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Ecophysiological responses of the seminal vesicle of Libyan jird (*Meriones libycus*) to the Saharan conditions: histological, morphometric and immunohistochemical analysis

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Summary. The Libyan jird (*Meriones libycus*) is a nocturnal Saharan Rodent submitted to a seasonal cycle of reproduction characterized by a short active period during spring and beginning of summer, and a long phase of sexual quiescence from the end of summer until the end of winter. During this cycle, the male reproductive organs, and more particularly seminal vesicles, experience some important weight and histological variations.

During the breeding period, the wall of each seminal vesicle describes several folds radiating inside a broad lumen filled with a very abundant secretion. The wall is limited with high columnar epithelial cells surrounded with extracellular matrix restricted to some connnective fibres located in the narrow axis of the folds and in the chorion. The fibro-muscular wall is narrow.

During sexual quiescence, the seminal vesicles regress. No secretion has been observed inside the lumen. The wall of lumen is now surrounded with a single cubic epithelium. The persistent epithelial folds possess a wide axis. The hypertrophied extracellular matrix is constituted with a very tight and abundant connective tissue. The fibro-muscular wall is thick.

A quantitative morphometric study was performed with automatic image analysis that allowed to quantify the seasonal variations of the histological components. The numerical values obtained agree with the histological images observed, the epithelial surface area (μm^2) is high in spring and significantly weak during sexual quiescence. The stroma and the fibro-muscular wall occupy an important surface area on sections during the resting period compared with the value collected during the active phase. The study of the apoptosis by TUNEL method revealed the presence of a considerable number of apoptotic nuclei in the epithelial fraction during the resting phase. The indirect immuno-histochemical method allowed us to visualize the presence of types I and III collagen in the extracellular matrix, weak during the period of breeding, intense and diffuse during the resting season like in castrated *Meriones libycus*.

Key words: Rodents, Desert, Reproduction, Seminal vesicle, Extracellular matrix, Stroma, Tissue remodeling, Apoptosis, Castration, Epithelial-stromal interactions, Collagen fibers

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Introduction

In the Saharan areas, the climatic conditions are extreme: precipitations are weak; temperatures are high and cause some abrupt seasonal and daily variations. These conditions lead to the scarcity and precariousness of trophic and hydrous resources. Nevertheless, these apparently hostile countries shelter many species of rodents which succeeded in overcoming the constraints

of their habitat, thanks to their multiple and varied adaptive faculties (Petter et al., 1984; Sicard, 1992; Ouali and Bensalem, 1996; Bozinovic et al., 2003; Shanas and Haim, 2004). The reproduction, ensuring the perennity of the species is the most sensitive function in front of the food and hydrous deficits, because the needed energy is particularly high. The Saharan rodents are adapted to breed when the conditions are able to satisfy the energy and hydrous request of pregnant and nursing females, young growing animals that need also a lenient temperature to live. When the conditions become unfavourable, the Saharan rodents do not show any reproductive activity and their consumption is mainly directed towards the vital physiological activities in order to ensure the survival of individuals (Khokhlova et al., 2000).

The majority of the rodents living in the arid and semi-arid regions, breed according to a seasonal cycle, and they are characterized by weight, structural, ultrastructural and biochemical variations (El-Bakry et al., 1998). During the active period, the reproductive organs show a considerable weight growth, the structure and the ultrastructure are developed and both testicular and plasma testosterone are high. In the sand rat *Psammomys obesus* (Khammar, 1987; Gernigon-Spychalowicz, 1995; Boufermes, 1997), desert jird *Meriones crassus* (Boufermes, 1997), Libyan jird *Meriones libycus* (Belhocine, 1998; Smaï, 1998) or still *Meriones shawi* (Zaime et al., 1992), the phase of quiescence is typically characterized by a reduction in the size of the reproductive organs (seminal vesicle and prostate) induced by a marked decrease in testicular and plasma testosterone levels. In temperate areas, the small mammals, like the hedgehog (Saboureau, 1992), the gilded hamster (Frungieri et al., 1999) and the field vole, (Kriegsfeld and Nelson, 1999) also present many comparable seasonal variations.

In *Meriones crassus* all the histological components of seminal vesicles are well affected during the seasonal cycle (Belhocine and Gernigon-Spychalowicz, 1994). During the active period, the epithelial zone presents a remarkable development, with a lot of folds surrounded with high secreting epithelial cells. These folds converge regularly in a broad lumen filled with an abundant secretion. The extracellular matrix is limited to some connective fibres and the fibro-muscular wall is slightly developed. During the period of sexual quiescence, the atrophied epithelial zone is characterized by a reduction in number and size of secreting epithelial cells, the disordered epithelial folds become less numerous without any secretion in the lumen. The stroma is hypertrophied, massively infiltrated with an excessive connective tissue penetrating inside the axis of the folds involving its widening. A great thickening of the fibro-muscular wall is also observed (Belhocine, 1998).

In *Meriones libycus*, *Meriones crassus* and *Gerbillus gerbillus*, the castration applied in spring involves some histological effects in the seminal vesicle, comparable to those observed during the season of sexual quiescence, one month after the operation (Belhocine and Gernigon-Spychalowicz, 1996; Belhocine et al., 1996). Similar effects of castration have been observed in bulbo-urethral gland of the mouse and guinea-pig (Parr et al., 1993; Raeside et al., 1997), in the prostate, the seminal vesicle and the coagulating gland of the rat (Holterhus et al., 1993; Wahlquist et al., 1996).

