazil. e-mail: taboga@ibilce.unesp.br

Introduction

The prostate is an accessory gland located in the genital tract of mammals that, together with the seminal vesicle, produces the bulk of the seminal fluid (Hayward et al., 1996). Some female species also have a prostate gland in their reproductive system, but its function remains unclear. However, strong evidence suggests that female prostate may have function in the transport of male gametes (Zaviacic, 1999).

In both males and females, the prostate is composed of a glandular epithelium immersed in a fibromuscular stroma that is rich in extracellular matrix (ECM) elements such as collagen fibers (Vilamaior et al., 2000), proteoglycans (Kofoed et al., 1990), laminin (Carvalho and Line, 1996), fibronectin and elastin (Carvalho et al., 1997). In addition to a structural role, the ECM and related molecules have been recognized as key regulatory components during the morphogenesis and cell differentiation of many branched organs (Timpl, 1989; Bruni-Cardoso et al., 2008), and they play important roles in cellular processes such as migration, adhesion, proliferation, differentiation and apoptosis (Hay, 1991; Alberts et al., 2008).

The ECM constantly undergoes changes in response to cellular and hormonal stimuli during many

physiological situations such as morphogenesis (Werb and Chin, 1998) and cyclic female reproductive organ activity (Green and Lund, 2005; Zhang and Nothnick, 2005). Furthermore, during pathological conditions such as the inflammatory response (Arroyo and Iruela-Arispe, 2010), cardiac and vascular diseases (Dobaczewski et al., 2010) and prostate tumor progression (Tuxorn et al., 2001; Stewart et al., 2004; Niu and Xia, 2009), the ECM is extensively remodeled. Thus, tight regulation of ECM degradation and resynthesis is essential for the maintenance of tissue homeostasis.

The matrix metalloproteinases (MMPs) are metal-dependent endopeptidases responsible for the first step in ECM degradation, and they have a wide range of substrates. These enzymes are secreted as latent zymogen molecules called proMMPs that must be proteolytically processed to become active. In addition, the activity of MMPs can be inhibited by a group of four glycoproteins that are known as tissue inhibitors of metalloproteinases (TIMPs) (Matrisitan, 1990; Basset et al., 1997; Hullboy et al., 1997; Stamenkovic, 2000; Wilson et al., 2002; Visse and Nagase, 2003).

MMP-2 and MMP-9, also known as gelatinases, are key members of the MMP family, and they preferentially degrade basement membrane components, especially type IV collagen and laminin. The expression of gelatinases has been associated with many pathological conditions, particularly cancer metastasis and angiogenesis. As they play a pivotal role in normal physiological processes and pathological stages, an imbalance between TIMPs and MMPs appears to participate in tumor progression and the metastatic process in several tissues and organs (Wilson et al., 2002; Zhang et al., 2004; Delella et al., 2007). In addition, it is known that the expression of MMPs and TIMPs is regulated by hormones, especially androgens (Hulboy et al., 1997; Wilson et al., 2002; Felisbino et al., 2007).

Due to its suitability for studies of prostate development and pathogenesis in males and the high frequency of prostate occurrence in females, the Mongolian gerbil (*Meriones unguiculatus*) is a rodent that has been widely used in studies of the reproductive tract (Pinheiro et al., 2003; Corradi et al., 2004; Oliveira et al., 2007; Góes et al., 2007; Rochel et al., 2007; Campos et al., 2008; Scarano et al., 2008), especially for studies related to the female prostate (Custódio et al., 2004, 2008; Santos et al., 2006, 2007, 2008; Fochi et al., 2008).

The above-mentioned studies have demonstrated morphological similarities between the ventral lobe of the male prostate and the female prostate, but more detailed studies, especially concerning stromal components, are necessary. Thus, this investigation presents a characterization of MMP-2 and TIMP-2 distribution in both the female prostate and the ventral lobe of the male prostate of the Mongolian gerbil. In addition, the effect of hormonal manipulation on the prostatic stroma was also assessed.

Materials and methods

Animals and experiments

Male and female adult gerbils (*Meriones unguiculatus*, Gerbilinae: Muridae) weighing between 70 and 90 g (15-18 weeks old) were maintained under controlled conditions of luminosity (12 h light: 12 h dark) and temperature (25°C) with water and ration *ad libitum*. The experiments were performed in accordance with the Ethical Principles for Animal Experimentation - Brazilian College of Animal Experimentation.

Ten males were submitted to bilateral orchiectomy under anesthesia with ketamine (800 μl/kg) and xylazine (200 μl/kg), and they were sacrificed at 7 or 21 days after surgery (Castrated groups, n=5). To determine the estrous cycle phase in gerbil females, daily vaginal smears were collected at 10:00 AM for 12 consecutive days. Ten females in the proestrus phase received intradermal injections of Testosterone Cypionate (5 mg/kg, diluted in corn oil, 0.1 ml per dose) every other day and were sacrificed 7 or 21 days after the beginning of the treatment (Testosterone-treated groups, n=5). Both males and females without experimental manipulation constituted the Control groups (n=5).

