Histol Histopathol (2010) 25: 619-636

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Immunoexpression of gelatinase (MMP-2 and MMP-9) in the seminal vesicles and ventral prostate of Libyan jird (*Meriones libycus*) during the seasonal cycle of reproduction

M. Belhocine[1,2], T. Gernigon-Spychalowicz[2], M-P Jacob[3], Y. Benazzoug[4] and J-M Exbrayat[5]

[1]Department of Agronomic Sciences, Faculty of Exact Sciences and of Natural and Life Sciences, Abdelhamid Ibn Badis University, Mostaganem, Algeria, [2]Laboratory of Arid Areas Research (LRZA), Reproduction of Small Vertebrates, Faculty of Biological Sciences (FSB), El Alia, Algiers, Algeria, [3]INSERM U698, Bichat-Claude Bernard Hospital, Paris Cedex 18, France and [4]Laboratory of Cellular and Molecular Biology (BCM), Extracellular Matrix, Faculty of Biological Sciences (FSB), USTHB, El Alia, Algiers, Algeria and [5]University of Lyon, Laboratory of General Biology, Catholic University of Lyon (UCLy), Laboratory of Reproduction and comparative Development (EPI+E), Lyon Cedex, France

Summary. An immunohistochemical study of matrix metalloproteinases (MMP-2 and MMP-9) or gelatinase (gelatinase A and gelatinase B) was performed on the seminal vesicles and ventral prostate of the Libyan jird (*Meriones libycus*) collected in the Beni-Abbes area during breeding period (spring and early summer), during resting phase (late summer, autumn, winter) and from castrated animals in the spring. The work was done using the indirect immunohistochemistry protocol by amplification with streptavidin-biotin-peroxidase and AEC as chromogen.

In the seminal vesicles, during the breeding period, an important immunohistochemical signal of MMP-2 and MMP-9 was observed in epithelial cells and smooth muscle cells (SMC) without any immunoexpression in the extracellular matrix (ECM) and secretion. During resting phase and in thirty days castrated *Meriones libycus*, the MMP-2 and MMP-9 immunoexpression was weak in the epithelial cells and persisted with the same intensity in the SMC. The ECM, with no immunostaining in active season, showed a pronounced immunoresponse of both the two gelatinase. Three days after castration, the MMP-9 immunohistochemical reaction in epithelial cells and SMC was as intense as during active season. A prolonged castration of 50 and 90 days resulted in the maintenance of the MMP-9 immunostaining in epithelial cells and SMC and its disappearance from the ECM, suggesting a slow process of regression.

During the breeding period, in the ventral prostate, MMP-2 immunostaining was more important in the SMC than in epithelial cells. The MMP-9 immunoexpression pattern was the opposite, the epithelial cells showed a higher immunoreaction than SMC. ECM and secretion lacked MMP-2 and MMP-9 immunostaining. The ventral prostate lumen contained a granular secretion without any gelatinase immunolabelling and was hollowed by empty circular forms reflecting the disappearance of the product in these areas. Part of the secretion showed a positive MMP-2 and MMP-9 immunoreaction. The latter was subsequently filled and seemed involved in the progression of the secretion in the tubules, preventing their filling. In resting phase and in animals castrated since thirty days, the immunoreactivity of both the two gelatinases was maintained in the epithelial cells and in the SMC, and was absent in the ECM. The gelatinases are involved in the seasonal reproductive cycle of *Meriones libycus*.

Key words: Reproduction, Metalloproteinases, Seminal vesicles, Prostate, Rodent

Offprint requests to: Pr. Jean-Marie Exbrayat, Université de Lyon, Laboratoire de Biologie Générale, Université Catholique de Lyon (UCly) and laboratoire de Reproduction et Développement Comparé, Ecole Pratique de Hautes Etudes (EPHE), 25, rue du Plat, 69288 Lyon Cedex 02, France. e-mail: jmexbrayat@univ-catholyon.fr. Dr. Belhocine Mansouria, Laboratoire de Recherche sur les Zones Arides (LRZA), Reproduction des Petits Vertébrés, Faculté des Sciences Biologiques (FSB), USTHB, 16111 El Alia, Alger, Algérie. e-mail: manbelhocine@hotmail.com

Introduction

Rodents inhabiting temperate regions and those living in arid and semi-arid areas are adapted to not breed when climatic conditions are inappropriate for the growth of young animals. The stopping of reproductive activity is characterized by structural and functional atrophy of reproductive organs correlated with a decrease of hormone production (Belhocine and Gernigon 1994; Young et al., 2000; Aguilera-Merlo et al., 2005, 2009; Schradin, 2008). Generally the process of regression generates organs characterized by extracellular matrix accumulation, fibromuscular wall hypertrophy and epithelial cells apoptosis leading to epithelial compartment involution (Belhocine, 1998, 2008; Belhocine et al., 2007; Carballada et al., 2007); the effects of castration are similar (Belhocine and Gernigon-Spychalowicz, 1996; Justulin et al., 2006; Oliveira et al., 2007; Goes et al., 2007). Recrudescent organs show an opposite structure with a dispersed stroma, a thin fibromuscular wall and a largely dominant epithelial compartment (El-Bakry et al., 1998; Aguilera-Merlo et al., 2005; Belhocine et al., 2007). These structural seasonal variations reflect a tissue remodeling that likely occurs through the reactions of degradation and re-synthesis of proteins of the extracellular matrix involving an enzyme system represented by proteases. In the Siberian hamster (*Phodopus sungorus*) proteases represented by matrix metalloproteinases (MMPs) were observed in regressing and recrudescent ovary and uterus caused by photoperiodic effects (Salverson et al., 2008; Shahed and Young, 2008).

Research about the MMPs is numerous. These enzymes are involved in tissue remodeling associated with normal physiological processes, such as reproduction, embryonic development and morphogenesis (Vu and Werb, 2000; Walsh et al., 2007), postnatal development of organs (Hulboy et al., 1997; Hu et al., 2004), healing (Mohan et al., 2002), bone and cartilage remodeling (Varghese, 2006; Krane and Inada, 2008), growth of the follicle hair, angiogenesis, hippocampal synaptic physiology and plasticity (Bozdagi et al., 2007; Wang et al., 2008a); and physiopathological process such as cancer, during which they are highly expressed (Ii et al., 2006; Cai et al., 2007), cardiovascular diseases (Lijnen, 2001; Kuzuya and Iguchi, 2003), lung diseases (Greenlee et al., 2007; Coraux et al., 2008), central nervous system disorders (Rivera et al., 2004), cirrhosis and fibrosis of the liver, arthritis, atherosclerosis and skin ulcerations (Murphy and Nagase, 2008).

The enzyme system of MMPs has been extensively studied in the female reproductive organs. Indeed, these organs undergo a cyclic tissue remodeling synchronized with the cyclical hormonal fluctuations; the MMPs play a crucial role in this remodeling and in the ovarian and uterine physiology. In the ovaries, they take part in all phases of growth and follicular atresia, ovulation, formation and luteolysis of the corpus luteum (Curry and Osteen, 2003; Ohnishi et al., 2005, Ribeiro et al., 2006; Kliem et al., 2007; Berisha et al., 2008). The MMPs are involved in the extracellular matrix remodeling of the endometrium associated with the proliferative phase, secretory step, menstrual stages, and during implantation (Curry and Osteen, 2003; Daimon and Wada, 2005), gestation, cervical dilation and postpartum involution (Manase et al., 2006; Kizaki et al., 2008). In mammary glands, they contribute to development phases, lactation and involution after stopping lactation by removing suckling young or by suspending milking (dry period) (Green and Lund, 2005; Rabot et al., 2007).

The MMPs are Zn^{2+} and Ca^{2+} dependent extracellular endopeptidases able to degrade all components of the ECM and non-matrix proteins, such as growth factors and their receptors, cytokines and chemokines, antimicrobial peptides, adhesion proteins such as E-cadherin and ß integrin, the cell surface proteoglycannes and a variety of enzymes (Nagase et al., 2006; Page-McCaw et al., 2007). Through their proteolytic action, they can produce bioactive fragments, release growth factors immobilized within the matrix (Nakamura et al., 2005; Ito et al., 2009) and may also allow, disable or modify the activity of signaling molecules leading to a microenvironment favorable to gene expression, cell migration, cell differentiation, growth and cell proliferation, cell survival and apoptosis. They can also change the tissue architecture through their action on proteins of the ECM and the cleavage of intercellular junctions and the basal lamina (Stamenkovic, 2003). Extracellular signals are transmitted to cells through receptors that anchor cells to the ECM which are the integrins (Larsen et al., 2006).

The MMPs are grouped into collagenases (MMP-1, -8, -13 and -18 in *Xenopus*), gelatinases (MMP-2, -9), stromelysin (MMP-3, -10, -11), matrilysin (MMP -7, -26), the membrane-type MMPs (MT-MMPs) including the transmembrane MMPs (MMP-14, -15, -16, -24) and glycosylphosphatidylinositol-anchored MMPs (MMP-17, -25). Other MMPs (MMP-12, -19, -20, -21, -23, -27, -28) are not still classified (Visse and Nagase, 2003; Bernal et al., 2005; Illman et al., 2006, 2008; Rodgers et al., 2009).

The MMPs are produced as zymogens, the majority of which is activated in the extracellular space by other proteolytic enzymes (serine proteinases, cysteine proteinases, plasmin and other MMPs) and non-proteolytic agents, such as denaturants, species reactive oxygen and reactive agents sulfhydriles. Several MMPs, such as MMP-11 and MMP-28 and the membrane-associated MMPs, are activated intracellularly by furin (Illman et al., 2003). It is the active form of membrane-type MMPs which is expressed at the cell surface. The pro-MMP-2 is activated at the cell surface by the active MMP-14 in cooperation with the TIMP-2. This later serves as an intermediary between the MMP-14 and pro-MMP-2. All membrane-type MMPs are able to activate pro-MMP-2, with the exception of MMP-17 (Longin et al., 2001; Itoh et al., 2008).

The expression of MMPs is controlled by hormones, cytokines, growth factors and by cell-cell and cell-extracellular matrix interactions. This regulation operates on several levels: gene transcription and mRNA stability, translation, intracellular transport, activation of zymogens, autodegradation, selective endocytosis, availability and accessibility to the substrate. The active MMPs are inhibited by a specific class of inhibitors, tissue inhibitors of metalloproteinases (TIMPs) comprising four members, TIMP-1, -2, -3, -4 which are able to react with all the MMPs (Brew et al., 2000; Stetler-Stevenson, 2008). A new mechanism for control of MMPs has recently been identified, namely their location at the cell surface. This is the concept of compartmentalization allowing to concentrate and to confine proteolysis in a given space (Ra and Parks, 2007). This cell surface docking permits the avoidance of the dissemination of the enzyme and the extent of proteolytic action, and could also protect them against attack by TIMPs (Yu and Stamenkovic, 1999, 2000; Yu and Woessner, 2000; Yu et al., 2002; Berton et al., 2007). Additionally, epigenetic mechanisms, such as DNA methylation or histone acetylation, may also contribute to MMPs regulation (Clark et al., 2007, 2008; Fanjul-Fernández et al., 2009).

The importance of MMPs and TIMPs in male reproductive function has been demonstrated in humans and mammals, although a limited number of studies have addressed the subject, the role of protease in male reproduction remains poorly understood compared to work performed in females. MMPs and TIMPs were detected in the seminal plasma of man (Shimokawa et al., 2002, 2003; Tentes et al., 2007). The MMPs have a role in gamete fusion during fertilization and acrosome reaction (Farach et al., 1987; Roe et al., 1988; Diaz-Perez and Meizel, 1992; Wolfsberg et al., 1993). The matrilysin (MMP-7) was identified in the male reproductive organs of the mouse and could be involved in sperm maturation by cleaving cell surface antigens or by acting on other proteins involved in the maturation of spermatozoa (Wolfsberg et al., 1993, Wilson et al., 1995). Overexpression of MMP-7 leads to structural disorder of reproductive organs and coincides with impaired fertility in males and premature maturation of the mammary gland in females (Rudolph-Owen et al., 1998). A deficiency of MMP-9 (gelatinase B) causes a decrease in fertility (Dubois et al., 2000). Prostatic epithelial and stromal cells placed in primary culture produce MMPs (MMP-2 and MMP-9) and TIMPs (TIMP-1 and TIMP-2) (Wilson et al., 2002). In exocrine glands, such as prostate and tubular structures, MMPs are produced by epithelial cells and discharged into the lumen to keep these structures clear by cleaving others secreted proteins which may cause blockage (Wilson et al., 1995; Saarialho-Kere et al., 1995; Hulboy et al., 1997). Hashimoto et al. (1997) suggest that MMP-7 may participate in the function of exocrine gland of normal prostate by preventing glandular obstruction.