During the resting phase, or in absence of testosterone following castration, as the secreting epithelial zone regresses, the surrounding stroma and fibro-muscular wall become denser. The reverse occurs in the active season. These observations reflect some reciprocal physiological interactions between the epithelial zone and stroma. This phenomenon, usually known during the embryonic development was described initially by Tenniswood (1986) in the adults and also found in the hormone dependent organs including the seminal vesicle (Cunha et al., 2004). Such interactions have also been observed in the female reproductive organs such as the uterus during menstruation and gestation (Shynlova et al., 2004), the ovary (Rodgers et al., 2003) and the mammary gland (Schedin et al., 2004). This phenomenon was also observed in pathological cases such as cancer (Takao et al., 2003).

The epithelial zone of the seminal vesicle of Libyan jird (*Meriones libycus*) regresses during the resting season and after hormonal deprivation caused by castration. In the seminal vesicle of castrated *Psammomys obesus* (Gernigon-Spychalowicz et al., 1994), the prostate of the castrated rat (Omezzine et al., 2003a), the seminal vesicle (Tanji et al., 2003) and the bulbo-urethral gland (Tsuji et al., 1998) of the castrated mouse, like in the coagulating gland of the Hamster subjected to an antagonist of the 17 ß-œstradiol, the diethylstilboestrol (DES) (Nonclercq et al., 1999) the epithelial atrophy is comparable with that noted in the Libyan jird and is manifested by a reduction of the size and number of the epithelial cells; these last die by apoptosis. This phenomenon also occurs in the epithelial zone of the female reproductive organs like the uterus and the vagina of the mouse after ovariectomy (Sato et al., 2003), the ovary during its cyclic activity (Amsterdam et al., 2003) and in the mammary glands of mouse (Prince et al., 2002).

To put at light the structural rearrangements of the seminal vesicle of *Meriones libycus* during the seasonal cycle of reproduction, we present here results about some histological, morphometric and immuno-histochemical variations. The implication of apoptosis in seminal vesicle remodeling has also been revealed using the TUNEL method (TdT-mediated dUTP Nick End Labelling).

Materials and Methods

Animals

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. So, it benefies from the shade

procured by plants (Petter, 1961).

The animals were collected in Beni-Abbes area (W. Béchar) situated in northwestern Algerian Sahara during the years 2000 and 2001. 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* taken 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 from barley. They were kept for about 24 hours in the laboratory and then killed always in late evening (17h 00 – 19h 00) to study seasonal histological variations. In breeding season (spring) five (05) *Meriones libycus* were castrated by abdominal incision under ether anaesthesia. Controls males (05) and castrated animals were held in the laboratory in virtually identical conditions of light (according to the photoperiod of season) and temperature 25°C equivalent to those in their burrows, they had free access to food (grilled grains). The sacrifice took place by decapitation 30 days later. Body weight of all animals used in this study varies between 80 and 90 g and their number varies between 5 and 11 (see Table1).

Histology

The seminal vesicles have been isolated from the adult animals taken from the seasonal group and from individuals castrated for one month in spring. The both seminal vesicles were quickly excised, carefully freed from surrounding fat, weighed and then fixed in Bouin-Hollande's fluid for histological and morphometric study. The studies using immunohistochemical and TUNEL methods were carried out on several seminal vesicles fixed with 10% formalin.

The seminal vesicles were dehydrated in a series of ethanol of increasing degree (70%, 95%, 100%). After one night (12 hours) of impregnation in paraffin in incubator at 60°C, the seminal vesicles were embedded in paraffin using Leuckart's bars. The blocks of paraffin were cut on a Minot's vertical microtome. After dewaxing and rehydration, the sections (5 to 7 μm thick) were stained with Romeis's azan, Van Gieson's trichroma and Masson's trichroma, and then they were dehydrated in ethanol. The montage of slides was performed by use of a neutral resin (Euckitt). The slides were observed with various magnifications (x4, x10, x40, x100) on light microscope and microphotographies were taken on Zeiss's photomicroscope.

Morphometry

In order to quantify the microscopic observations, a morphometric study of the histological images was performed and the numerical data were exposed to a statistical processing. This study was carried out by automatic image analysis thanks to the image analysis software "IPS4". The slides were observed on a photomicroscope to which a color video camera was connected to transmit the image to the screen of a computer. The measurements expressed in μm^2 were taken on the seasonal and the castrated groups. The quantified histological parameters were the epithelial surface, the lumen surface, the surface of the connective portion (extracellular matrix) and the surface of fibro-muscular wall. Analysis consists to making the thresholding of the parameters; each one was isolated according to its color except for the wall whose contour was traced with electronic pencil. The number of analyzed slides corresponds to the number of animals used in this study and both seminal vesicles were examined (1 slide by seminal vesicle and 2 seminal vesicles by animal). Slides of each lot were observed at objective 4 on 5 fields of observation, 50 measurements were performed for each group (seasonal and castrated animals). The values obtained were directly sent to Stat 2005 software in order to realize an automatic statistical analysis. The averages and standard deviations of each parameter were calculated. For each parameter, the average obtained during breeding period was compared with Student's test T to that observed during quiescence and in castrated animals.

Immunohistochemistry

Type I and III collagens of the extracellular matrix have been visualised by means of immunohistochemical method. The antibodies used were polyclonal antibodies (rabbit anti-bovine collagen type I and III polyclonal antibody, CHEMICON International) and the followed protocol is the protocol of immunohistochemistry KIT LSBA2 (DAKO) peroxidase according to the indirect method.