Histological processing and stereological analysis

The prostates of the female gerbils and the prostatic ventral lobes of the males were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 24 h and were embedded in Paraplast (Sigma-Aldrich Co, USA). Histological sections (5 μm) were stained with Gömori's Trichrome for stereological analysis to evaluate the relative proportion (relative volume) of each prostatic tissue component, including the epithelium, lumen, smooth muscle cells (smc), collagen fibers and non-muscular stroma, as described by Huttunen and collaborators (1981). A total of 30 microscopic fields (200x magnification) from at least three animals per group were randomly digitalized and analyzed using Weibel's multipurpose grid with 130 points and 60 test lines (Weibel, 1963). In summary, the relative values were determined by counting the number of intersections of the grid that overlapped each tissue compartment and dividing them by the total number of points. Absolute volumes could not be determined because it was not possible to isolate the female prostate from the adhering tissue to determine its accurate weight.

Immunohistochemistry

For MMP-2 and TIMP-2 detection, paraplast sections (5 μm) from all experimental groups were pretreated with the citric acid monohydrate antigen retrieval method. Next, they were immunostained with mouse monoclonal antibodies against MMP-2 (sc-13595, Santa Cruz Biotechnology, Inc) and TIMP-2 (sc-21735, Santa Cruz Biotechnology, Inc) at a 1:100

dilution in TBS containing 3% bovine serum albumin (BSA) overnight at 4°C, according to the manufacturer's protocol. The primary antibody was detected using a peroxidase-conjugated Polymer (Novolink Polymer, Leica Microsystems, Inc., USA), and the peroxidase activity was revealed using 3,3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich Co, USA). The sections were counterstained with Harris hematoxylin. Negative controls were obtained by omitting the primary antibody incubation step.

Hormonal dosage

Circulating serum testosterone and estradiol levels were determined using immunochemical assays. Serum was separated by centrifugation and stored at -20°C for subsequent assays. Measurements were performed in triplicate, using automated equipment (Vitros-ECi; Johnson & Johnson, orthoclinical Diagnostics Division, Amershan, UK) for detection by ultrasensitive chemiluminescence. The sensitivity was 0.1-150 ng⁄ml for testosterone and 0.1-3.814 pg/ml for estradiol. For testosterone, the intra- and inter-assay variations were 1% and 1.1%, respectively, and for estradiol they were 2.1% and 1.5%.

Statistical analysis

The data were evaluated by analysis of mean ± standard deviation (SD). Statistical analyses were performed with Statistica 6.0 software (StatSoft, Inc., Tulsa, OK, USA). One-way Analysis of Variance (ANOVA) was performed to determine possible differences among groups, and the Tukey HSD test was employed to determine the significance of the differences. A p-value of ≤0.05 was considered statistically significant.

Results

Serum steroid hormones

Serum steroid hormone levels are shown in Table 1. In males, serum testosterone levels drastically dropped after surgical castration, with values ranging from 1.5 ng/ml in the control group to below 0.2 ng/ml at day 7 and 21 of the experiment. The levels of estradiol showed a slight decrease only after 21 days of castration (11.3 pg/ml), which was not statistically significant. In females, exogenous testosterone administration resulted in sustained high levels of circulating testosterone, with values reaching as high as 14 ng/ml and 12 ng/ml after 7 and 21 days of treatment, respectively. These values were significantly higher than the levels in the control females in the proestrus phase (0.2 ng/ml). The circulating estradiol levels did not show significant alterations with T treatment.

Morphological and stereological analysis

The stereological data of the prostates of normal, castrated male and testosterone-treated female gerbils are shown in Table 2. The stereological assessment of the male ventral prostate tissue compartments revealed significant alterations for all evaluated components of the Castrated groups when compared to the Controls

Table 1. Serum levels of testosterone (T) and estradiol (E) of male gerbils intact or castrated for 7 or 21 days and of female gerbils in proestrus phase or treated with testosterone for 7 or 21 days.

	MALES			FEMALES		
	C	7	21	C	7	21
T (ng/ml)	1.5±0.2	0.2±0.03*	0.2±0.04*	0.22±0.04	14.2±0.5*	12.2±1.2*
E (pg/ml)	15.3±1.7	14.8±2.3	11.3±1.2	23.9±3.1	17.5±2.9	24.1±5.8

n=10 samples/group. Values are means ± SE. Asterisks represent significant differences relative to the control group.*Tukey HSD Test, p≤0.05.

Table 2. Descriptive prostatic stereology statistics of male and female adult gerbils in different hormonal situations.

	MALE VENTRAL PROSTATE			FEMALE PROSTATE		
	Control	Castration (7 d)	Castration (21 d)	Control	Testosterone (7 d)	Testosterone (21 d)
Epithelium	14.5±5.7	10±6.05*	28.5±8.2*	26.2±11	26.7±4.5	27.7±10.7
Lumen	72.6±9.8	78.3±9.7	28.3±15*	41.1±19	27.2±8.3*	36.3±22.7
Collagen fibers	4.4±2.2	3.4±2.64	15.7±7.3*	9.6±4.5	16.3±4.2*	15.5±10*
Smooth muscle cells	7±3.9	6.8±2.8	21.9±5.5*	16.2±8.1	22±5.6	13.9±12
Other	1.4±1.7	1.5±1.42	5.5±2.9*	6.7±6.6	7.6±3.3	6.5±5.5

Values (%) are means ± SD. The asterisks (*) indicate statistically significant differences relative to controls. *Tukey HSD Test, p≤0.05.

especially at 21 days after castration.