In the testis, during spermatogenesis, germ cells must cross the blood-testis barrier to migrate from basal to the adluminal compartment (Yan et al., 2008; Cheng and Mruk, 2009; Li et al., 2009). Membrane junctions between the germ cells and Sertoli cells must be broken or restructured to allow germ cells to move in the seminiferous epithelium and reach the lumen of seminiferous tubules and, during spermiation, spermatozoa are detached from the Sertoli cells. Obviously a protease system is required to perform all these cellular events. This enzyme system is represented by serine proteases and MMPs. These are produced by Sertoli cells, germ cells, Leydig cells and peritubular cells (Ebisch et al., 2007; Huang et al., 2007; Le Magueresse-Battistoni, 2007; Liu, 2007).

In the organs in perpetual cyclical or seasonal structural changes orchestrated by hormones, growth factors, cytokines and cell-stroma interactions, the proteolytic action of MMPs generates an extracellular matrix, whose structure and composition are permissive to the establishment of such a specific physiological state, depending on the nature of the stimulus.

The aim of this work was to display immuno-histochemically the presence of gelatinase A (MMP-2) and gelatinase B (MMP-9) in the Libyan jird (*Meriones libycus*) seminal vesicles and ventral prostate during the seasonal reproductive cycle and after castration. We attempted to elucidate their involvement in the physiology of reproduction and tissue remodeling affecting these glands during seasonal reproduction. By a careful analysis of the seasonal fluctuations and effects of varying time of castration, we aimed to demonstrate an adaptive strategy in the physiological expression of the enzyme system constituted with matrix metalloproteinases in order to circumvent the unfavorable Saharan conditions.

Materials and methods

Animals

The Libyan jird (*Meriones libycus*) is a nocturnal herbivorous and granivore Saharan Rodent belonging to the Gerbillidae family. It lives in a superficial burrow arranged under the most important bushes to benefit from the shade produced by the plant. In the Algerian Sahara, Libyan jird is submitted to a seasonal reproductive cycle characterized by a short breeding period programmed in spring and beginning of summer, and a long phase of sexual quiescence from late summer until late winter.

The animals were collected during the years 2003 and 2005 in the Beni-Abbes area (W. Béchar) situated in northwestern Algerian Sahara. The capture took place by trapping them in the middle of each season. The trap was a latticed cage crammed with dates and roasted barley. The traps were deposited at night fall near the opening of inhabited burrows that were recognizable according to the fresh traces. These traps were recovered very early in the morning and the captured *Meriones libycus* were

transported to the laboratory. Adult males were separated from females and the immature animals and then placed in a collective cage and nourished with grains of barley. They were kept for about 24 to 48 hours in the laboratory and then euthanized always in late evening (17h 00 – 19h 00) because of their nocturnal activity. The study was performed on three groups: seasonal group (37 individuals) to study the immunohistochemical seasonal variations of MMPs, castrated group (14 individuals) and control group (14 individuals) (see table 1 for animal number and their distribution in each group) to study the effects of testosterone deprivation on MMPs expression. *Meriones libycus* were castrated in breeding season (spring) by abdominal incision under ether anaesthesia. Control males and castrated animals were held in the laboratory in conditions of light (according to the photoperiod of season) and temperature (25°C) equivalent to those found in their burrows; they had free access to food (grilled grains). They were euthanized 3, 30, 50 and 90 days later.

Table 1. Summary of *Meriones libycus* number used in each season, as well as the number of *Meriones libycus* castrated in spring and the number of controls.

Animal group	Animal number
seasonal	
Spring	14
Summer	8
Fall	6
Winter	9
Castrated Meriones	
For 3 days	3+3 controls
For 30 days	5+5 controls
For 50 days	3+3 controls
For 90 days	3+3 controls

Immunohistochemistry

Both seminal vesicles and ventral prostate were quickly excised, carefully freed from surrounding fat and weighed. For histology and immunohistochemistry, tissue samples (approximately 5 mm thick) were fixed in Bouin-Hollande's fluid for 48 h or in 10% neutral buffered formalin for 24 h, dehydrated in a graded series of ethanol, cleared in cyclohexane, and embedded in paraffin. Serial sections (5 μm) were cut with a Leitz microtome and mounted on sterile water coated superfrost glass slides. The gelatinases (MMP-2 and MMP-9) were immunohistochemically visualized by the streptavidin-biotin-peroxydase complex (ABC) technique (Hsu et al., 1981). The antibodies used were: anti-MMP-2 (Rabbit anti-human MMP-2 polyclonal antibody [AbCys]) and anti-MMP-9 (Rabbit anti-human MMP-9 polyclonal antibody [AbCys]).

Following deparaffinization and hydration of the slides with distilled water, the sections were rinsed in phosphate buffer saline (PBS) and surrounded with Pappen and then treated for 5 min with hydrogen peroxide

Table 2. Summary of the intensity of the immunostaining for each antibody, tissue area, and stage of reproductive cycle and in castrated *Meriones libycus*.

			Seasonal group		Castrated animal group			
Organs	MMPs	Tissue area	Breeding period	Resting phase	Three-day castrated	Thirty-day castrated	Fifty-day castrated	Ninety-day castrated
Seminal vesicle	MMP-2	E-cell	+++	++		++		
		SMC	+++	+++		+++		
		ECM	–	+++		+++		
		S	–	–		–		
	MMP-9	E-cell	+++	++	+++	++	+++	++
		SMC	+++	+++	+++	+++	++	++
		ECM	–	+++	–	+++	–	–
		S	–	–	–	–	–	–
Ventral prostate	MMP-2	E-cell	++	++		++		
		SMC	+++	++		++		
		ECM	–	–		–		
		Initial S	–	–		–		
	MMP-9	E-cell	+++	+		++		
		SMC	+	+++		+++		
		ECM	–	–		–		
		Initial S	–	–		–		

From – (no staining) to +++ (very strong staining). MMPs: matrix metalloproteinases; E-cell: epithelial cell; SMC: smooth muscle cell; ECM: extracellular matrix; S: secretion.

diluted at 3% in PBS (PBS-H_2O_2 at 3%) to eliminate endogenous peroxydase activity. The slides were then rinsed in PBS buffer. Non specific protein binding was blocked by incubation with PBS-BSA at 1% for 10 min at room temperature. Sections were then incubated for 1 h at room temperature with primary antibodies, polyclonal anti-MMP-2, diluted to: 1:50 in PBS and polyclonal anti-MMP-9, diluted to 1:100 in PBS. The slides were then washed 3 times in PBS for 10 min and incubated with secondary biotinylated antibody directed against the primary one for 1 h at room temperature. After washing the slides 3 times in PBS during 10 min, the sections were reacted with biotin-peroxydase complex during 1 h at ambient temperature and then were rinsed 3 times in PBS. Enzymatic reaction was revealed by the application of substrate-chromogen solution during 10 to 15 min at room temperature, AEC was used such as a chromogen. All incubations were carried out in wet chambers to prevent evaporation. Sections were counterstained with Mayer's haematoxylin and mounted in aqueous medium with the AQ VectaMount (VECTOR laboratories) that does not require polymerization in an oven. Controls were performed by 1) substituting the primary antibody with buffer; 2) replacing the secondary antibody with buffer. Observation and photographs were made on a photo-microscope Nikon Eclipse E400 equipped with a Nikon digital camera DXM1200.

Results

Immunohistochemical seasonal variations of MMP-2 in Libyan jird seminal vesicles

In seminal vesicles of *Meriones libycus*, MMP-2 immunostaining was observed in epithelial cells, smooth muscle cells (SMC) and extracellular matrix (ECM).

During the breeding period, MMP-2 immuno-reactivity was important in epithelial cells and SMC, showing the same intensity in both cells (Fig. 1a,b). The extracellular matrix included in the epithelial folds axis showed a negative immunoreaction (Fig. 1a). The secretion is of fluid and homogeneous appearance and did not show any MMP-2 immunolabelling (Fig. 1a). Negative controls prepared without primary antibody did not show any immunostaining (Fig. 1a,b insert).

During the resting phase, the epithelial cells showed a lessened MMP-2 immunostaining (Fig. 2a); this latter remained strong in the SMC (Fig. 2c). The epithelial folds axis widened and was filled with a strong accumulation of the ECM. In the extracellular space a higher MMP-2 immunoreactivity appeared than that of epithelial cells (Fig. 2b). The morphological aspect of MMP-2 immunostaining and its distribution in extracellular space resembles that of fibrillar aspect of extracellular matrix protein, such as type I and III collagen, demonstrated previously by histochemical and immunohistochemical methods. This is an illustration of MMP-2 fixation on ECM proteins (Fig. 2b). Thirty days after castration, the MMP-2 immunostaining is identical to that during sexual quiescence (Fig. 2d,e). All negative controls prepared without primary antibody were devoid of immunostaining (Fig. 2a-e in insert).

Immunohistochemical seasonal variations of MMP-9 in Libyan jird seminal vesicles

In *Meriones libycus* seminal vesicles, MMP-9 immunoexpression was located in both epithelial cells and SMC, like MMP-2.

During the breeding period, both the epithelial cells and SMC strongly expressed MMP-9 and with the same intensity (Fig. 3a,b). The ECM of epithelial folds and secretion did not express any signal (Fig. 3a).

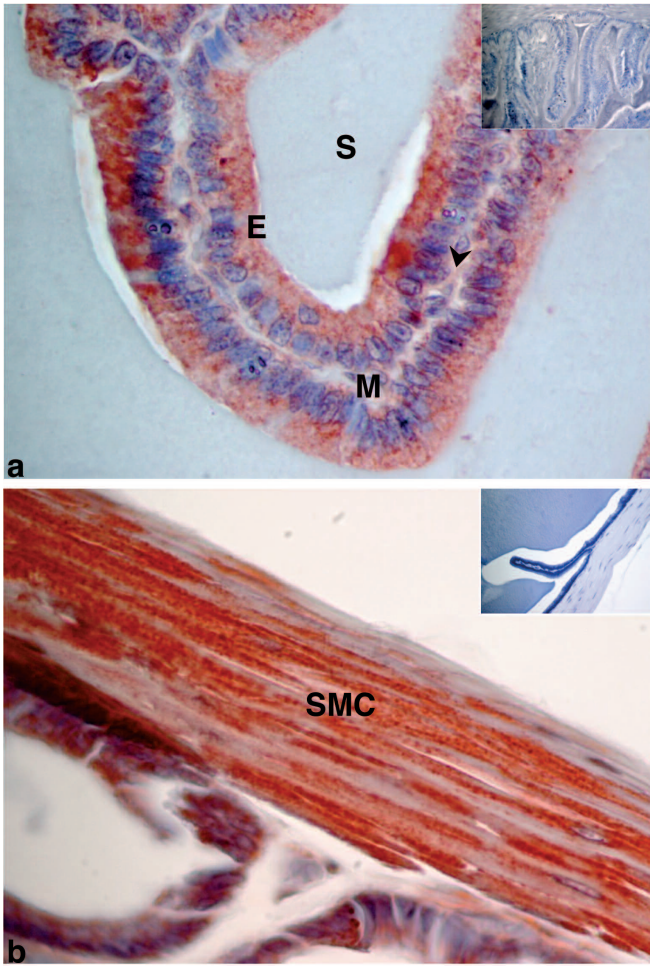

Fig. 1. MMP-2 immunohistochemistry in the adult Libyan jird (*Meriones libycus*) seminal vesicles in breeding period (spring and early summer). **a.** An important immunohistochemical signal was observed in epithelial cells (E); secretion (S) and the extracellular matrix (M) included in the narrow epithelial folds axis (arrowhead) were not immnolabelled. **b.** In the smooth muscle cells (SMC) immunostaining is similar to that of the epithelial cells. In insert are presented the negative controls prepared without primary antibody. a, b, x 1250

During the resting period, MMP-9 immunoexpression was reduced in epithelial cells and maintained in SMC with the same intensity as in the active season (Fig. 3c,d). A high proportion of ECM with a significant MMP-9 immunostaining was housed in the dilated epithelial folds axis (Fig. 3c).