After dewaxing and hydration of the slides with distilled water, the sections were surrounded with PAP-PEN, and then treated with hydrogen peroxide diluted at 3% in distilled water to inhibit endogenous peroxidasic activity. The slides were then rinsed in PBS buffer. The primary antibody intended to reveal our structure is applied during 30 minutes at ambient temperature. The slides were then rinsed in PBS three times for 5 minutes and treated with the secondary biotinyled antibody directed against the primary one during 30 minutes at ambient temperature. This step was followed by the application of sterptavidine-peroxidase during 30 minutes and the enzymatic reaction was revealed by the use of the substrate-chromogen solution during 10 to 15 minutes at ambient temperature. The sections were stained with aqueous hematoxylin in order to increase the contrast of specific staining, and they were then mounted in aqueous medium (crystal mount). After microscopic examination the required structures were coloured in red. To verify the specificity of primary antibody some negative slides were also prepared, 1) by applying the primary antibody and replacing the secondary one with PBS buffer, and 2) replacing the

primary antibody with PBS.

TUNEL

The apoptotic nuclei were revealed with the TUNEL method according to the protocol of the kit TUNEL-Phosphatase-Alkaline developed by Boehringer-Mannheim. After dewaxing and hydration with distilled water, the sections were incubated during 30 minutes at 37°C with proteinase K (20 μg/ml in distilled water), in order to separate the chromatin from histones. The TUNEL solution was then applied during one hour at 37°C. After 3 rinsing with PBS buffer, the anti-fluorescein antibody was applied during 10 minutes at 37°C, and then the sections were rinsed 3 times in PBS buffer. The cuts were then treated with the substrate solution during 10 min at laboratory temperature. After rinsing with PBS the slides were stained with aqueous haematoxylin in order to increase the contrast, and then mounted with crystal mount, a hydrophilic medium. The apoptotic nuclei and fragments were coloured in dark brown after microscopic observation.

Results

Seasonal Variations of seminal vesicle weight

In spring, the weight of seminal vesicle was 686.67 mg. This weight decreased during the period of sexual quiescence from the end of the summer until the end of winter and also in castrated *Meriones libycus* to reach respectively 144.71 mg and 185.06 mg representing a ponderal loss of 79% during quiescence and 73% in castrated animals. These variations were statistically significant (P<0.001) (Tables 1, 2, Fig. 1).

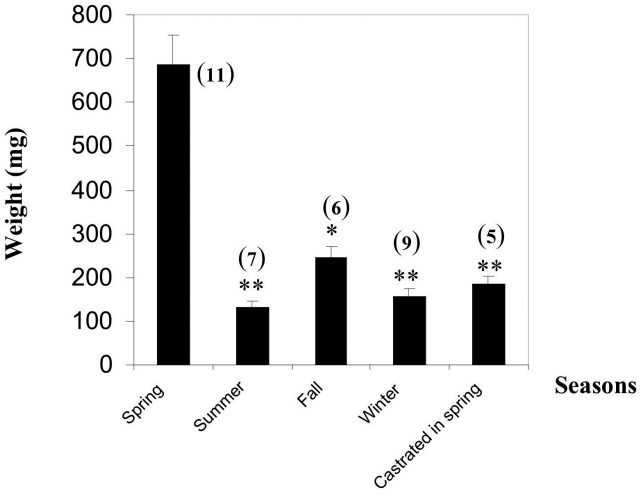

Fig. 1. Seasonal variations of seminal vesicle weight of Libyan jird (*Meriones libycus*) Collected from Beni-Abbes area in 2000 and 2001 and the effects of castration practised In spring for one month on seminal vesicle weight. The number between brackets indicates the number of animals of each group. *: the difference is significant 0,001<P<0,01; **: the difference is very significant P<0,001.

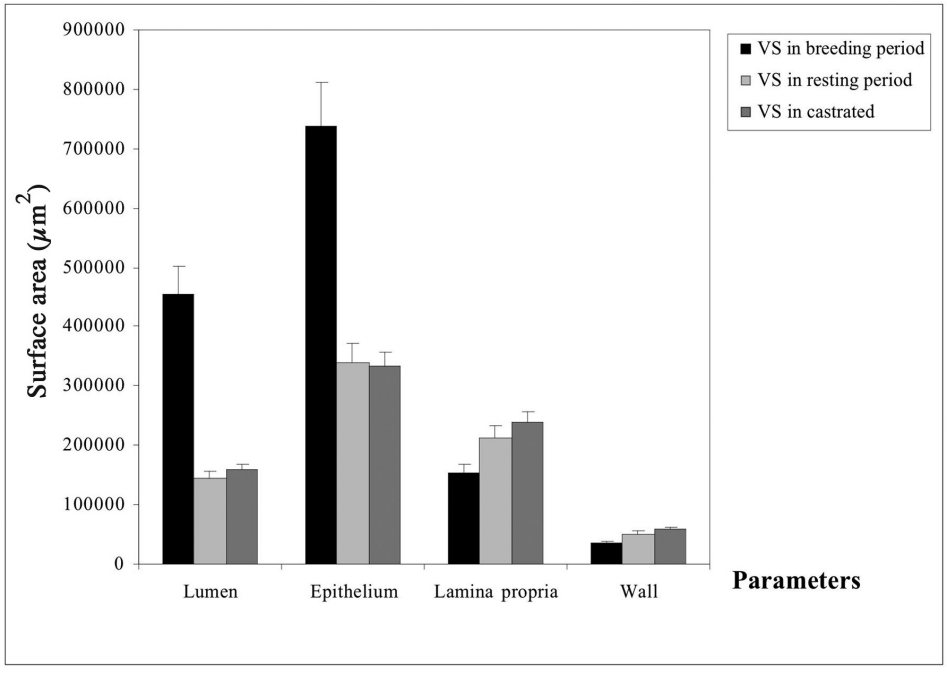

Fig. 2. Morphometric seasonal variations of histological constituents of Libyan jird (*Meriones libycus*) seminal vesicle collected in Beni-Abbes area in 2000 and 2001 in seasonal and castrated groups. The differences are highly significant P<0,001