In the 7-day castrated group (Fig. 1B), the drop in the androgen levels led to a drastic reduction in the height of epithelial cells and consequently in their secretory activity. The volume occupied by the epithelial compartment showed a significant decrease in this group, from 14.5% in the intact animals to 10% in the 7-day castrated group.

After 21 days of castration (Fig. 1C,G), the extended decline in T levels promoted the involution of the whole organ, with both epithelial regression and stromal reorganization. Furthermore, the appearance of epithelial folds, connective tissue condensation and fibromuscular wall thickening was observed. The acini also suffered a reduction in luminal size, and an apparent increase in the amount of blood vessels with reduced diameter was observed. Stereology showed that epithelial volume significantly increased, and the morphology of epithelial cells changed from pavimentous, as found in the 7-day castration group, to cubic or columnar. In this group, the increase in epithelial volume density was counterbalanced by the reduction of the volume occupied by the lumen, which was reduced from 72% in control animals to 28% in the Castrated group, showing

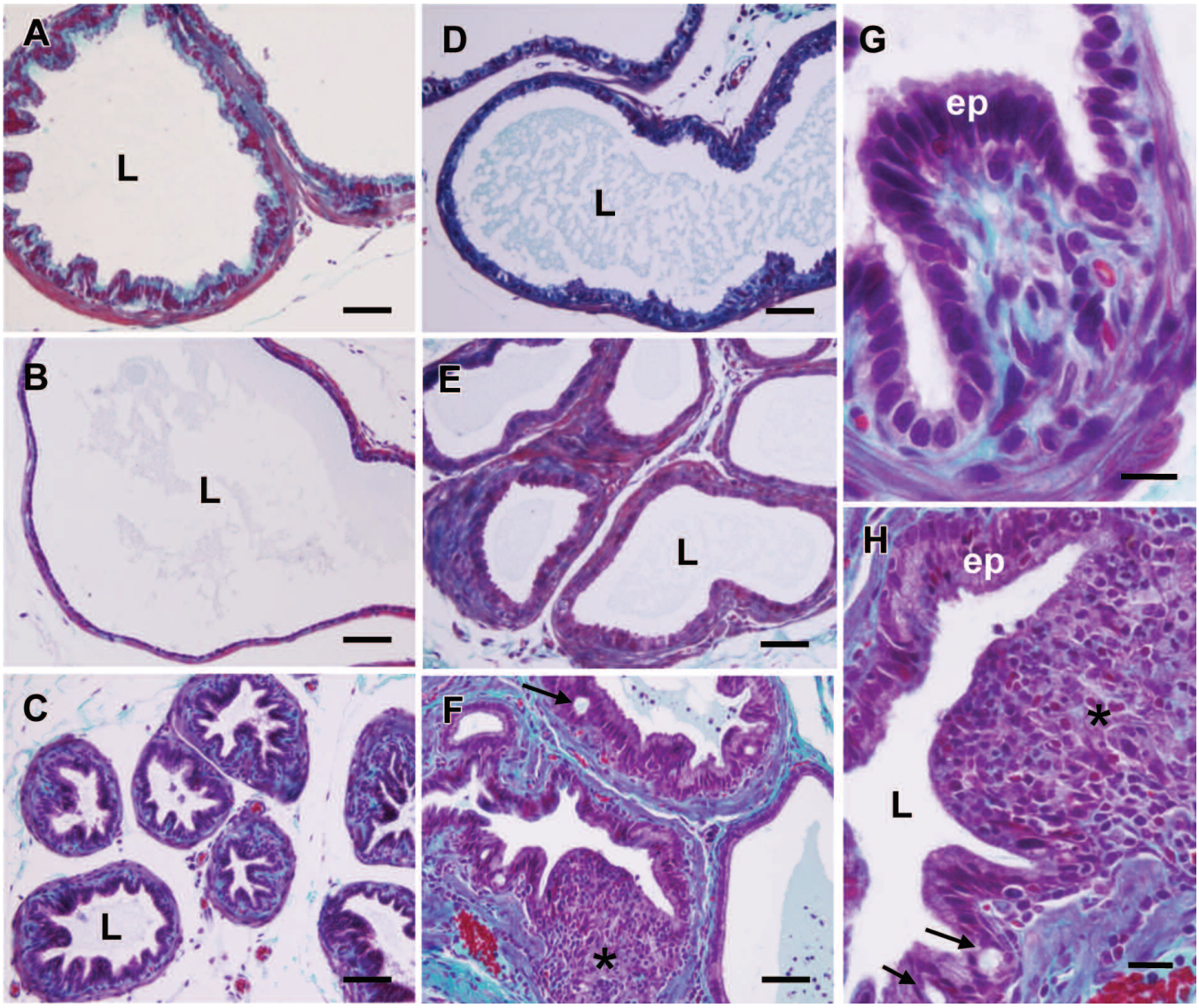

Fig. 1. Histology of the ventral prostate of male gerbils and the prostate of female gerbils stained with Gömori's Trichrome. Male ventral prostate controls **(A)** castrated for 7 (**B**) or 21 days (**C, G**). Female prostate controls (**D**) and females treated with testosterone for 7 (**E**) or 21 days (**F, H**). In **G**, detail of a ventral male prostate after 21 days of castration showing epithelial in-folding with subepithelial accumulation of collagen fibers stained in green. In **H**, a female prostate treated with testosterone for 21 days showing microacini (arrows) and epithelial proliferative lesions (*). ep: epithelium; L: lumen. Bars: A-F, 50 μm; G, 10 μm; H: 20 μm.