In castrated *Meriones libycus*, three days after castration, MMP-9 immunostaining of the epithelial

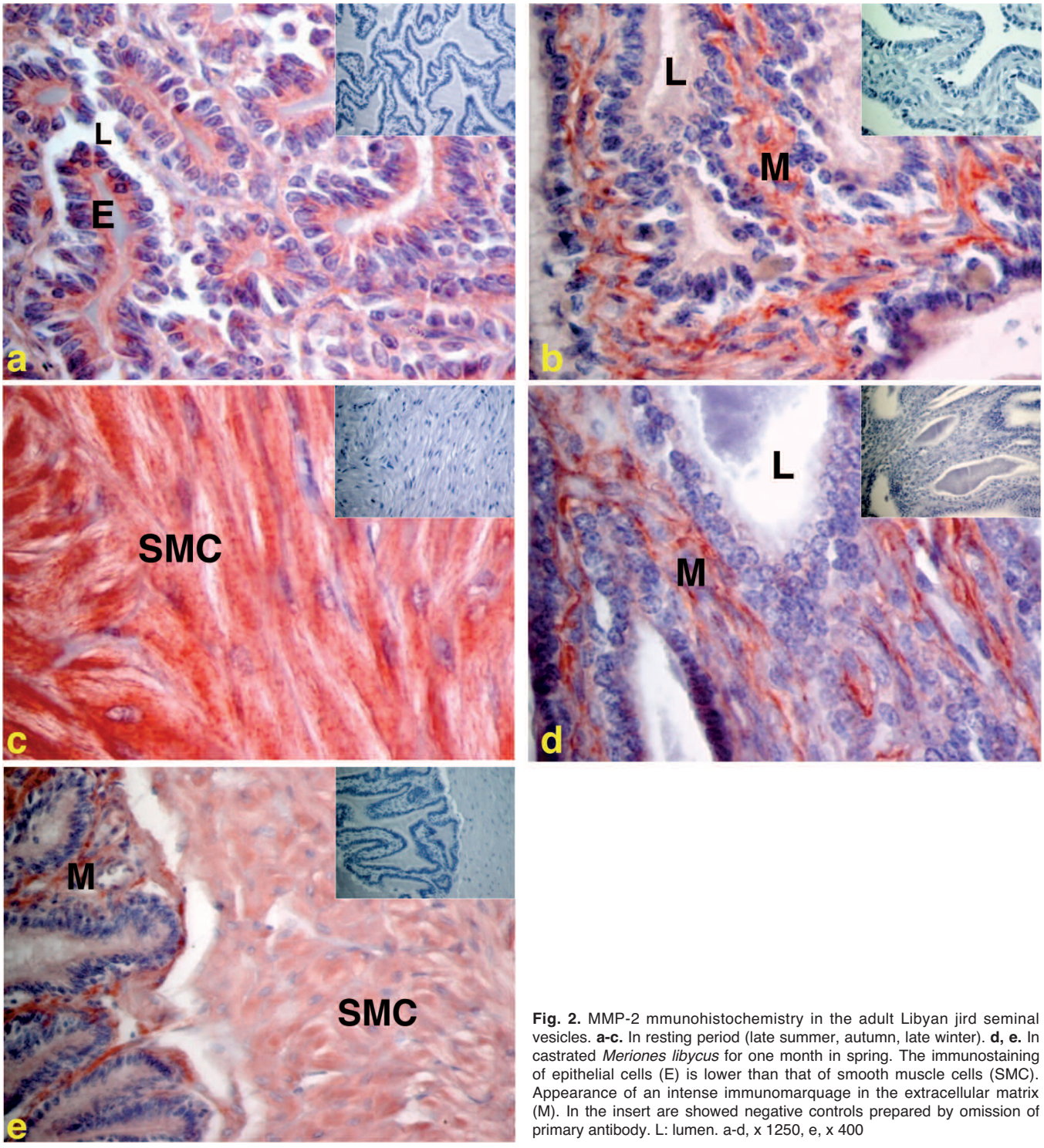

Fig. 2. MMP-2 mmunohistochemistry in the adult Libyan jird seminal vesicles. **a-c.** In resting period (late summer, autumn, late winter). **d, e.** In castrated *Meriones libycus* for one month in spring. The immunostaining of epithelial cells (E) is lower than that of smooth muscle cells (SMC). Appearance of an intense immunomarquage in the extracellular matrix (M). In the insert are showed negative controls prepared by omission of primary antibody. L: lumen. a-d, x 1250, e, x 400

cells was as strong as in active period, and in the narrow axis of the epithelial folds, ECM remained without immunostaining (Fig. 4a). 30 days after castration, MMP-9 immunostaining, low in the epithelial cells, appeared with a strong intensity in the ECM infiltrated in the extended epithelial folds axis. Immunostaining distribution in the extracellular space was comparable to that of type I and III collagen previously demonstrated immunohistochemically, signifying a sequestration of MMP-9 in the ECM (Fig. 4b). After a long-term castration of 50 days (Fig. 4c) and 90 days (Fig. 4d) epithelial cells showed a significant immunoresponse comparable to that expressed in the active phase, despite their atrophied state, and in the dilated axis of the epithelial folds ECM lost its immunoexpressions (Fig. 4c,d). In the SMC MMP-9 immunostaining persisted in all castrated Meriones (Fig. 4e,f).

No immunoreaction was detected in any of the negative controls prepared by omission of the primary antibody and presented in the insert of figures (Fig. 4a-f in insert).

Immunohistochemical seasonal variations of MMP-2 in Libyan jird ventral prostate

In the ventral prostate, during the breeding period, MMP-2 was highly expressed in SMC, but the signal was low in the epithelial cells (Fig. 5a). The secretion texture was granular and devoid of immunostaining (Fig. 5a). A part of the secretion showed a positive MMP-2 immunostaining, which seems to digest the initial unmarked secretion (Fig. 5b-d). The presence of the empty circular areas of varying size in the secretion is a demonstration of this digestion (Fig. 5a,e).

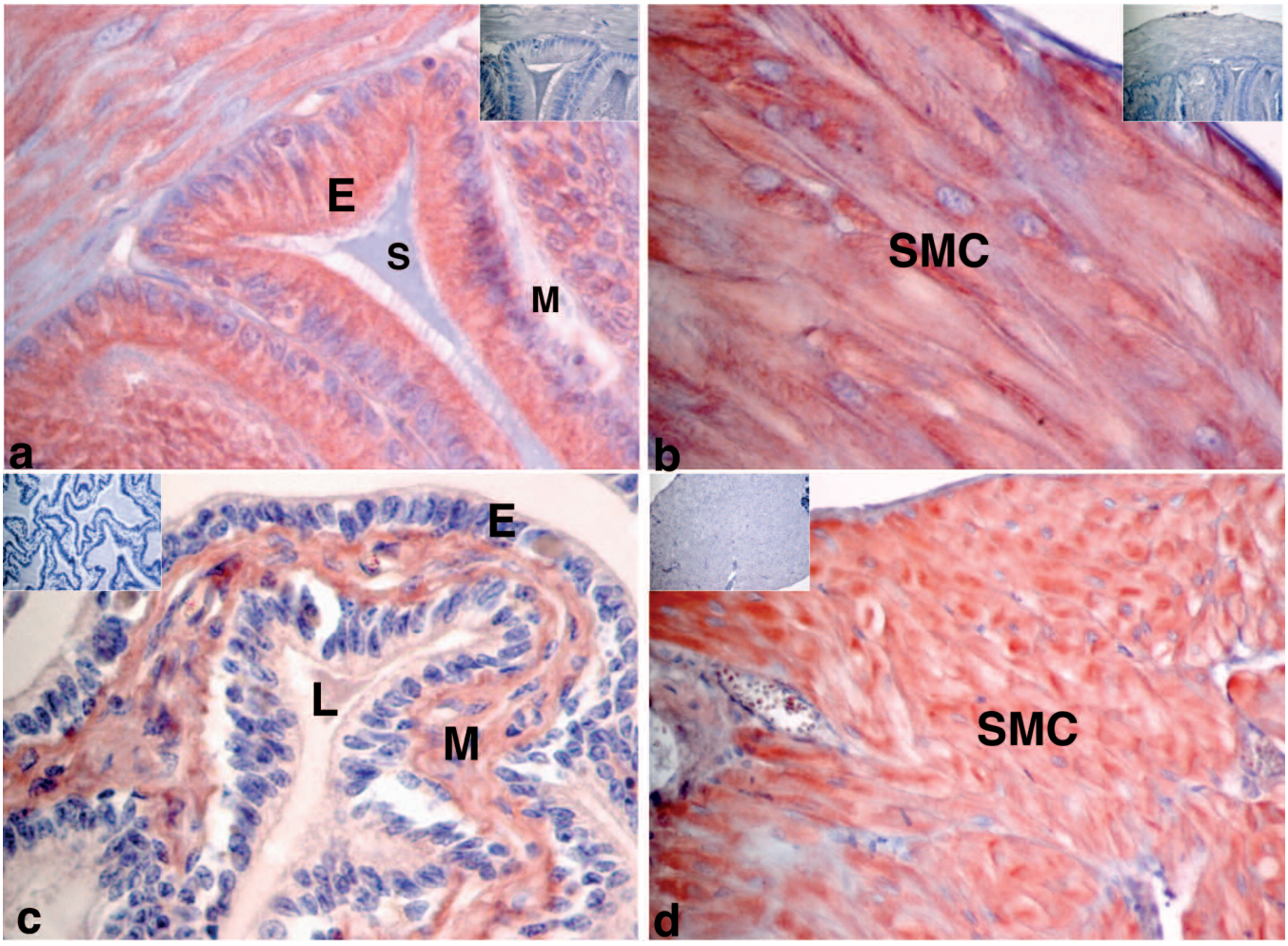

Fig. 3. MMP-9 immunohistochemistry in the adult *Meriones libycus* seminal vesicles. **a, b.** In breeding season (spring and early summer). Epithelial cells (E) and smooth muscle cells (SMC) expressed a similar important immunostaining. In the extracellular matrix (M) and secretion (S) the immunoresponse is negative. **c, d.** In resting phase (late summer, autumn, late winter). Epithelial cells (E) immunostaining decreases and that of smooth muscle cells (SMC) persists with a comparable value to that in active season. An important immunoreactivity appears in the extracellular matrix (M) which was absent in active phase. In the insert are presented the negative controls prepared without primary antibody. L: lumen. x 1250