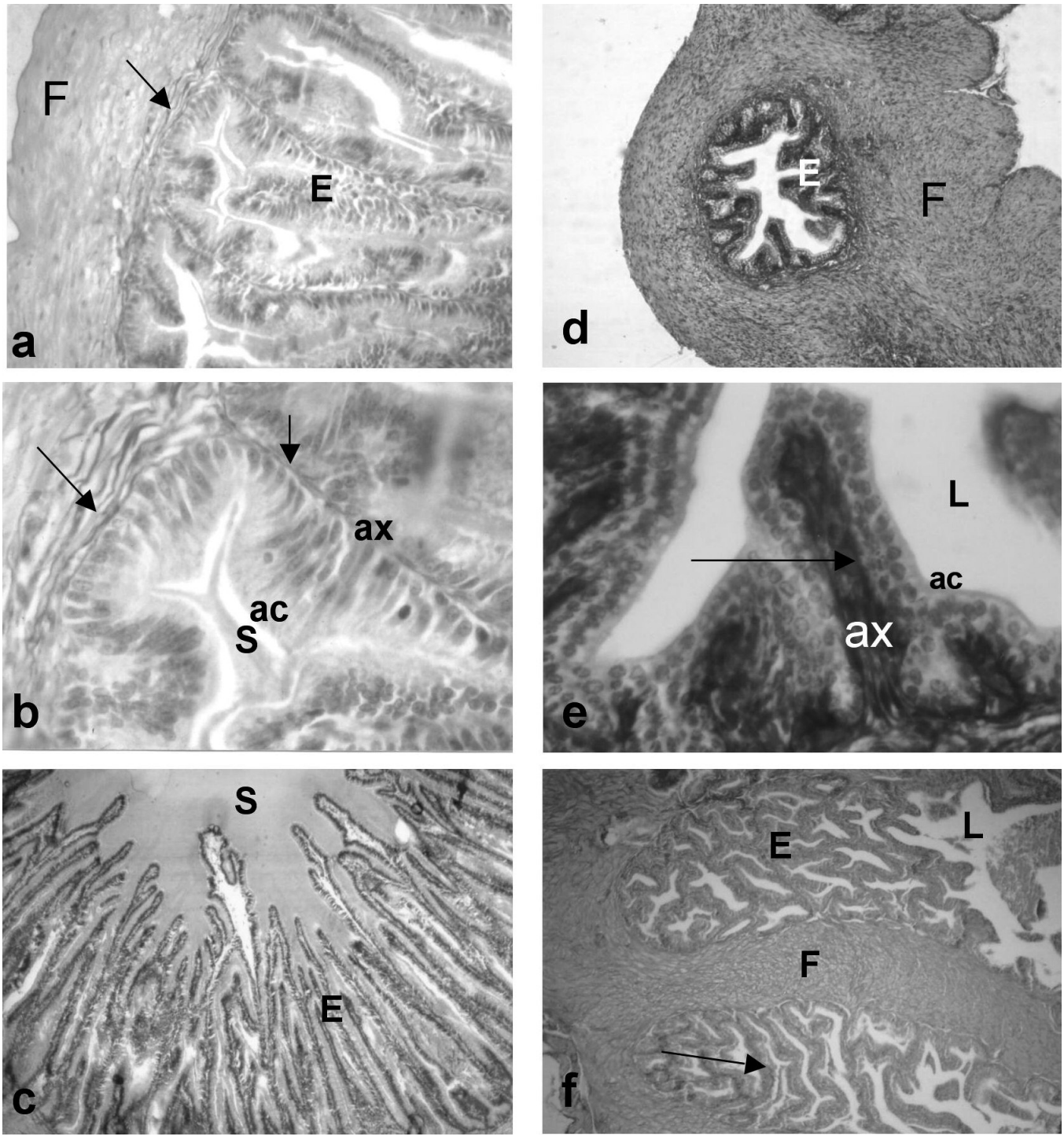

Fig. 3. Histological seasonal changes of seminal vesicle of Libyan jird (*Meriones libycus*). **a, b, c:** in breeding period (spring and onset of summer). **d, e:** in resting phase (end of summer, fall, winter). **f:** in castrated Libyan jird (*Meriones libycus*) in spring during one month. F: fibromuscular wall, E: epithelial folds, S: secretion, ax: epithelial folds axis, ac: apical cytoplasme of epithelial cells, L: lumen, arrows: connective fibers. staining: **a, b, c:** Van-Gieson; **d, e, f:** Azan of Heidenhain. a, x 200; b, e, x 500; c, d, f, x 75

Histological and morphometric seasonal variations

During breeding, the seminal vesicles were bordered with a thin fibro-muscular wall representing 35 000 μm^2 in total surface area (Table 3; Figs. 2, 3a). This fibro-muscular wall was separated from the epithelium by some connective fibres dispersed on a limited surface area of 153000 μm^2 (Table 3; Figs. 2, 3a,b). The cylindrical epithelium was constituted with cells measuring 23 μm in height with an abundant supranuclear cytoplasm without any specialisations measuring 13 μm (Fig. 3b). This zone whose surface area was 738000 μm^2 (Table 3, Fig. 2), formed many folds converging to a dilated lumen (455000 μm^2 in surface area) (Table 3) filled with abundant secretion (Fig. 3c). The axis of these folds was narrow and contained very few connective fibers (Fig. 3b).