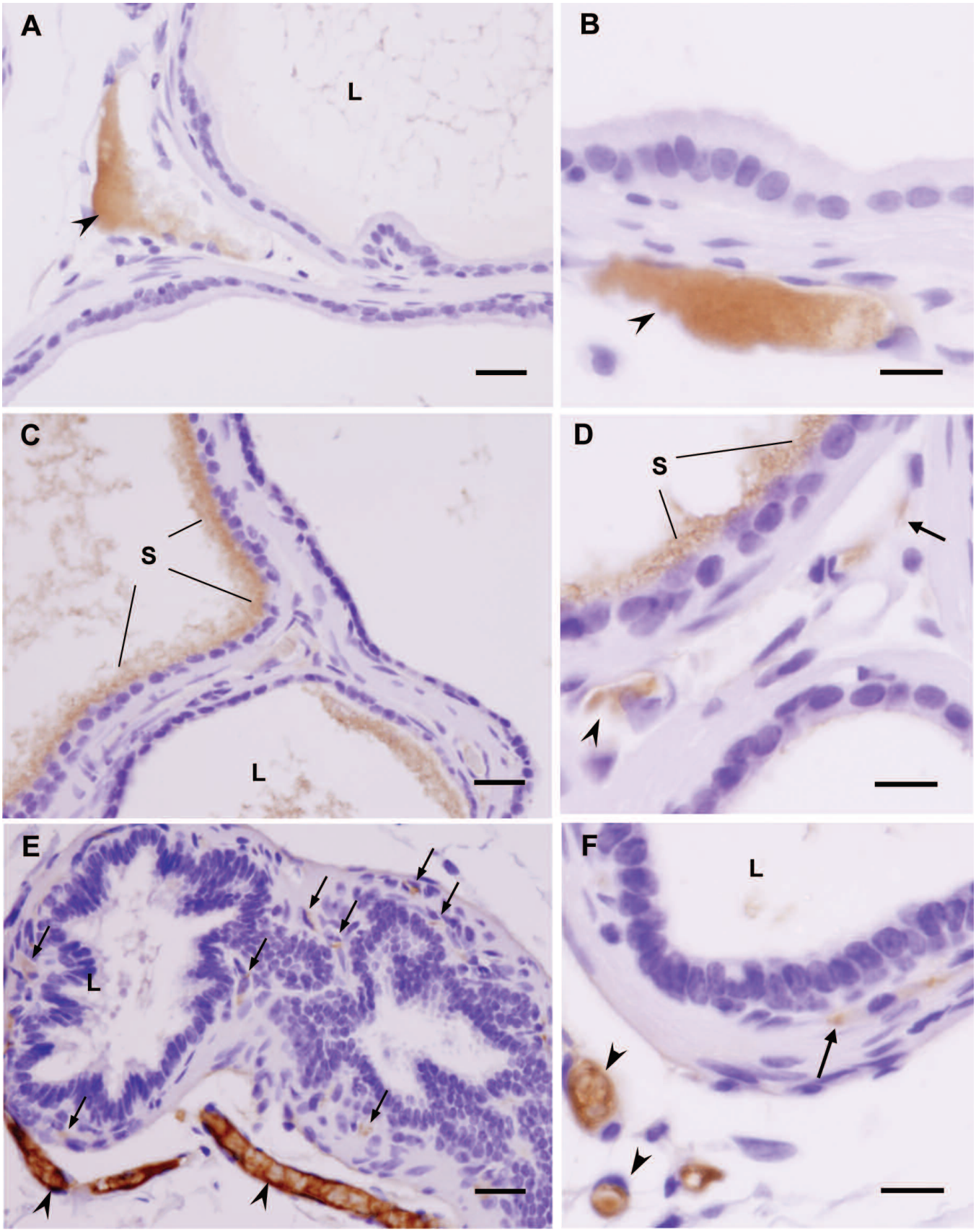

Fig. 2. Immunohistochemical reaction for active MMP-2 in male gerbil ventral prostates. Controls (**A, B**) and castrated for 7 (**C, D**) or 21 days (**E, F**). The immunoreaction was more intense after 21 days of castration, with strong immunostaining in the blood vessels and various MMP-2-positive regions throughout the stroma. S: secretion; arrow heads: MMP-2-positive blood vessels; arrows: positive stromal cells. Bars: A, C, E, 20 μm; B, D, F, 10 μm.

great involution of the whole organ after 21 days of androgen suppression (T). The results also demonstrated that the stromal compartment occupies approximately 13% of the ventral lobe in intact adult male gerbils, with 7% including the smc and 4% including collagen fibers (Table 2). These values were not significantly changed after 7 days of castration (6.8% smc and 3.4% collagen fibers), but they significantly increased in the 21-day Castrated group, rising to 22% smc and to 15.7% collagen fibers (Table 2, Fig. 1G). These results demonstrated great sensitivity of the stromal components to the absence of testosterone in the male ventral prostate, because together they occupied approximately 40% of the total organ after 21 days of castration versus only 12% in the intact animals of the control group.