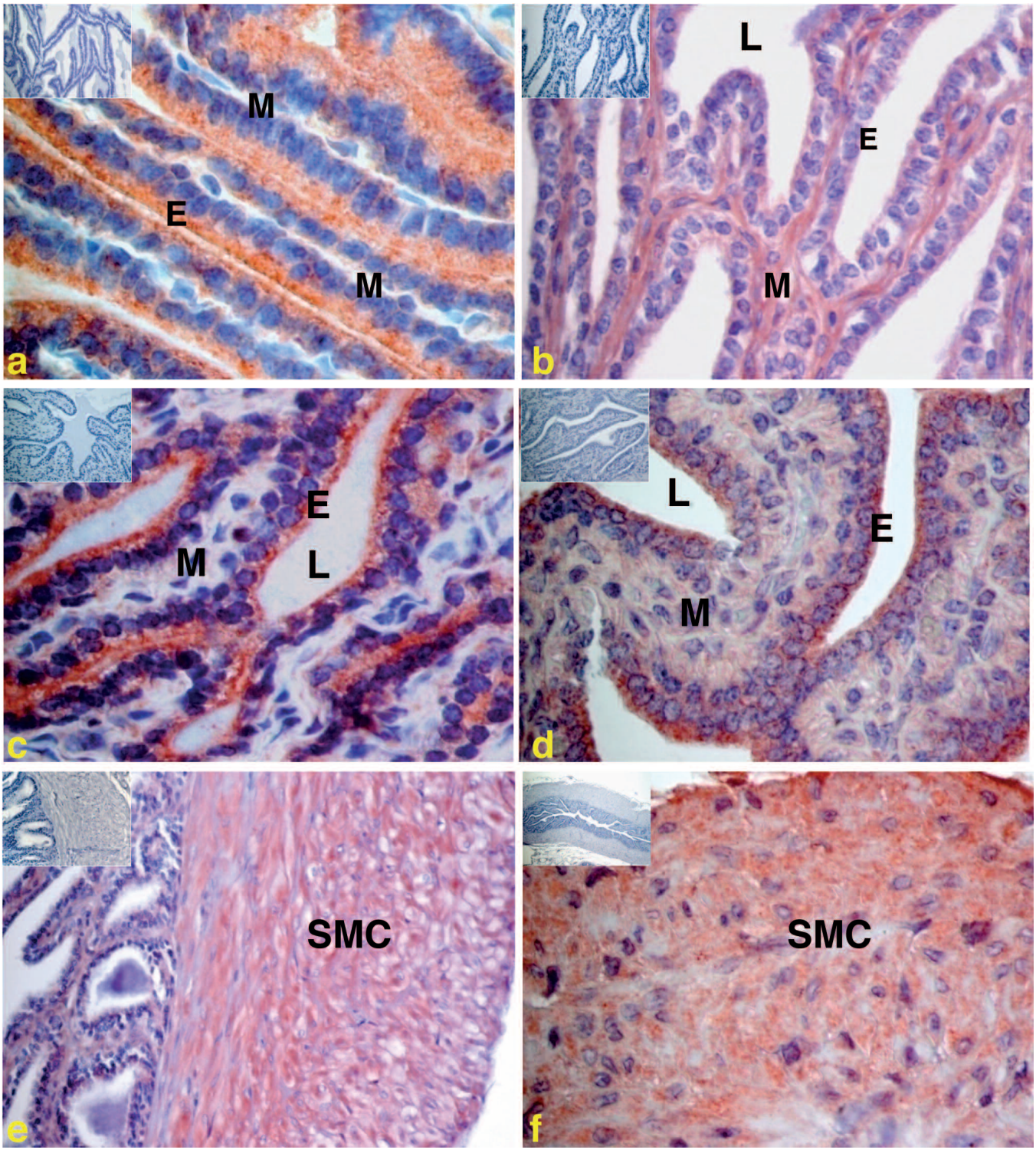

Fig. 4. MMP-9 immunohistochemistry in the adult castrated *Meriones libycus* seminal vesicles. **a.** *Meriones libycus* castrated for three days. Epithelial cells (E) are too strongly immunolabelled as in active phase, the extracellular matrix (M) is devoid of immunoreactivity. **b.** *Meriones libycus* castrated for thirty days. Epithelial cells (E) show an attenuated immunolabelling and the extracellular matrix (M) manifest an obvious immunostaining which was not present in active season. **c.** *Meriones libycus* castrated for fifty days. **d.** *Meriones libycus* castrated for three months. Epithelial cells (E) show a significant immunoreactivity with disappearance of immunostaining in the extracellular matrix (M). **e, f.** Fibromuscular wall of *Meriones libycus* castrated for thirty days **(e)** and for three months **(f)**. The immunohistochemical response persists greatly in smooth muscle cells (SMC). The inserts illustrate negative controls prepared without primary antibody. L: lumen. a-d, f, x 1250; e, x 400

During the resting period and 30 days after castration, the ventral prostate showed a remarkable regression characterized by a manifest epithelial atrophy and hyper-growth of the fibromuscular wall with no secretion into the lumen of tubules. MMP-2 immunoexpression was maintained similarly in the epithelial cells and the SMC (Fig. 5f,g). The negative controls presented in the insert of the figure are not

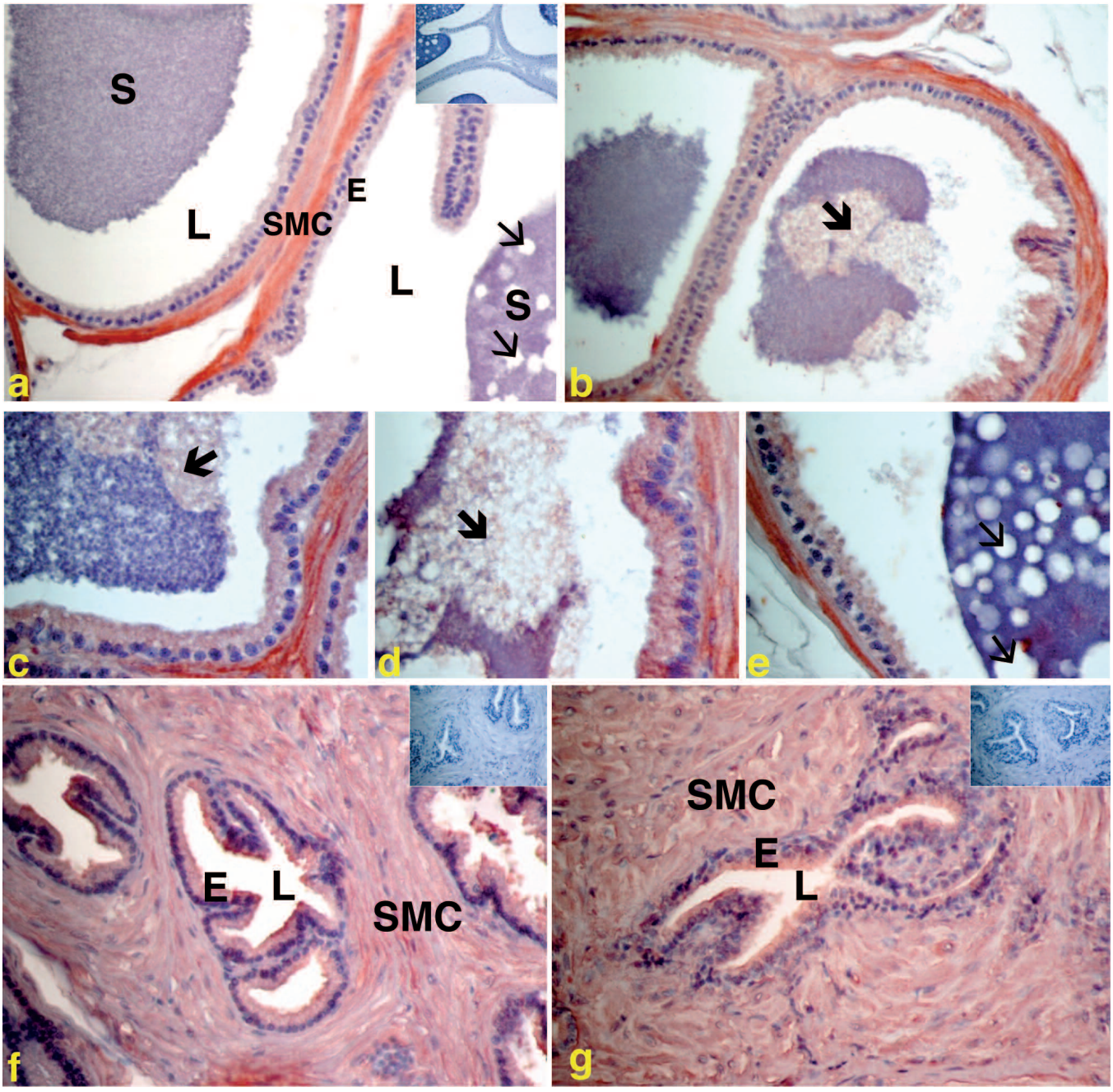

Fig. 5. MMP-2 immunohistochemistry in the adult *Meriones libycus* ventral prostate. **a-e.** In breeding period (spring and early summer). **a.** The immunoreactivity is elevated in smooth muscle cells (SMC) and lowest in the epithelial cells (E), secretion (S) is not immunostained, clear circular areas (arrow) with varying diameter are dug in the secretion. **b-d.** In the lumen (L) a part of the secretion is immunolabelled (arrow), this morphological aspect explains a kind of digestion of secretion to permit its movement and to prevent obstruction of tubules. **e.** The appearance of empty circular structure (arrow) clearly illustrates the leave of the secretion from these zones. **f.** In resting phase (end of summer, autumn, late winter). **g.** *Meriones libycus* castrated for one month in spring. Note the significant epithelial regression and the considerable development of the fibromuscular wall with the persistence of immunohistochemical signal with a similar rate in epithelial cells (E) and smooth muscle cells (SMC). In the lumen (L) secretion is absent. In the insert, the negative controls prepared without primary antibody lacked immunomarquage. a, b, f, g, x 400; c-e, x 1250

immunostained.

Immunohistochemical seasonal variations of MMP-9 in Libyan jird ventral prostate

In the ventral prostate of *Meriones libycus* during the breeding period, the profile of MMP-9 immunostaining was opposite to that of MMP-2. The epithelial cells showed a high immunohistochemical signal contrarily to the SMC, which were weakly or not at all immunomarked (Fig. 6a). The secretion was granular and did not show any positive immunohistochemical

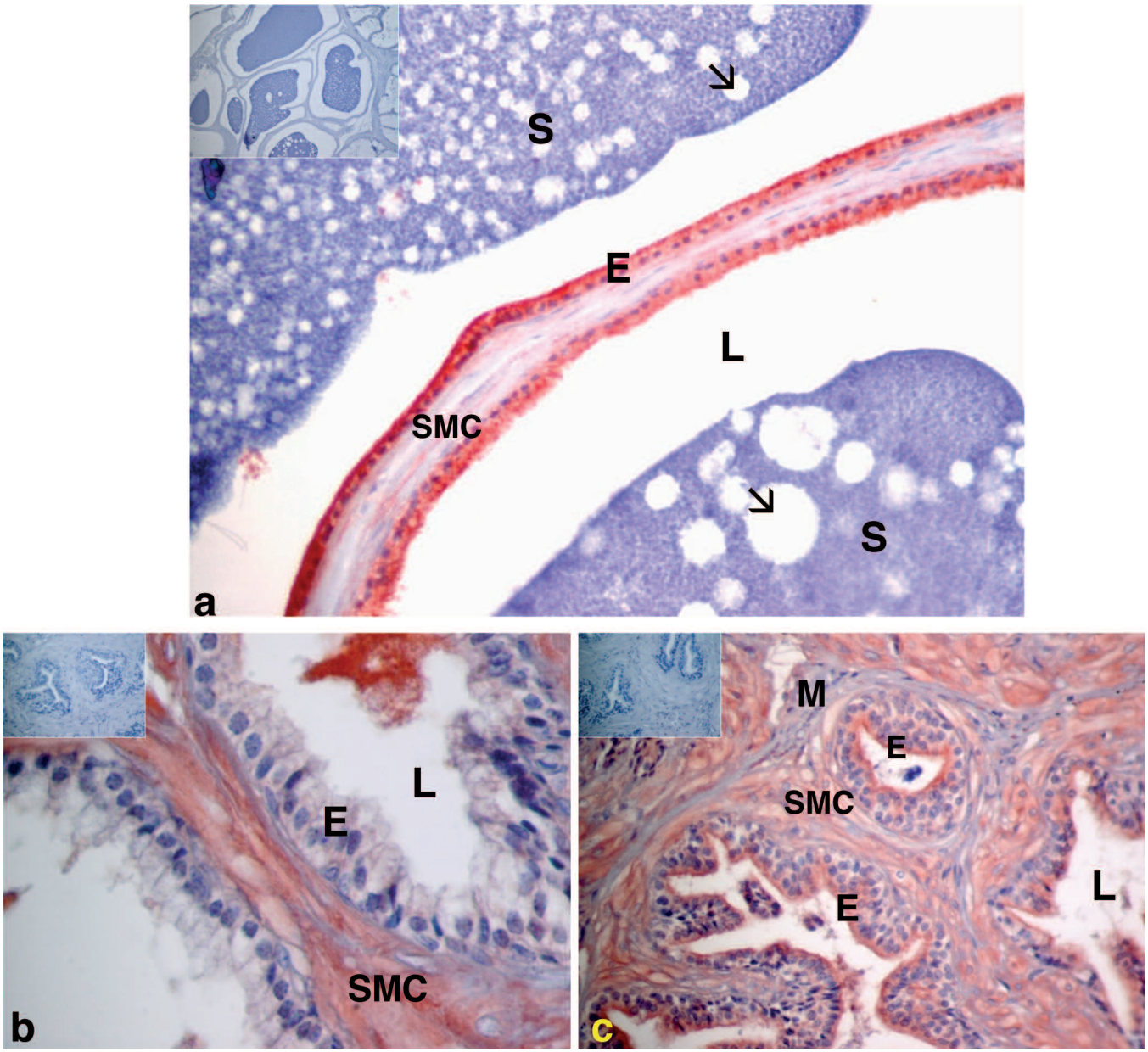

Fig. 6. MMP-9 immunohistochemistry in the adult *Meriones libycus* ventral prostate. **a.** During breeding period (spring and early summer), the immunohistochemical signal is concentrated in the epithelial cells (E); smooth muscle cells (SMC) show a very low immunoresponse and in the secretion immunostaining is absent. As for MMP-2, unfilled circular form of variable size are shaped in the secretion, due to the action of the MMP-9 by detaching the secretion in small fractions. **b.** In quiescent period (end of summer, autumn, late winter) epithelial cells (E) illustrate a very small or absent immunostaining, in contrast smooth muscle cells (SMC) show a significant immunoreaction. **c.** In *Meriones libycus* castrated for thirty days, in the epithelial cells (E) the immunostaining is strongly concentrated in the apical pole and smooth muscle cells (SMC) also show an equal strong immunoreactivity. The extracellular matrix (M) remains without immunostaining. In the insert the negative controls prepared without primary antibody are not immunostained. L: lumen. a, c, x 400; b, x 1250

signal. Like for MMP-2, in this secretion clear areas of circular shape and varying size were observed, due to a detachment of secretion by a small fraction caused probably by MMP-9 action (Fig. 6a).