During the resting period and in castrated animals for one month in spring, the fibro-muscular wall became

Table 1. Weight averages of the body and the seminal vesicle weight of Libian jird (*Meriones libycus*) taken from seasonal and castrated animals.

Seasons	Animals number	Body weight average (g)	Seminal vesicle weight (mg)
Spring	11	81.75	686.67
Summer	8	67.10	131.77
Fall	6	83.50	245.50
Winter	9	89.60	156.86
Castrated in spring	5	80.00	185.06

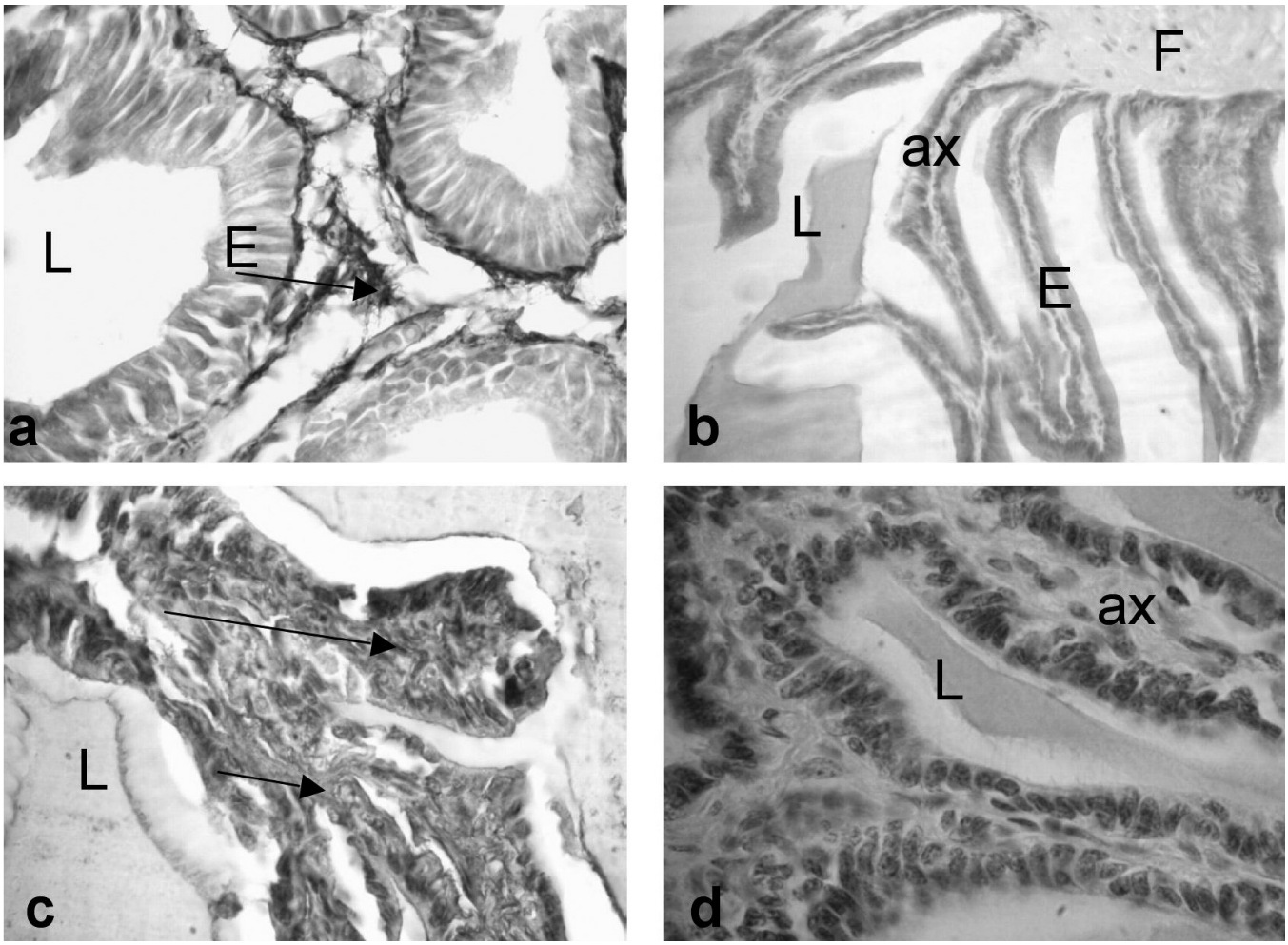

Fig. 4. Immunohistochemical seasonal variations of collagen type I in seminal vesicle of Libyan jird (*Meriones libycus*). **a, b:** in breeding period (spring and onset of summer). The collagen I fibers are thin and follows the contour of epithelium. **c, d:** in resting periode (end of summer, fall, winter). The collagen I fibers are in the form of thick and undulated beams and fill the widened axis of the epithelial folds. **b, d:** negative control prepared without primary antibody. Note the absence of immunohistochemical staining. E: epithelium, ax: epithelial folds axis, L: lumen, F: fibro-muscular wall, arrows: collagen I fibers. a, c, d, x 1250; b, x 400

well developed and measured about 54000 μm^2 in surface area (Table 3; Figs. 2, 3d). The interstitial compartments and the widened axis of the epithelial folds are filled with hypertrophied connective tissue (Fig. 3e,f). The surface area of this connective space is about 226000 μm^2 (Table 3; Fig. 2). The epithelium regressed, measuring only 336000 μm^2 (Table 3; Fig. 2). The number of epithelial folds decreased (Fig. 3d) and the epithelial cells of which the number was now reduced became cubic and measured only 11.6 μm in height with a short supranuclear cytoplasm of 3.7 μm in length (Fig. 3e). The lumen was narrow, measuring 151000 μm^2 in surface area (Table 3, Fig. 2).