In the prostates of females in the proestrus phase, the acinar compartment (epithelium+lumen) composed the majority of the prostate volume (~60%) and remained relatively constant throughout the treatment with testosterone (T), as shown in Table 2. The remaining volume was shared by the smc, collagen fibers and other connective tissue components, which were, respectively, 16%, 9.6% and 6.7% of the total volume. Stereological evaluation showed a significant increase in the amount of collagen fibers after 7 and 21 days of T treatment compared to the control group (Table 2). In addition, the administration of T for 21 days led to the disorganization of the female prostate. Tissue components were disrupted, stromal remodeling occurred, epithelial lesions such as prostatic intraepithelial neoplasia (PIN) developed and microacini formed in the epithelium (Fig. 1F, H).

Immunohistochemical analysis

Immunohistochemical analysis of MMP-2 and TIMP-2 in the male ventral prostate

In the male ventral prostate, an immunohistochemical reaction to active MMP-2 occurred only in

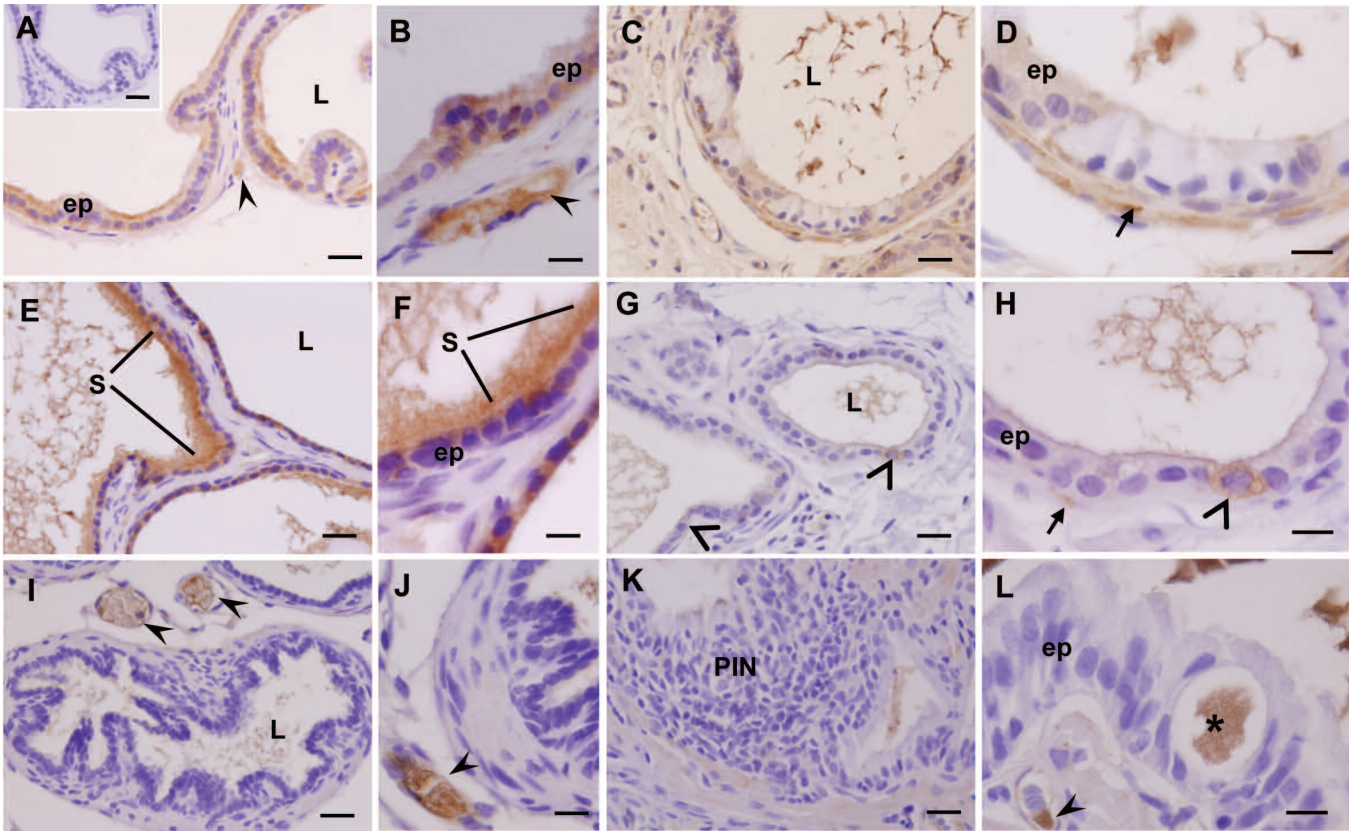

Fig. 3. Immunohistochemical reaction for TIMP-2 in male gerbil ventral prostates and female gerbil prostates. Ventral prostates from control males (**A, B**) and from males castrated for 7 (**E, F**) or 21 days (**I, J**). Prostates from intact females (**C, D**) and from females treated with testosterone for 7 (**G, H**) or 21 days (**K, L**). In **L** and **K**, epithelial alterations in female prostates resulting from 21-day T treatment; microacini filled with immunostained secretions (*) and prostatic intraepithelial neoplasias (PINs), respectively. The inset in A shows a negative control for TIMP-2 immunohistochemical reaction. ep: epithelium; L: lumen; S: secretion; PIN: prostatic intraepithelial neoplasia; asterisk: microacinus with TIMP-2 positive secretion; arrows: TIMP-2-positive stromal cells; full arrowhead: TIMP-2-positive blood vessels; empty arrowhead: TIMP-2-positive epithelial cells. Bars: A, C, E, G, I, K, 20 μm; B, D, F, H, J, L, 10 μm.