During the resting phase the epithelial cells exhibited a slight immunoreaction; contrarily, SMC were strongly immunolabeled (Fig. 6b). In thirty days castrated *Meriones libycus*, the immunostaining was concentrated in the SMC and the apical pole of epithelial cells, the extracellular matrix was devoid of immunostaining (Fig. 6c). Negative controls prepared without primary antibody are shown in the insert of the figure without immunoreactivity.

Discussion

The MMPs studied were MMP-2 (gelatinase A) and MMP-9 (gelatinase B). These MMPs were found in epithelial cells, smooth muscle cells (SMC) and the extracellular matrix (ECM) throughout the seasonal cycle of reproduction and in all the castrated *Meriones*. The profile of expression of these gelatinases seems unaffected by seasonal hormonal fluctuations marked by a weak testicular androgen contents and a minimal plasmatic testosterone in the non-breeding period, the value being high in the active season (Boufermes, 1997; Mataoui, 1999). This result suggested that the synthesis and production of these enzymes were constitutive and not controlled by testosterone, or that the site of regulation is not at the level of gene transcription or translation of mRNA. Testosterone appears to control the dynamic of gelatinases between the different compartments of these glands as in the prostate of castrated dog (Shidaifat et al., 2007).

In fact, Androgens play an important role in the development, growth and maintenance of differentiated functions of the prostate and seminal vesicle (Farnsworth, 1999; Hayward and Cunha, 2000). In addition to being regulated by steroid sex hormones, seminal vesicle and prostate are dependent upon reciprocal stromal/epithelial interactions, mediated by several molecules, such as growth factors (Cunha et al., 1996, 2004). Epidermal growth factor (EGF), keratinocyte growth factor (KGF), insulin-like growth factor (IGF), basic fibroblast growth factor (bFGF) and transforming growth factor alpha (TGF-α) can stimulate growth of epithelial cells (Culig et al., 1996). In contrast, transforming growth factor-ß (TGF-ß) has been shown to have inhibitory effects on prostatic stromal cells and epithelial cells (Kim et al., 1996; Pannek et al., 1999); it is the only known negative regulator of prostatic growth (Farnsworth, 1999; Hayward and Cunha, 2000).TGF-ß is negatively regulated in the prostate and seminal vesicle by androgen (Kyprianou and Isaacs, 1988; Desai and Kondaiah, 2000) and is implicated as a mediator in castration-induced cell death in the prostate (Kyprianou and Isaacs, 1988, 1989) and in extracellular matrix production in prostate cells (Butter et al., 2001). A series of cytokines and growth factors including aFGF, bFGF, platelet-derived growth factor, epidermal growth factor, tumor necrosis factor-α, TGF-α and TGF-ß, have been previously found to play important roles in up-regulating expression of MMPs (Okada et al., 1990; Uria et al., 1998; Konrad et al., 2009). In canine, long-term castration (6 month) induce a slight shift in TGF-ß immunolocalization from the stromal cells to the remaining glandular cells of the atrophied prostatic acini, and exerts no significant effects on MMP-13 expression. These changes in the immunolocalization of TGF-ß were associated with a two fold increase in its mRNA expression. MMP-13 is upregulated by androgen in prostate cancer at the protein level (Pang et al., 2004). It seems that in absence of androgens, MMP-13 expression is maintained by TGF-ß, which might act to suppress prostatic cell proliferation and to maintain a low cellular turnover and survival of the atrophied gland after castration (Shidaifat et al., 2007). In human prostate cancer cell lines, TGF-ß1 stimulate transcription of MMP-9 through increased mRNA stability and induces higher secreted levels of MMP-2 through increased stability of the secreted 72-kDa proenzyme (Sehgal et al., 1999). Limaye et al. (2008) suggested that the induction of MMP-2, MMP-9, TIMP-1 and TIMP-2 mRNAs post-castration could be at least in part due to post-transcriptional stabilization. In primary cultures of human prostatic stromal and epithelial cells, stromal cells were found to constitutively express MMP-2 and TGF-ß1 induced MMP-2 and MMP-9 in normal epithelial cells (Wilson et al., 2002; Zhang et al., 2002).

This network of interactions orchestrated by androgens, stromal-epithelial relationship and growth factors can take place in the seminal vesicles and ventral prostate of *Meriones libycus* to regulate the production of gelatinases. TGF-ß is postulated to have an important role in this process (Itoh et al., 1998).

Moreover, our data can indicate that the presence of these enzymes in the fully active phase of seasonal reproductive cycle is evidence of their role in cellular processes related to the reproductive activity of this rodent in addition to its function in seasonal tissue remodeling.

In the seminal vesicles, during the breeding season, MMP-2 and MMP-9 were immunolocalized in epithelial cells and SMC with a similar immunostaining level; this demonstrated that these two cell types could cooperate to produce MMP-2 and MMP-9 for the same purpose or for different use, such as secretory production by epithelial cells and stromal remodeling by SMC. These enzymes were absent in the ECM and secretion. During the resting phase and in thirty-day castrated *Meriones libycus*, MMP-2 and MMP-9 expressions were sustained in SMC with the same intensity as in the active season, and in epithelial cells, but with a reduced immunolabelling. In contrast, the ECM that was not immunostained in the active season expressed a more pronounced MMP-2 and MMP-9 immunostaining, most likely to promote extracellular matrix remodeling.

These observations showed a relocation of MMP-2

and MMP-9, from epithelial cells or SMC where they were probably produced, to the surrounding stroma in which they would also be produced by fibroblasts in order to participate in extracellular matrix remodeling. We can also assume that MMP-2 and MMP-9 synthesis by fibroblasts is repressed by testosterone and is induced in deficiency or absence of this hormone. It seems that the production of gelatinases in these three cell types (epithelial cells, fibroblasts, smooth muscle cells) is regulated by androgens with different mechanisms.

The concentration of the immunostaining in the SMC could also mean a synthesis of MMP-2 and MMP-9 in SMC that yield it to the epithelial cells after diffusion into the ECM. In the active season, the enzyme can be rapidly absorbed, but during the period of sexual quiescence and in the absence of testosterone epithelial cell atrophy would cause a slowdown or an inability to remove the enzyme from extracellular space, leading to its accumulation.

After three days, castration had no impact on MMP-9 immunohistochemical signal; as in the active phase, the epithelial cells strongly expressed MMP-9 and the extracellular space remained without MMP-9 immunostaining. Thirty days after castration, MMP-9 immunoexpression decreased in epithelial cells but became higher in the stroma. After a long-term castration of 50 and 90 days, MMP-9 completely disappeared from stromal tissue and remained well expressed in epithelial cells and SMC, suggesting the continuation of the regression process and tissue remodeling as in dairy animals mammary glands in which involution proceeds more slowly with less pronounced morphological changes (Rabot et al., 2007). Atrophied epithelial cells would have enough time to absorb the enzyme from the extracellular space after a long time castration and to stoke it until the startup of recrudescence in the active phase.

The concentration of gelatinase in the extracellular space during the non-breeding season and after androgen ablation also means that its transport into epithelial cells is a controlled mechanism. It was shown that MMP-2 and MMP-9 were not able to be active unless they were anchored to the cell surface and that cell surface docking was required for the ability of these enzymes to promote a normal or pathologic tissue remodeling (Yu and Stamenkovic, 1999, 2000). Retention of MMPs on the cell surface may therefore provide a general mechanism for cellular regulation of MMP activity. It may help concentrate the proteolytic activity at points of contact between the cell and the ECM (Nagase, 1997). Anchoring MMPs to the cell surface or extracellular matrix would not only prevent them from rapidly diffusing away but would also enable the cell to keep them under close regulatory control. If the MMPs bind to the matrix, this could provide a reservoir for subsequent rapid tissue degradation (Yu and Woessner, 2000).

The patterns of MMP-2 and MMP-9 immunolabelling in seminal vesicles during the seasonal reproductive cycle and after castration were similar: gelatinase A and B would be produced by the SMC. They could diffuse in the extracellular matrix where they were quickly captured by the epithelial cells for use in cellular processes. These enzymes could also be produced by epithelial cells. In the resting phase and in testosterone withdrawal, the reduced number of epithelial cells induced by apoptosis and the slowdown of cell metabolic and secretory activities lead to MMP-2 and MMP-9 concentration in the SMC and in the stroma, and appeared with a low level in epithelial cells following an impairment of their capacity for internalization. The gelatinase conservation in the extracellular space during the resting phase and in hormonal deprivation is a demonstration for their sequestration in the extracellular matrix, allowing a regulated use by the cells, as is the case of MMP-7 (Yu and Woessner, 2000; Ra et al., 2009) and TIMP-3 (Yu et al., 2000). Binding of gelatinases A and B to type-I collagen and other matrix components was observed by Allan et al. (1995). These authors suggest that this binding to collagenous matrix confer retention, stability and bioactivity for prolonged periods thus facilitating its role in pericellular proteolysis. The long persistence of gelatinase in ECM and their disappearance from the extracellular compartment after a prolonged castration suggests that the regression of seminal vesicles is not a quick process like in laboratory rodents such as the rat (Pereira et al., 2006), but a slow and continuous one. The resting period of *Meriones libycus* is elongated; it begins in late summer and continues until late winter; this phase coincides with water and energetic deficiency and an extremely significant regression of seminal vesicles, which would need a high energy consumption and a long time to be achieved. Prolonged and slowed atrophy would allow an equal distribution of energy between the various physiological activities of the organism and therefore an energy economy. The maintenance of these enzymes in the resting phase and in castrated *Meriones libycus* suggests a form of physiological adaptation. Because of the shortening of the breeding season associated with an elevated rate of epithelial compartment proliferation, once the recrudescence started, there would not be de novo synthesis of the enzyme, allowing a quick transition to the active phase of the cycle, and it is a physiological plasticity to cope with the precarious energy resources in the Saharan environment. So, the enzyme system of MMPs is perfectly adapted to work in energetic economy. Such explanations were also postulated for the case of Siberian hamster (*Phodopus sungorus*) ovaries and uterus, of which the MMPs/TIMPs system is expressed during photoperiod-induced regression and recrudescence. It has been demonstrated that during a short photoperiod the atrophy was extended up to 8 weeks and the return to fully activated organs is prompt and was observed after a week of exposure to a long-day

photoperiod (stimulating). This rapid (~7 days) response to stimulating photoperiod suggests that mechanisms are in place to immediately exploit conditions conducive to breeding (Salverson et al., 2008; Shahed and young, 2008).

This cooperation between the glandular epithelial cells and SMC in the production of gelatinases was also demonstrated for TIMP-4 between the stromal cells and epithelial cells of the endometrium; TIMP-4 is produced by stromal cells, discharged into the ECM and then picked up by the epithelial cells in which it accumulates in apical granules before being released into the uterine fluid (Pilka et al., 2006). An example of epithelial-stromal transition was also demonstrated for MMP-7 in the ventral prostate of castrated rats; the MMP-7 produced by epithelial cells was found in the stroma near the basal lamina 3 days after castration, and disappeared completely from epithelial cells to be concentrated in the stroma 21 days after castration, suggesting its involvement in the remodeling of the epithelial-stromal interface caused by castration, probably through degradation of components of the basal lamina (Felisbino et al., 2007). In intact dogs MMP-13 was immunolocalized to the cytoplasm of the glandular cells of the prostate gland, 6 months after castration, it was localized to epithelial and stromal cells; this spatial immunolocalization appeared to suggest a biological role of MMP-13 that is remained to be determined (Shidaifat et al., 2007).

In the ventral prostate, during the breeding period, SMC also show a significant immunostaining for MMP-2 with a slight immunoexpression in epithelial cells, suggesting a high rate of MMP-2 synthesis in the SMC, a similar result was obtained in human primary cultured normal prostatic cells (Zhang et al., 2002). The connective stroma and secretion lacked MMP-2 immunostaining. MMP-9 immunoexpression follows an opposite profile; the epithelial cells show a higher immunostaining than SMC with absence of immunolabelling in the stroma tissue and secretion. During the resting phase and in castrated *Meriones libycus*, the ventral prostate showed strong MMP-2 and MMP-9 expression in epithelial cells and in the SMC with no marking in the ECM.