Seasonal variations of collagen

The use of immunohistochemical methods for the study of the extracellular matrix showed the presence of both type I and III collagen in the connective fraction infiltrated in the axis of the epithelial folds and in the interstitial spaces of the seminal vesicle. The immunoreactivity for collagen I fibers was stronger than that of collagen III (Figs. 4a,c, 5a,b). During the breeding period, the immunohistochemical labelling of both type I and III collagen was closely observed to the basal part of the epithelium and infiltrated in the network

Table 2. Weight loss in percentage (%) compared to spring and probabilities values of Libian jird (*Meriones libycus*) seminal vesicle of seasonal and castrated animals.

Compared lots	Differences in %	Probabilities (P)
Spring/Summer	-80.80	P<0.001
Spring/Autumn	-64.25	0.001<P<0.01
Spring/Winter	-77.15	P<0.001
Spring/Castrated	-73.00	P<0.001

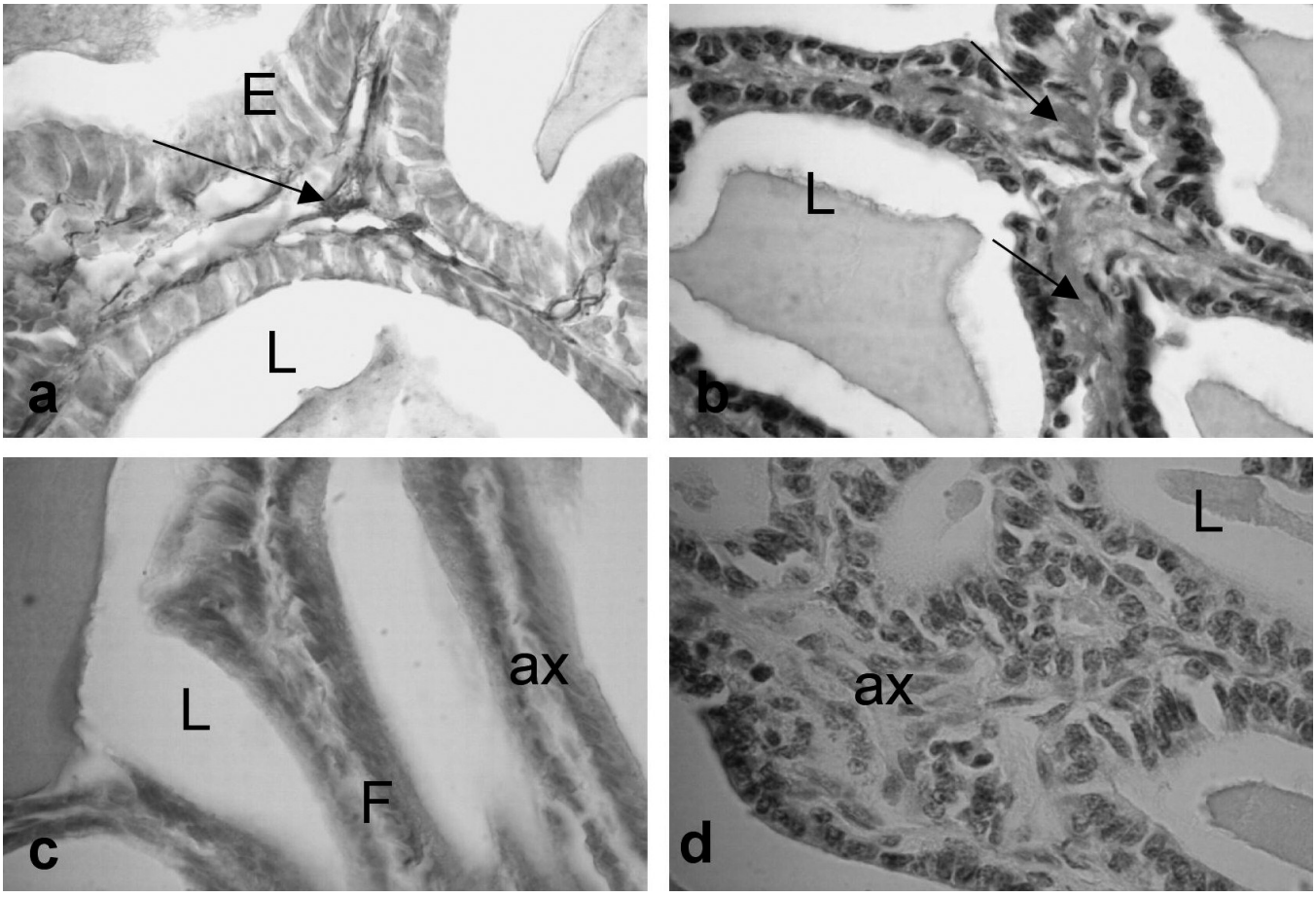

Fig. 5. Immunohistochemical seasonal variations of collagen type III in seminal vesicle of Libyan jird (*Meriones libycus*). **a, b:** in breeding period (spring and onset of summer). The collagen III fibers are thin and follow the contour of epithelium. **c, d:** in resting period (end of summer, fall, winter). The collagen III fibers are in the form of thick and undulated beams and fill the widened axis of the epithelial folds. NB: The immunoreactivity is less intense than collagen I. **b, d:** negative temoin prepared without primary antibody. Note the absence of immunohistochemical staining. E: epithelium, F: epithelial folds, ax: epithelial folds axis, L: lumen, arrows: collagen III fibers. a, b, c, d, x 1250

Table 3. Numerical values of the histological components of Libyan jird (*Meriones libycus*) seminal vesicle taken from seasonal and castrated lots.