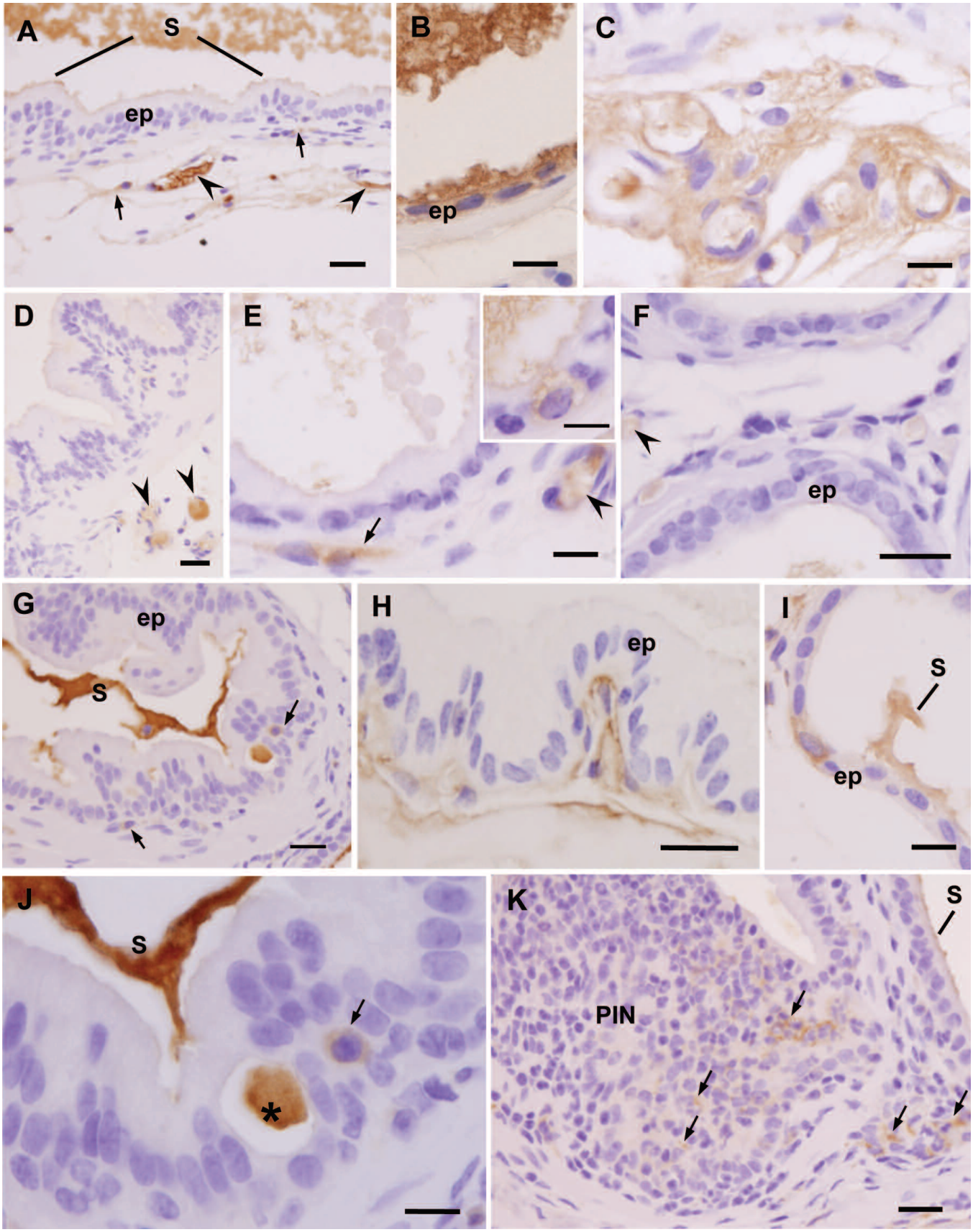

Fig. 4. Immunohistochemical reaction for active MMP-2 in the female gerbil prostate. Female prostates from intact animals (**A-C**) and from animals treated with T for 7 (**D-F**) or 21 days (**G-K**). In **E**, the inset shows positive epithelial cells among negative cells in the group treated with T for 7 days. In **J** and **K**, respectively, microacini filled with immunolabeled secretion (*) and prostatic intraepithelial neoplasias (PIN), both epithelial alterations present in the 21-day group showing MMP-2-positive reaction. ep: epithelium; S: secretion; PIN: prostatic intraepithelial neoplasia; *: microacinus; arrows: positive stromal cells; arrowheads: positive blood vessels. Bars: A, D, F, G, H, K, 10 μm; B, C, E, I, J, 20 μm.

blood vessels in the intact animals of the control group, with no positive reaction in the epithelial cells. The immunoreaction was specific to blood plasma, (diffuse immunolabeling) because the reaction was negative in erythrocytes and other blood cells (Fig. 2A,B). In the 7-day castrated group, the immunoreaction for active MMP-2 occurred in the lumen secretions of only some acini and was located on the apical border of the epithelial cells. Marked immunolabeling was also observed in some stromal fibroblasts and in blood plasma (Fig. 2C,D). After 21 days of castration, the immunoreaction for MMP-2 was more intense than in the other male groups, with strong MMP-2 immunostaining in the blood vessels and various stromal regions. Moreover, several MMP-2-positive fibroblasts were observed in regions with epithelial disruptions (Fig. 2E,F).