In the active phase, the ventral prostate lumen contained a mixture of two granular products, and the most abundant did not express MMP-2 or MMP-9. In this mass there were several empty circular areas of varying size reflecting the disappearance of the product in these zones. A portion of the mixture showed positive MMP-2 and MMP-9 immunoreaction. Epithelial cells of the ventral prostate produced a secretion which contained neither MMP-2 nor MMP-9. This granular secretion was released into the lumen, and tended to accumulate. So, it could not flow easily and might close the tubules. Thereafter, the epithelial cells secreted a MMP-2 and MMP-9 rich substance which mixed with the initial secretion and proceeded to digesting it. Consequently, small fragments of secretion were removed and empty circular areas were observed in the secretion. The MMPs facilitated the progression of secretion in the tubules and participated in maintaining the flow of secretion. Identical results were reported by Saarialho-Kere et al. (1995) for MMP-7 after immunolocalisation in the exocrine glands, such as prostate, mammary gland, parotid glands, liver, pancreas and in serous acini of peribronchial glands of the lungs, conducting airways and in the eccrine and apocrine glands of the skin. These authors postulated that MMP-7 served a role in preventing the obstruction of these glands and was called enzyme pipe-cleaner (Saarialho-Kere et al., 1995; Hashimoto et al., 1997; Hulboy et al., 1997; Dunsmore et al., 1998).

The seminal vesicle secretion has a smooth and fluid texture without MMP-2 and MMP-9 immunoreactivity. This texture allowed it to move easily in the lumen and did not need help to circulate. Since these enzymes were not found in the secretion, the seminal vesicle epithelial cells needed these enzymes in cellular processes related to reproductive activity, such as secretory activity and intracellular transport of proteins and their isolation in secretory vesicles.

The simultaneous occurrence of MMP-2 and MMP-9 in epithelial cells and SMC of the seminal vesicles and ventral prostate provided evidence of reciprocal communication between epithelial cells and smooth muscle cells in the production of gelatinase. The hypothetical role of SMC in the maintenance of the differentiated state of epithelial cells advanced by Cunha et al. (1996, 2004) and Cunha (2008) was confirmed.

The role of gelatinase is multiple, without excluding a crucial function in physiological and physio-pathological tissue remodeling. In the seminal vesicles where they are produced without being discharged into the secretion, they are probably involved in physiological processes of the elaboration of the secretion. Their constant presence during the seasonal reproductive cycle despite seasonal hormonal fluctuations demonstrated by Boufermes (1997) and Mataoui (1999) and in castrated *Meriones libycus* completely deprived of testosterone does not offer any evidence of an active state. The latter depended on the local composition of the microenvironment where they were located, and despite their presence they would respond only when needed and would be maintained in latency. In the ventral prostate they participate in progression and maintaining the flow of secretion along the tubule. Finally, these enzymes could simply have a function in tissue and cell homeostasis to limit an exaggerated epithelial cell growth in the active phase, or an excessive stromal proliferation during sexual quiescence, as they may be involved in apoptosis.

During the first weeks of rat ventral prostate postnatal development MMP-2 and MMP-9 are expressed in the epithelium and in the epithelium-stroma interface, suggesting their involvement in epithelial

growth (Bruni-Cardoso et al., 2008). In the adult rat MMP-2, MMP-9, TIMP-1 and TIMP-2 produced in the ventral prostate are implicated in the castration-induced atrophy and remodeling of this gland (Limaye et al., 2008). TIMP-2 immunodetected in epithelial cells of the rat prostate lobes was postulated to regulate MMPs activity in the seminal plasma or in glandular homeostasis (Delella et al., 2007). The testicular and epididymal fluid of domestic animals contain a large amount of MMP-2, MMP-3 and MMP-9, with TIMP-2 reflecting their contribution in the fertilization by cleaving egg physical barriers (McCauley et al., 2001; Métayer et al., 2002).

Many studies postulated the implication of MMPs in fertilization, spermatogenesis, sperm coagulation and liquefaction, and sperm maturation and capacitation. The involvement of MMPs in the process of fertilization mainly in acrosome reaction in mammals has been studied by Diaz-Perez and Meizel (1992) using metalloproteinases inhibitors. The work of Farach et al. (1987) on sea urchin highlighted the role of MMPs in the process of fusion of the acrosome membrane with the sperm membrane during the acrosome reaction. Thereafter, in the same animal, the involvement of MMPs was revealed in sperm and egg membrane fusion, specifically in establishing continuity between the two membranes by an unknown mechanism which remained to be elucidated (Roe et al., 1988, Kato et al., 1998).

In rodent, semen coagulation forming the vaginal plug, these enzymes would participate in the formation of the latter by cleaving certain proteins to facilitate their incorporation into the vaginal plug or to limit, on the contrary, their hydrolysis to allow the release of sperm immobilized in the vaginal plug. An impaired protease system would lead to inadequate semen coagulation and deficient vaginal plug formation upon copulation (Van Dreden et al., 2007; España et al., 2007). Semen coagulation after ejaculation is caused by the semenogelin, a major protein expressed exclusively in the seminal vesicles and is the major coagulum-forming protein. Semen liquefaction is provoked by PSA (Prostatic Specific Antigen); this protease, secreted by the prostate, plays a prominent role in the process of liquefaction by causing semenogelin proteolysis (Su and Wang, 2009). Breakdown of the coagulum releases the progressively motile spermatozoa so that they can be further transported to the ovum (Jonsson et al., 2006; Wang et al., 2008b; Yoshida et al., 2008). Gelatinase would be involved in the process of semenogelin digestion by PSA (Shimokawa et al., 2003).

In gelatinase B deficient mice during a test period of 3 months, the number of mice born per breeding pair is significantly lower than in wild-type mice. In addition, the individual litters in knockout mice are smaller, and the percentage of infertile breeding pairs is elevated in the gelatinase B–deficient mice, whereas maximal litter numbers were observed in wild-type mice (Dubois et al., 2000). MMPs enzyme system was also related to fertility problems in men and women (Baumgart et al., 2002; Tentes et al., 2007; Skrzypczak et al., 2007).

Conclusion

In *Meriones libycus* during the seasonal reproductive cycle the seminal vesicles and ventral prostate produce gelatinase A (MMP-2) and gelatinase B (MMP-9) for use in their cell activities. These MMPs are also useful for the flow of secretion in the ventral prostate. The production of gelatinase may be constitutive or controlled by a mechanism that is remained to be elucidated. The mechanism of production and regulation of these gelatinases is adapted to the insecure and irregular food availability of the Sahara. This enzyme system of MMPs in place constantly in the sexual glands would permit a prompt start of the recrudescence physiological process, maintenance of physiological cell activities during the active season and the establishment of organ regression during sexual quiescence. This enzyme system is endowed with a great physiological plasticity enabling it to take the best advantage from the energy resources of the Saharan environment, despite climate seasonal fluctuations that condition the energy intake required for these physiological processes of the reproductive function in arid areas. These MMPs may be involved in the formation of the vaginal plug, sperm coagulation and liquefaction, sperm maturation and fertilization.

Acknowledgements. The authors thank all the personnel of Beni-Abbes station in the Algerian Sahara. The members of the Research Laboratory in Arid Regions (LRZA) and those of the Laboratory of General Biology of the Catholic University of Lyon are also thanked. I address particularly my sincere thanks to members of INSERM U 689 of hospital Bichat-Claude Bernard for their warm welcome. This study performed in the laboratory of general biology in the Catholic University of Lyon was supported by the Algerian-French scholarship program (BAF).

References

Aguilera-Merlo C., Muñoz E., Dominguez S., Scardapane L. and Piezzi R. (2005). Epididymis of viscacha (*Lagostomus maximus maximus*): morphological changes during the annual reproductive cycle. Anat. Rec. A. Discov. Mol. Cell. Evol. Biol. 282, 83-92.

Aguilera-Merlo C., Fogal T., Sator T., Dominguez S., Sosa M., Scardapane L. and Piezzi R. (2009). Ultrastructural and biochemical seasonal changes in epididymal corpus and cauda of viscacha (*Lagostomus maximus maximus*). J. Morphol. 270, 805-814.

Allan J.A., Docherty A.J., Barker P.J., Huskisson N.S., Reynolds J.J. and Murphy G. (1995). Binding of gelatinases A and B to type-I collagen and other matrix components. Biochem J. 309, 299-306.

Baumgart E., Lenk S.V., Loening S.A. and Jung K. (2002). Quantitative differences in matrix metalloproteinase MMP-2, but not in MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1 or TIMP-2, in seminal plasma of normozoospermic and azoospermic patients. Hum. Reprod. 17, 2919-2923.

Belhocine M. (1998). Etude histo-cytologique des variations

saisonnières de l'appareil reproducteur mâle d'un rongeur déserticole nocturne, le Mérion du désert (*Meriones crassus*) et son espèce sympatrique, le Mérion de Libye (*Meriones libycus*). Thèse de Magister. 175p, ISN, USTHB, Alger.

Belhocine M. (2008). Modulation de la matrice extracellulaire au cours de la reproduction du Mérion: approche au niveau des vésicules séminales. Thèse de Doctorat. 156p, FSB, USTHB, Alger.

Belhocine M. and Gernigon T. (1994). Histologie comparée des variations saisonnières de l'appareil reproducteur mâle de deux rongeurs sahariens: *Psammomys obesus* et *Meriones crassus*. Bull. Soc. Zool. Fr. 119, 325-333.

Belhocine M. and Gernigon-Spychalowicz T. (1996). Effets de la castration sur l'appareil génital mâle et la surrénale des Mérions sahariens. Bull. Soc. Zool. Fr. 12, 107-110.

Belhocine M., Gernigon-Spychalowicz T., Robert A.M., Schoevaert D., Bennazzoug Y. and Exbrayat J.M. (2007). Ecophysiological responses of the seminal vesicle of Libyan jird (*Meriones libycus*) to the Saharan conditions: histological, morphometric and immunohistochemical analysis. Histol. Histopathol. 22, 603-615.

Berisha B., Steffl M., Welter H., Kliem H., Meyer H.H., Schams D. and Amselgruber W. (2008). Effect of the luteinising hormone surge on regulation of vascular endothelial growth factor and extracellular matrix-degrading proteinases and their inhibitors in bovine follicles. Reprod. Fertil. Dev. 20, 258-268.

Bernal F., Hartung H.P. and Kieseier B.C. (2005). Tissue mRNA expression in rat of newly described matrix metalloproteinases. Biol. Res. 38, 267-271.

Berton A., Selvais C., Lemoine P., Henriet P., Courtoy P.J., Marbaix E. and Emonard H. (2007). Binding of matrilysin-1 to human epithelial cells promotes its activity. Cell. Mol. Life. Sci. 64, 610-620.

Boufermes R. (1997). Etude comparative des variations saisonnières testiculaires et thyroïdiennes chez trois espèces de Rongeurs déserticoles, le Mérion (*Meriones crassus*), la Gerbille (*Gerbillus gerbillus*) et le Rat des sables (*Psammomys obesus*). Thèse de Magister. 150p, ISN, USTHB, Alger.

Bozdagi O., Nagy V., Kwei K.T. and Huntley G.W. (2007). In vivo roles for matrix metalloproteinase-9 in mature hippocampal synaptic physiology and plasticity. J. Neurophysiol. 98, 334-344.

Brew K., Dinakarpandian D. and Nagase H. (2000). Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim. Biophys. Acta 1477, 267-283.

Bruni-Cardoso A. Vilamaior P.S., Taboga S.R. and Carvalho H.F. (2008). Localized matrix metalloproteinase MMP-2 and MMP-9 activity in the rat ventral prostate during the first week of postnatal development. Histochem. Cell. Biol. 129, 805-815.

Butter S., Laping N.J., Pullen M., Grygielko E., Olson B. and Brooks D.P. (2001). The role of transforming growth factor-ß and its receptors in human prostate smooth muscle cell fibronectin production. Eur. J. Pharmacol. 422, 47-52.

Cai K.Q., Yang W.L., Capo-Chichi C.D., Vanderveer L., Wu H., Godwin A.K. and Xu X.X. (2007). Prominent expression of metalloproteinases in early stages of ovarian tumorigenesis. Mol. Carcinog. 46, 130-143.

Carballada R., Jara M. and Esponda P. (2007). Photoperiod-induced apoptosis in the male genital tract epithelia of the golden hamster. Int. J. Androl. 30, 73-79.

Cheng C.Y. and Mruk D.D. (2009). An intracellular trafficking pathway in the seminiferous epithelium regulating spermatogenesis: a biochemical and molecular perspective. Crit. Rev. Biochem. Mol. Biol. (in press).

Clark I.M., Swingler T.E. and Young D.A. (2007). Acetylation in the regulation of metalloproteinase and tissue inhibitor of metalloproteinases gene expression. Front. Biosci. 12, 528-535.