Parameters (μm^2)	Average of the value obtained (μm^2)		
	SV in breeding period	SV in resting phase	SV in castrated animals
Lumen sur	455000±250.25	143000±48.56	158000±57.59
Epithelial sur	738000±256.39	340000±145.75	332000±123.41
ECM sur	153000±56.23	213000±86.23	239000±110.11
Wall sur	35000±26.34	50000±36.52	58000±39.12

Conclusion: The values obtained in breeding period are compared with those obtained in sexual quiescence and in castrted animals. For a threshold of 0.05 the average differences of the value of all the parameter studied are highly significant (P<0.001). Sur: surface; SV: seminal vesicle; ECM: extracellular matrix.

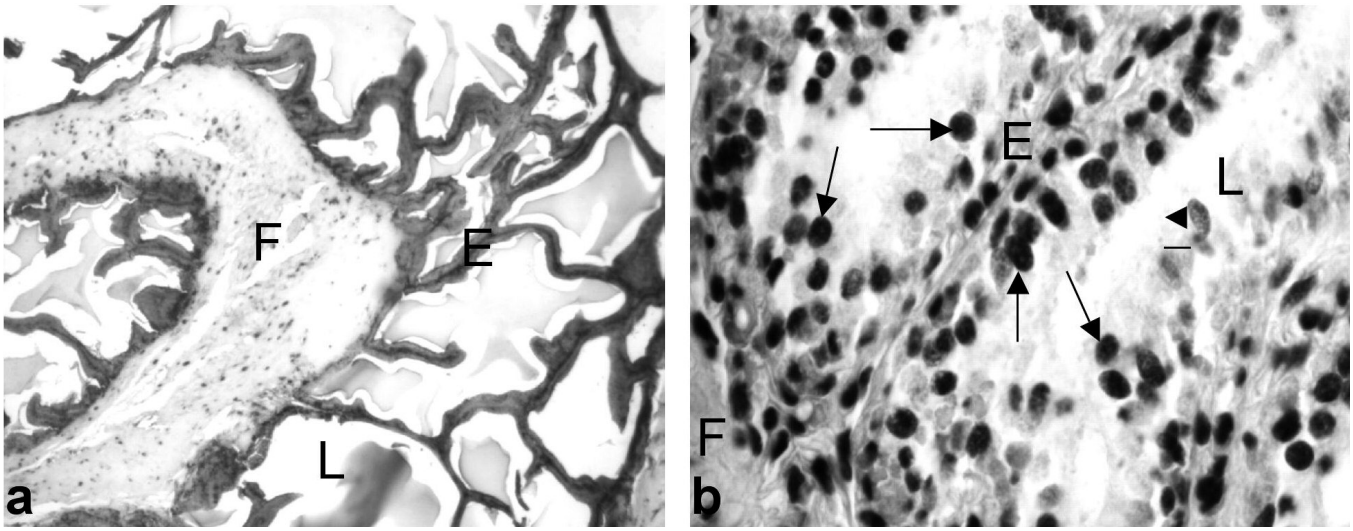

Fig. 6. TUNEL labelling applied on seminal vesicle of Libyan jird (*Meriones libycus*). **a:** in breeding period (spring and the beginning of summer). Note the absence of apoptotic nuleus in the epithelium. **b:** in resting phase (late summer, autumn, winter). Note the presence in the epithelium of a high number of apoptotic nuclei with very dense chromatin (arrow) which largely dominate the non apoptotic nuclei (arrowhead) which show a clear nucleus with decondensed chromatin. F: fibromuscular wall, E: epithelial folds, L: lumen, arrows: apoptotic nuclei. a, x 400; b, x 1250

of interstitial space, the collagen fibers were rectilinear (Figs. 4a, 5a). During the period of quiescence, both type I and III collagen fibers, gathered in thickness and undulated beams filled the interstitial space entirely (Figs. 4c, 5b). The negative controls did not show any immunostaining (Figs. 4b,d, 5c,d).

Results of the TUNEL method

During the breeding period, the TUNEL method did not detect any apoptotic nucleus in the epithelium (Fig. 6a). In period of sexual quiescence, this same method revealed the presence of numerous apoptotic nuclei in the epithelial zone. The chromatin of these nuclei was very condensed (Fig. 6b); the non-apoptotic nuclei were clear and scattered among the apoptotic nuclei (Fig. 6b). Based on the microscopic observation, the difference between the period of reproduction and the phase of sexual quiescence are very obvious, the apoptotic nuclei are more numerous in resting season and the results are apparent (Fig. 6a,b).

Discussion

The Libyan Jird (*Meriones libycus*), a nocturnal Saharan Rodent, experiences a seasonal cycle of reproduction divided into two distinct phases. A short breeding period programmed at spring and beginning of summer. At this phase, associated with an increasing development of the reproductive organs, the animals profit from maximum food and hydrous abundance as well as leniency of the temperatures of these seasons. The phase of sexual quiescence starts at the end of summer and continues until the end of winter. This long period of quiescence is accompanied with a well accentuated regression of the reproductive organs, making possible *Meriones libycus* to use the little available energy to its vital physiological activities, as it

was underlined by Khokhlova et al. (2000) and El-Bakry et al. (1999). The female *Meriones libycus* studied at the same time as the males present some identical seasonal variations (Smaï, 1998). The reproduction of the sympatric species *Meriones crassus*, living in the same biotope, proceeds according to a comparable seasonal cycle (Boufermes, 1997; Belhocine, 1998).