Alternatively, immunolabeling for the tissue inhibitor TIMP-2 was positive in the epithelial cells of the ventral prostate in the male control group, but the staining was non-homogeneous because not all of the secretory epithelial cells showed a positive immunoresponse. Additionally, among the positive cells, a marked difference in the immunolabeling intensity was observed (Fig. 3A,B). Furthermore, the majority of blood vessels showed a positive immunoreaction in blood plasma in this group. In the prostates of males castrated for 7 days, every evaluated acini showed a TIMP-2-positive reaction in the epithelial cells that varied from weak to strong. Even the pavimentous epithelial cells that were observed in this group were TIMP-2-positive, and in some regions, this enzyme was found in prostatic secretions and in blood plasma (Fig. 3E,F). After 21 days of castration, immunolabeling for TIMP-2 appeared to be preferentially plasmatic, with weak or absent reactions in other prostatic components (Fig. 3I,J).

Immunohistochemical analysis of MMP-2 and TIMP-2 in the female prostate

The immunolocalization of active MMP-2 in control female prostates was quite diverse, occurring in some epithelial cells, in the secretions and in the stromal compartment (Fig. 4A-C). In most acini, MMP-2 was present in the apical border of the luminal secretory cells (Fig. 4B); however, in the stroma, the immunoreaction was visualized in both the interior of blood vessels and in connective tissue fibroblasts (Fig. 4A,C). After 7 days of T administration (Fig. 4D-F), the expression of active MMP-2 appeared to decrease and was found only in some positive fibroblasts in the stroma (Fig. 4E) and in blood vessels (Fig. 4D,F); however, only a few positive secretory cells were observed in the epithelium, as shown in detail in Figure 4E. The prostatic secretion released into the lumen also presented a weak reaction to active MMP-2 in this group. The reaction for active MMP-2 in the 21-day T group (Fig. 4G-K) appeared to be stronger than that in other groups, both in the epithelium (Fig. 4I) and in stroma (Fig. 4H). A strong reaction was observed in the basal membrane (Fig. 4H) and in the secretion discharged into the lumen (Fig. 4G, J).

TIMP-2 immunolocalization in the female control group was similar to that in the ventral prostates of control males, as shown by positive reactions in the cytoplasm of epithelial cells of all acini but with non-homogeneous distribution (Fig. 3C,D). In some regions, the reaction was strong, but in others regions, the secretory cells showed no reaction. TIMP-2 was also found in areas of non-muscular stroma, especially in blood vessels. After 7 or 21 days of T administration, the expression of TIMP-2 was weak, only occurring in the prostatic secretion and in the apical border of the secretory cells (Fig. 3G,H,K,L). Only a few epithelial cells demonstrated TIMP-2-positive staining in the epithelium. The stromal compartment and the blood plasma of the testosterone-treated female groups also showed a negative reaction to the TIMP-2 antibody.

Discussion

This study demonstrates that both male and female gerbil prostates are sensitive to the circulating levels of androgens, which may cause important alterations in the prostate gland, especially regarding the ECM. However, males and females showed different responses to altered serum testosterone levels.

The experimental protocol employed in this study resulted in circulating levels of testosterone that were 7x lower in males and more than 50x higher in females than the levels measured in untreated animals. As the levels of serum estradiol showed no significant alterations in either males or females, we assume that the results obtained here are due to the effects caused by the excess of testosterone in females or its absence in males.

A female prostate has been reported in humans (Zaviacic, 1999; Wimpissinger et al., 2007) and in several rodent species (Price, 1939; Shehata, 1980; Gross and Didio, 1987; Flamini et al., 2002), and its morphology and biochemistry show similarity to the ventral lobe of the male prostate (Santos and Taboga, 2006). In addition, the prostate gland in females has been reported to be susceptible to benign and malignant pathologies similar to the conditions developed by the male gland (Uzoaru et al., 1992; Dodson et al., 1995; Sloboda et al., 1998; Zaviacic et al., 2000; Custodio et al., 2004; Wimpissinger et al., 2009). The occurrence of pathological conditions in the female prostate of humans and rodents that are similar to the conditions affecting men such as cancer, benign prostatic hyperplasia and prostatitis (Sloboda et al., 1998; Zaviacic, 1999; Chan et al., 2000; Pongtippan et al., 2004; Santos et al., 2006; Custódio et al., 2008; Wimpissinger et al., 2009), indicates that it is an organ highly dependent on hormonal action, in particular to testosterone levels, as demonstrated in this study. Excess T induced stromal alterations in the female prostates treated for 21 days,

including an increase in the amount of collagen and smooth muscle cells along with overexpression of MMP-2. The epithelium was also impaired by androgen activity, resulting in the appearance of pathological epithelial lesions such as PINs and microacini.