Clark I.M., Swingler T.E., Sampieri C.L. and Edwards D.R. (2008). The regulation of matrix metalloproteinases and their inhibitors. Int. J. Biochem. Cell. Biol. 40, 1362-1378.

Coraux C., Roux J., Jolly T. and Birembaut P. (2008). Epithelial cell-extracellular matrix interactions and stem cells in airway epithelial regeneration. Proc. Am. Thorac. Soc. 5, 689-694.

Culig Z., Hobisch A., Cronauer M.V., Radmayr C., Hittmair A., Zhang J., Thurnher M., Bartsch G. and Klocker H. (1996). Regulation of prostatic growth and function by peptide growth factors. Prostate 28, 392-405.

Cunha A.G., Cooke P.S. and Kurita T. (2004). Role of stromal-epithelial interactions in hormonal responses. Arch. Histol. Cytol. 67, 417-434.

Cunha G.R. (2008). Mesenchymal-epithelial interactions: past, present, and future. Differentiation 76, 578-586.

Cunha G.R., Hayward S.W., Dahiya R. and Foster B.A. (1996). Smooth muscle-epithelial interactions in normal and neoplastic prostatic development. Acta Anat. (Basel) 155, 63-72.

Curry T.E Jr. and Osteen K.G. (2003). The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. Endocr. Rev. 24, 428-465.

Daimon E. and Wada Y. (2005). Role of neutrophils in matrix metalloproteinase activity in the preimplantation mouse uterus. Biol. Reprod. 73, 163-171.

Delella F.K., Justulin L.A. Jr and Felisbino S.L. (2007). Tissue inhibitor of metalloproteinase-2 (TIMP-2) location in the ventral, lateral, dorsal and anterior lobes of rat prostate by immunohistochemistry. Cell Biol. Int. 31, 229-234.

Desai K.V. and Kondaiah P. (2000). Androgen ablation results in differential regulation of transforming growth factor-beta isoforms in rat male accessory sex organs and epididymis. J. Mol. Endocrinol. 24, 253-260.

Díaz-Pérez E. and Meizel S. (1992). Importance of mammalian sperm metalloendoprotease activity during the acrosome reaction to subsequent sperm-egg fusion: inhibitor studies with human sperm and zona-free hamster eggs. Mol. Reprod. Dev. 31, 122-130.

Dubois B., Arnold B. and Opdenakker G. (2000). Gelatinase B deficiency impairs reproduction. J. Clin. Invest. 106, 627-628.

Dunsmore S.E., Saarialho-Kere U.K., Roby J.D., Wilson C.L., Matrisian L.M., Welgus H.G. and Parks W.C. (1998). Matrilysin expression and function in airway epithelium. J. Clin. Invest. 102, 1321-1331.

Ebisch I.M., Steegers-Theunissen R.P., Sweep F.C., Zielhuis G.A., Geurts-Moespot A. and Thomas C.M. (2007). Possible role of the plasminogen activation system in human subfertility. Fertil. Steril. 87, 619-626.

El-Bakry H.A., Zahran W.M. and Bartness T.J. (1998). Photoperiodic responses of four wild-trapped desert rodent species. Am. J. Physiol. 275, R2012-R2022.

España F., Navarro S., Medina P., Zorio E. and Estellés A. (2007). The role of protein C inhibitor in human reproduction. Semin. Thromb. Hemost. 33, 41-45.

Fanjul-Fernández M., Folgueras A.R., Cabrera S. and López-Otín C. (2009). Matrix metalloproteinases: Evolution, gene regulation and functional analysis in mouse models. Biochim. Biophys. Acta 2009 (in press).

Farach H.A. Jr, Mundy D.I., Strittmatter W.J. and Lennarz W.J. (1987).

Evidence for the involvement of metalloendoproteases in the acrosome reaction in sea urchin sperm. J. Biol. Chem. 262, 5483-5487.

Farnsworth W.E. (1999). Prostate stroma: physiology. Prostate 38, 60-72.

Felisbino S.L., Justulin Junior L.A., Carvalho H.F. and Taboga S.R. (2007). Epithelial-stromal transition of MMP-7 immunolocalization in the rat ventral prostate following bilateral orchiectomy. Cell Biol. Int. 31, 1173-1178.

Gernigon T., Berger M. and Lécher P. (1994). Seasonal variations in the ultrastructure and production of androgen-dependent proteins in the seminal vesicles of a Saharan rodent (*Psammomys obesus*). J. Endocrinol. 142, 37-46.

Góes R.M., Zanetoni C., Tomiosso T.C., Ribeiro D.L. and Taboga S.R. (2007). Surgical and chemical castration induce differential histological response in prostate lobes of Mongolian gerbil. Micron 38, 231-6.

Green K.A. and Lund L.R. (2005). ECM degrading proteases and tissue remodeling in the mammary gland. Bioessays 27, 894-903.

Greenlee K.J., Werb Z. and Kheradmand F. (2007). Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. Physiol. Rev. 87, 69-98.

Hashimoto K., Yano A., Tanabe T., Usui T., Kihira Y. and Matsuo Y. (1997). Localization and expression of matrix metalloproteinase-7 in human prostate. Nippon Hinyokika Gakkai Zasshi. 88, 852-857.

Hayward S.W. and Cunha G.R. (2000). The prostate: development and physiology. Radiologic Clinics North America. 38, 1-14.

Hsu S.M., Raine L. and Fanger H. (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 29, 577-580.

Hu J., Zhang X., Nothnick W.B. and Spencer T.E. (2004). Matrix metalloproteinases and their tissue inhibitors in the developing neonatal mouse uterus. Biol. Reprod. 71, 1598-1604.

Huang D.H., Zhao H., Tian Y.H., Li H.G., Ding X.F. and Xiong C.L. (2007). Gene expression changes of urokinase plasminogen activator and urokinase receptor in rat testes at postnatal stages. Asian J. Androl. 9, 679-683.

Hulboy D.L., Rudolph L.A. and Matrisian L.M. (1997). Matrix metalloproteinases as mediators of reproductive function. Mol. Hum. Reprod. 3, 27-45.

Ii M., Yamamoto H., Adachi Y., Maruyama Y. and Shinomura Y. (2006). Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. Exp. Biol. Med. (Maywood). 231, 20-27.

Illman S.A., Keski-Oja J., Parks W.C. and Lohi J. (2003). The mouse matrix metalloproteinase, epilysin (MMP-28), is alternatively spliced and processed by a furin-like proprotein convertase. Biochem. J. 375, 191-197.

Illman S.A., Lehti K., Keski-Oja J. and Lohi J. (2006). Epilysin (MMP-28) induces TGF-beta mediated epithelial to mesenchymal transition in lung carcinoma cells. J. Cell. Sci. 119, 3856-3865.

Illman S.A., Lohi J. and Keski-Oja J. (2008). Epilysin (MMP-28)-structure, expression and potential functions. Exp. Dermatol. 17, 897-907.

Ito T.K., Ishii G., Saito S., Yano K., Hoshino A., Suzuki T. and Ochiai A. (2009). Degradation of soluble VEGF receptor-1 by MMP-7 allows VEGF access to endothelial cells. Blood 113, 2363-2369.

Itoh N., Patel U., Cupp A.S. and Skinner M.K. (1998). Developmental and hormonal regulation of transforming growth factor-ß1 (TGF-ß1), -2, and -3 gene expression in isolated prostatic epithelial and stromal cells: epidermal growth factor and TGF-ß interactions. Endocrinology 139, 1378-1388.

Itoh Y., Ito N., Nagase H. and Seiki M. (2008). The second dimer interface of MT1-MMP, the transmembrane domain, is essential for ProMMP-2 activation on the cell surface. J. Biol. Chem. 283, 13053-13062.

Jonsson M., Lundwall A., Linse S., Frohm B. and Malm J. (2006). Truncated semenogelin I binds zinc and is cleaved by prostate-specific antigen. J. Androl. 27, 542-547.

Justulin L.A Jr., Ureshino R.P., Zanoni M. and Felisbino S.L. (2006). Differential proliferative response of the ventral prostate and seminal vesicle to testosterone replacement. Cell. Biol. Int. 30, 354-364.

Kato K.H., Takemoto K., Kato E., Miyazaki K., Kobayashi H. and Ikegami S. (1998). Inhibition of sea urchin fertilization by jaspisin, a specific inhibitor of matrix metalloendoproteinase. Dev. Growth Differ. 40, 221-230.

Kim I.Y., Ahn H.J., Zelner D.J., Park L., Sensibar J.A. and Lee C. (1996). Expression and localization of transforming growth factor-beta receptors type I and type II in the rat ventral prostate during regression. Mol. Endocrinol. 10, 107-115.

Kizaki K., Ushizawa K., Takahashi T., Yamada O., Todoroki J., Sato T., Ito A. and Hashizume K. (2008). Gelatinase (MMP-2 and -9) expression profiles during gestation in the bovine endometrium. Reprod. Biol. Endocrinol. 6, 66.

Kliem H., Welter H., Kraetzl W.D., Steffl M., Meyer H.H., Schams D. and Berisha B. (2007). Expression and localisation of extracellular matrix degrading proteases and their inhibitors during the oestrous cycle and after induced luteolysis in the bovine corpus luteum. Reproduction 134, 535-547.

Konrad L., Scheiber J.A., Schwarz L., Schrader A.J. and Hofmann R. (2009). TGF-beta1 and TGF-beta2 strongly enhance the secretion of plasminogen activator inhibitor-1 and matrix metalloproteinase-9 of the human prostate cancer cell line PC-3. Regul. Pept. 155, 28-32.

Krane S.M. and Inada M. (2008). Matrix metalloproteinases and bone. Bone 43, 7-18.

Kuzuya M. and Iguchi A. (2003). Role of matrix metalloproteinases in vascular remodeling. J. Atheroscler. Thromb. 10, 275-282.

Kyprianou N. and Isaacs J.T. (1988). Identification of a cellular receptor for transforming growth factor-beta in rat ventral prostate and its negative regulation by androgens. Endocrinology 123, 2124-2131.

Kyprianou N. and Isaacs J.T. (1989). Expression of transforming growth factor-beta in the rat ventral prostate during castration-induced programmed cell death. Mol. Endocrinol. 3, 1515-1522.

Larsen M., Artym V.V., Green J.A. and Yamada K.M. (2006). The matrix reorganized: extracellular matrix remodelling and integrin signaling. Curr. Opin. Cell Biol. 18, 463-471.

Le Magueresse-Battistoni B. (2007). Serine proteases and serine protease inhibitors in testicular physiology: the plasminogen activation system. Reproduction 134, 721-729.

Li M.W., Mruk D.D., Lee W.M. and Cheng C.Y. (2009). Cytokines and junction restructuring events during spermatogenesis in the testis: an emerging concept of regulation. Cytokine Growth Factor Rev. 20, 329-338.

Lijnen H.R. (2001). Plasmin and matrix metalloproteinases in vascular remodeling. Thromb. Haemost. 86, 324-333.

Limaye A.M., Desai K.V., Chavalmane A.K. and Kondaiah P. (2008). Regulation of mRNAs encoding MMP-9 and MMP-2, and their

inhibitors TIMP-1 and TIMP-2 by androgens in the rat ventral prostate. Mol. Cell. Endocrinol. 294, 10-18.

Liu Y.X. (2007). Involvement of plasminogen activator and plasminogen activator inhibitor type 1 in spermatogenesis, sperm capacitation, and fertilization. Semin. Thromb. Hemost. 33, 29-40.

Longin J., Guillaumot P., Chauvin M.A., Morera A.M. and Le Magueresse-Battistoni B. (2001). MT1-MMP in rat testicular development and the control of Sertoli cell proMMP-2 activation. J. Cell. Sci. 114, 2125-2134.

Manase K., Endo T., Chida M., Nagasawa K., Honnma H., Yamazaki K., Kitajima Y., Goto T., Kanaya M., Hayashi T., Mitaka T. and Saito T. (2006). Coordinated elevation of membrane type 1-matrix metalloproteinase and matrix metalloproteinase-2 expression in rat uterus during postpartum involution. Reprod. Biol. Endocrinol. 4, 32.

Mataoui H. (1999). Effet de la castration sur le tractus génital mâle et sur l'activité thyroïdienne chez trois rongeurs sahariens: Le Mérion (*Meriones libycus*), la Gerbille (*Gerbillus gerbillus*) et le rat des sables (*Psammomys obesus*). Thèse de Magister. 186p, ENS, Kouba, Alger.