The suppression of androgens in the male leads to a process of regression known as prostate involution, which is mainly characterized by epithelial atrophy and a progressive decrease in the volume of the gland (Lee, 1996). This process of involution that occurs in the gerbil is similar to the process described for the mouse (Sugimura et al., 1986; Vilamaior et al., 2000; Antoniolli et al., 2004), which is characterized by a decrease in acinar size and epithelial atrophy, and these changes become clearer due to apoptosis (Rittmaster et al., 1995; Kurita et al., 2001; Campos et al., 2010) that occurs two weeks after castration. The stroma and epithelium have a direct interaction with each other, the so-called epithelial-stromal interaction; therefore, the stroma undergoes remodeling after castration, as shown by increases in both muscle cells and collagen fibers and a change in the composition of the basement membrane (Vilamaior et al., 2000; Cunha et al., 2004). According to some authors, alterations in the composition of the basement membrane and other components of connective tissue may influence cellular processes such as proliferation and differentiation in the prostate, and these changes may have fundamental importance in the etiology and progression of pathological processes (Tuxhorn et al., 2001). This hypothesis would explain the appearance of pathological epithelial alterations, as observed in the 21-day castration group. Moreover, a drastic reorganization of collagen fibers and dedifferentiation of smooth muscle cells of the prostatic stroma have been previously detected in the ventral lobe of rats after surgical castration (Carvalho and Line, 1996; Carvalho et al., 1997) or chemical castration using finasteride (Corradi et al., 2004); the authors suggest that stromal remodeling following androgen ablation involves transformation of the contractile phenotype of smooth muscle cells to a secretory phenotype, indicating that these smooth muscle cells may be key players in stromal remodeling. However, the results found in this study suggest that several factors in addition to smooth muscle cells may act in the process of prostatic regression, including the regulators of the extracellular matrix MMP-2 and TIMP-2.

The stereology results obtained in this study show a significant increase in the volume of collagen fibers in the ventral male prostate after 21 days of castration, although there was an apparent increase in the expression of MMP-2 in this group. Thus, these data show that the collagen degradation promoted by MMP-2 does not necessarily reduce the fiber content in the ventral male prostate, but it does cause a break in its structure, allowing tissue reorganization in this group when the prostate undergoes structural regression and the ECM is remodeled.

Metalloproteinases are secreted as latent molecules called proMMPs that must be proteolytically processed to become active. Some MMPs can become activated on the cell surface by complexing with metalloproteinases linked to the cell membrane (the MT-MMPs), which can accumulate in the boundary and express local gelatinolytic activity (Hullboy et al., 1997; Quaranta, 2000; Stamenkovic, 2000, Wilson et al., 2002; Murphy and Nagase, 2008; Belhocine et al, 2010). According to Itoh et al. (2008), pro-MMP-2 is activated at the cell surface by active MMP-14 in cooperation with TIMP-2. In some of the studied groups, MMP-2 and TIMP-2 expression occurred close to the apical membrane of the epithelial cells on the luminal side, and this expression may be related to the process of enzymatic metalloproteinase activation.

Our results demonstrate the presence of MMP-2 and TIMP-2 in blood plasma, with an increase in their expression after 21 days of castration. According to some authors, white blood cells produce MMPs when they are involved in inflammatory reactions, and tissue remodeling is considered to be an inflammatory reaction that attracts inflammatory cells. It has been shown in the literature that castration induces an increase in the number of stromal and intraepithelial macrophages and mast cells, which also express MMP-2 (Franck-Lissbrant et al., 1998; Kwong et al., 1999; Elkington et al., 2009; Justulin et al., 2010). According to Justulin Jr. et al. (2010), stromal cells such as macrophages and mast cells also contribute to the entire content of prostate MMPs and may also participate in the regulation of tissue remodeling during prostate atrophy.

The prostatic secretions of both the male and female controls of this study had a positive immunoreaction for MMPs and TIMPs, indicating the presence of these proteases in the fluid produced by the prostate secretory epithelial cells. Studies have shown that, in addition to acting as components of the extracellular matrix, MMPs and TIMPs have also been found in seminal plasma, and their presence in prostatic secretion has been demonstrated (Yin et al., 1990; Wilson et al., 1993; Delella et al., 2007; Belhocine et al., 2010). Belhocine and collaborators (2010) suggested that MMP-2 and MMP-9, found in the prostate of *Meriones libycus,* facilitate the process of secretion in the tubules and help to maintain the flow of secretion.

Recently, zymographic analysis of Wistar rat prostates showed that MMP-2 and MMP-9 activity increases after castration, and it is mainly the active forms of these enzymes that increase (Justulin Jr. et al., 2010). Accordingly, our results demonstrated that after 21 days of treatment, both castrated males and females treated with T showed more intense active MMP-2 immunoreactivity when compared to their controls. However, the tissue distribution of MMP-2 in the 21-day castrated group changed. MMP-2 was primarily located in stromal cells and in blood vessels rather than on the epithelium surface, and this distribution contributes to tissue remodeling. As expected, the opposite occurred with the TIMP-2 reaction, which was weak and

sometimes absent in both male and female prostates.

This finding demonstrates that T promotes an alteration in the MMP/TIMP balance; however, it acts antagonistically in both organisms because an excess of T appears to promote an increase in the expression of MMP-2 in female prostates. This increase assists the process of remodeling and causes pathological changes, whereas a drop in T levels exerts this effect on the ventral prostate lobe of male gerbils; this mechanism remains to be elucidated.

Thus, MMP-2 and TIMP-2 are key factors that contribute to the ability of both female and male gerbil prostates to respond to the alterations promoted by androgenic changes. This response involves ECM degradation and stromal remodeling. Further biochemical studies are extremely important because they could elucidate the behavior of these proteases in male and female diseases.

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