McCauley T.C., Zhang H.M., Bellin M.E. and Ax R.L. (2001). Identification of a heparin-binding protein in bovine seminal fluid as tissue inhibitor of metalloproteinases-2. Mol. Reprod. Dev. 58, 336-341.

Métayer S., Dacheux F., Dacheux J.L. and Gatti J.L. (2002). Comparison, characterization, and identification of proteases and protease inhibitors in epididymal fluids of domestic mammals. Matrix metalloproteinases are major fluid gelatinases. Biol. Reprod. 66, 1219-1229.

Mohan R., Chintala S.K., Jung J.C., Villar W.V., McCabe F., Russo L.A., Lee Y., McCarthy B.E., Wollenberg K.R., Jester J.V., Wang M., Welgus H.G., Shipley J.M., Senior R.M. and Fini M.E. (2002). Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. J. Biol. Chem. 277, 2065-2072.

Murphy G. and Nagase H. (2008). Progress in matrix metalloproteinase research. Mol. Aspects Med. 29, 290-308.

Nagase H. (1997). Activation mechanisms of matrix metalloproteinases. Biol. Chem. 378, 151-160.

Nagase H., Visse R. and Murphy G. (2006). Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc. Res. 69, 562-573.

Nakamura M., Miyamoto S., Maeda H., Ishii G., Hasebe T., Chiba T., Asaka M. and Ochiai A. (2005). Matrix metalloproteinase-7 degrades all insulin-like growth factor binding proteins and facilitates insulin-like growth factor bioavailability. Biochem. Biophys. Res. Commun. 333, 1011-1016.

Ohnishi J., Ohnishi E., Shibuya H. and Takahashi T. (2005). Functions for proteinases in the ovulatory process. Biochim. Biophys. Acta 1751, 95-109.

Okada Y., Tsuchiya H., Shimizu H., Tomita K., Nakanishi I., Sato H., Seiki M., Yamashita K. and Hayakawa T. (1990). Induction and stimulation of 92-kDa gelatinase/type IV collagenase production in osteosarcoma and fibrosarcoma cell lines by tumor necrosis factor alpha. Biochem. Biophys. Res. Commun. 171, 610-617.

Oliveira S.M., Leite Vilamaior P.S., Corradi L.S., Góes R.M. and Taboga S.R. (2007). Cellular and extracellular behavior in the gerbil (*Meriones unguiculatus*) ventral prostate following different types of castration and the consequences of testosterone replacement. Cell. Biol. Int. 31, 235-245.

Page-McCaw A., Ewald A.J. and Werb Z. (2007). Matrix metalloproteinases and the regulation of tissue remodeling. Nat. Rev. Mol. Cell. Biol. 8, 221-233.

Pang S.T., Flores-Morales A., Skoog L., Chuan Y.C., Nordstedt G. and Pousette A. (2004). Regulation of matrix metalloproteinase 13 expression by androgen in prostate cancer. Oncol. Rep. 11, 1187-1192.

Pannek J., Berges R.R., Sauvageot J., Lecksell K.L., Epstein J.I. and Partin A.W. (1999). Cell turnover in human seminal vesicles and the prostate: an immunohistochemical study. Prostate Cancer Prostatic Dis. 2, 200-203.

Pereira S., Martinez M., Martinez F.E. and Júnior W.M. (2006). Repercussions of castration and vasectomy on the ductal system of the rat ventral prostate. Cell. Biol. Int. 30, 169-174.

Pilka R., Noskova V., Domanski H., Andersson C., Hansson S. and Casslén B. (2006). Endometrial TIMP-4 mRNA is expressed in the stroma, while TIMP-4 protein accumulates in the epithelium and is released to the uterine fluid. Mol. Hum. Reprod. 12, 497-503.

Ra H.J., Harju-Baker S., Zhang F., Linhardt R.J., Wilson C.L. and Parks W.C. (2009). Control of promatrilysin (MMP7) activation and substrate-specific activity by sulfated glycosaminoglycans. J Biol. Chem. (in press).

Ra H.J and Parks W.C. (2007). Control of matrix metalloproteinase catalytic activity. Matrix Biol. 26, 587-596.

Rabot A., Sinowatz F., Berisha B., Meyer H.H.D. and Schams D. (2007). Expression and localization of extracellular matrix-degrading proteinases and their inhibitors in the bovine mammary gland during development, function, and involution. J. Dairy Sci. 90, 740–748.

Ribeiro L.A., Turba M.E., Zannoni A., Bacci M.L. and Forni M. (2006). Gelatinases, endonuclease and Vascular Endothelial Growth Factor during development and regression of swine luteal tissue. BMC Dev. Biol. 6, 58.

Rivera S., Jourquin J., Ogier C., Bernard A., Charton G., Tremblay E. and Khrestchatisky M. (2004). Le système MMP/TIMP dans le système nerveux. Medecine/Sciences 20, 55-60.

Rodgers U.R., Kevorkian L., Surridge A.K., Waters J.G., Swingler T.E., Culley K., Illman S., Lohi J., Parker A.E. and Clark I.M. (2009). Expression and function of matrix metalloproteinase (MMP)-28. Matrix Biol. 28, 263-272.

Roe J.L., Farach H.A. Jr, Strittmatter W.J. and Lennarz W.J. (1988). Evidence for involvement of metalloendoproteases in a step in sea urchin gamete fusion. J. Cell Biol. 107, 539-544.

Rudolph-Owen L.A., Cannon P. and Matrisian L.M. (1998). Overexpression of the matrix metalloproteinase matrilysin results in premature mammary gland differentiation and male infertility. Mol. Biol. Cell. 9, 421-435.

Saarialho-Kere U.K., Crouch E.C. and Parks W.C. (1995). Matrix metalloproteinase matrilysin is constitutively expressed in adult human exocrine epithelium. J. Invest. Dermatol. 105, 190-196.

Salverson T.J., McMichael G.E., Sury J.J., Shahed A. and Young K.A. (2008). Differential expression of matrix metalloproteinases during stimulated ovarian recrudescence in Siberian hamsters (*Phodopus sungorus*). Gen. Comp. Endocrinol. 155, 749-761.

Schradin C. (2008). Seasonal changes in testosterone and corticosterone levels in four social classes of a desert dwelling sociable rodent. Horm. Behav. 53, 573-579.

Sehgal I. and Thompson T.C. (1999). Novel regulation of type IV collagenase (matrix metalloproteinase-9 and -2) activities by transforming growth factor-beta1 in human prostate cancer cell lines. Mol. Biol. Cell 10, 407-416.

Shahed A. and Young K.A. (2008). Differential activity of matrix metalloproteinases (MMPs) during photoperiod induced uterine regression and recrudescence in Siberian hamsters (*Phodopus sungorus*). Mol. Reprod. Dev. 75, 1433-1440.

Shidaifat F., Gharaibeh M. and Bani-Ismail Z. (2007). Effect of castration on extracellular matrix remodeling and angiogenesis of the prostate gland. Endocr. J. 54, 521-529.

Shimokawa K., Katayama M., Matsuda Y., Takahashi H., Hara I., Sato H. and Kaneko S. (2002). Matrix metalloproteinase (MMP)-2 and MMP-9 activities in human seminal plasma. Mol. Hum. Reprod. 8, 32-36.

Shimokawa K., Katayama M., Matsuda Y., Takahashi H., Hara I. and Sato H. (2003). Complexes of gelatinases and tissue inhibitor of metalloproteinases in human seminal plasma. J. Androl. 24, 73-77.

Skrzypczak J., Wirstlein P., Mikoƒajczyk M., Ludwikowski G. and Zak T. (2007). TGF superfamily and MMP2, MMP9, TIMP1 genes expression in the endometrium of women with impaired reproduction. Folia. Histochem. Cytobiol. 45 (Suppl 1), 143-148.

Stamenkovic I. (2003). Extracellular matrix remodelling: the role of matrix metalloproteinases. J. Pathol. 200, 448-464.

Stetler-Stevenson W.G. (2008). Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. Sci. Signal. 1, re6.

Su S.F. and Wang Z.J. (2009). Advances in the study of Semenogelin I from human seminal vesicles. Zhonghua Nan Ke Xue. 15, 364-366.

Tentes I., Asimakopoulos B., Mourvati E., Diedrich K., Al-Hasani S. and Nikolettos N. (2007). Matrix metalloproteinase (MMP)-2 and MMP-9 in seminal plasma. J. Assist. Reprod. Genet. 24, 278-281.

Uría J.A., Jiménez M.G., Balbín M., Freije J.M. and López-Otín C. (1998). Differential effects of transforming growth factor-beta on the expression of collagenase-1 and collagenase-3 in human fibroblasts. J. Biol. Chem. 273, 9769-9777.

Van Dreden P., Carlo A. and Rousseau A. (2007). Human seminal plasma fibrinolytic activity. Semin. Thromb. Hemost. 33, 21-28.

Varghese S. (2006). Matrix metalloproteinases and their inhibitors in bone: an overview of regulation and functions. Front. Biosci. 11, 2949-2966.

Visse R. and Nagase H. (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ. Res. 92, 827-839.

Vu T.H. and Werb Z. (2000). Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev. 14, 2123-2133.

Walsh L.A., Carere D.A., Cooper C.A. and Damjanovski S. (2007). Membrane type-1 matrix metalloproteinases and tissue inhibitor of metalloproteinases-2 RNA levels mimic each other during *Xenopus laevis* metamorphosis. PLoS ONE 2, e1000.

Wang X.B., Bozdagi O., Nikitczuk J.S., Zhai Z.W., Zhou Q. and Huntley G.W. (2008a). Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. Proc. Natl. Acad. Sci. USA 105, 19520-19525.

Wang Z.J., Zhang W., Feng N.H., Song N.H., Wu H.F. and Sui Y.G. (2008b). Molecular mechanism of epididymal protease inhibitor modulating the liquefaction of human semen. Asian J. Androl. 10, 770-775.

Wilson C.L., Heppner K.J., Rudolph L.A. and Matrisian L.M. (1995). The metalloproteinase matrilysin is preferentially expressed by epithelial cells in a tissue-restricted pattern in the mouse. Mol. Biol. Cell 6, 851-869.

Wilson M.J., Sellers R.G., Wiehr C., Melamud O., Pei D. and Peehl D.M. (2002). Expression of matrix metalloproteinase-2 and -9 and their inhibitors, tissue inhibitor of metalloproteinase-1 and -2, in primary cultures of human prostatic stromal and epithelial cells. J. Cell. Physiol. 191, 208-216.

Wolfsberg T.G., Bazan J.F., Blobel C.P., Myles D.G., Primakoff P. and White J.M. (1993). The precursor region of a protein active in sperm-egg fusion contains a metalloprotease and a disintegrin domain: structural, functional, and evolutionary implications. Proc. Natl. Acad. Sci USA 90, 10783-10787.

Yan H.H., Mruk D.D., Wong E.W., Lee W.M. and Cheng C.Y. (2008). An autocrine axis in the testis that coordinates spermiation and blood-testis barrier restructuring during spermatogenesis. Proc. Natl. Acad. Sci. USA 105, 8950-8955.

Yoshida K., Kawano N., Yoshiike M., Yoshida M., Iwamoto T. and Morisawa M. (2008). Physiological roles of semenogelin I and zinc in sperm motility and semen coagulation on ejaculation in humans. Mol. Hum. Reprod. 14, 151-156.

Young K.A., Zirkin B.R. and Nelson R.J. (2000). Testicular regression in response to food restriction and short photoperiod in white-footed mice (*Peromyscus leucopus*) is mediated by apoptosis. Biol. Reprod. 62, 347-354.

Yu Q. and Stamenkovic I. (1999). Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. Genes Dev. 13, 35-48.

Yu Q. and Stamenkovic I. (2000). Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev. 14, 163-176.

Yu W.H. and Woessner J.F. Jr (2000). Heparan sulfate proteoglycans as extracellular docking molecules for matrilysin (matrix metalloproteinase 7). J. Biol. Chem. 275, 4183-4191.

Yu W.H., Yu S., Meng Q., Brew K. and Woessner J.F. Jr (2000). TIMP-3 binds to sulfated glycosaminoglycans of the extracellular matrix. J. Biol. Chem. 275, 31226-31232.

Yu W.H., Woessner J.F. Jr, McNeish J.D. and Stamenkovic I. (2002). CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. Genes Dev. 16, 307-323.

Zhang J., Jung K., Lein M., Kristiansen G., Rudolph B., Hauptmann S., Schnorr D., Loening S.A. and Lichtinghagen R. (2002). Differential expression of matrix metalloproteinases and their tissue inhibitors in human primary cultured prostatic cells and malignant prostate cell lines. Prostate 50, 38-45.

Accepted December 16, 2009