In the seminal vesicle of *Meriones libycus*, seasonal variations are very obvious and affect weight, histological, morphometric and biochemical aspects. During the breeding phase, a strong accumulation of secretion is observed in each seminal vesicle, correlatively to an increase of its weight. The histological and morphometric analysis illustrates a developed epithelial fraction occupying an important surface area of seminal vesicle. The epithelial cells are high, possessing all the organelles implicated in the reactions of synthesis, translating a great secreting activity (Belhocine, 1998). The epithelium forms diverticula which penetrate inside a very dilated lumen filled with an abundant secretion. The extracellular matrix is underdeveloped, the connective frame observed in the axis of the epithelial folds and in the interstitial compartments is limited to some dispersed fibers. This space is little extended in a limited surface area of seminal vesicle. The opposite occurs during the resting season when the seminal vesicles show a considerable weight fall and a more held external aspect and the epithelial zone is strongly atrophied so it spreads on a low space and presents restricted number of cubic epithelial cells. The consequence is the disappearance of the majority of epithelial folds, even if some of them are persistent but disordered and laid out in a narrow lumen. Contrarily, the extracellular matrix hypertrophies. The stroma extending on a large surface area is massively infiltrated by connective fibers accumulated in the widened axis of the epithelial folds and in the under-epithelial space (lamina propria). The fibro-muscular wall is strongly thickened. The castration applied in spring on *Meriones libycus* caused, one month later, some comparable weight loss with histological and morphometric effects in the seminal vesicle. Similar seasonal histological changes with persistence of the secretion and without modification of the fibro-muscular wall were noted in resting period in the seminal vesicle of the sand Rat *Psammomys obesus* (Gernigon-Spychalowicz et al., 1994; Gernigon-Spychalowicz, 1995), the gerbil *Gerbillus gerbillus* (Belkacemi, 1988) and the Hamster (Schindelmeiser et al., 1988). A castration using the bilateral ablation of both testes, deprives the remaining of the reproductive organs of androgens (testosterone), those react by a regression similar to that observed in the resting phase. So, during sexual quiescence, testicular steroidogenic activity falls down or it is inhibited. Indeed, Boufermes (1997) showed reduced plasma and testicular testosterone levels in *Meriones libycus* and in the desert Jird (*Meriones crassus*) during the resting season. In the sand Rat *Psammomys obesus* the seasonal, structural, ultrastructural and biochemical cycles studied by Gernigon-Spychalowicz (1995) coincided with the hormonal seasonal cycle demonstrated by Khammar (1987). The effects of castration detected in *Meriones libycus* and other Rodents of Sahara, bring more evidence of the androgenodependance of the seminal vesicle and agree with those observed in the Rat (Ono et al., 2003, 2004). In all the small mammals studied and particularly the laboratory rodents, the impact of castration is similar to that noted in *Meriones libycus* (Mata, 1995; Tsuji et al., 1998; Tanji et al., 2003).

The regression of the epithelial zone observed in the seminal vesicle of *Meriones libycus* in quiescence and after castration is characterized by the atrophy of secreting epithelial cells which lose their apical cytoplasm, becoming cubic with a high nucleoplasmic ratio. It is also characterized by a significant decrease of the number of the epithelial cells dying by apoptosis. An epithelial atrophy with loss of the epithelial cells by apoptosis was also noted in the seminal vesicle of the castrated mouse (Tsuji et al., 1998; Tanji et al., 2003) like in other glands such as the prostate in man (Staack et al., 2003; Scaltriti et al., 2004; Bozec et al., 2005), mouse (Kuhara et al., 2005) and rat (Izawa et al., 2001; Omezzine et al., 2003a; Garcia-Florez et al., 2005). The apoptosis is a natural phenomenon making it possible for the adult organism to remove the cells when they became redundant after organs involution and turnover. Apoptosis were also detected during the testicular and ovarian regression of the majority of the Rodents during seasonal activity (Young et al., 1999; Young and Nelson, 2001; Gao et al., 2003; Moffat-Blue et al., 2006) and in the epithelium of male genital tract (different regions of epididymis) and accessory sex glands (seminal vesicles, prostate and coagulating gland) of the golden hamster (*Mesocricetus auratus*) exposed to short photoperiod (Carballada et al., 2006). Some apoptotic nuclei were also observed in the germinal cells of the intra-uterine Rat exposed to flutamide, an anti-androgen (Omezzine et al., 2003b) like in all the organs experimenting a periodic activity such as the mammary glands (Furth et al., 1997; Flint et al., 2005; Seol et al., 2005; Watson, 2006), the ovaries (Sakamaki, 2003; Nnene et al., 2004; Peluffo et al., 2005) and the uterus (Kurita et al., 2001; Kuhara et al., 2005). According to Bemis and Schedin (2000) the apoptosis observed in the epithelial cells of the mammary gland, would be due to the loss of cell-cell or cell-matrix contacts caused by the proteolytic action of matrix metalloproteinases (MMPs). On the other hand, Lund et al. (1996) and Watson (2006) stipulated that the apoptosis starts in absence of MMPs in the secreting epithelial cells of the mammary gland of the mouse.

The stroma and fibro-muscular wall of the seminal vesicle of *Meriones libycus* are well developed during the resting season and after castration. The connective and fibro-muscular growth could compensate the epithelial loss. Such a phenomenon was also noted in the

seminal vesicle, the prostate and coagulating gland of the castrated rat (Holterhus et al., 1993; Wahlqvist et al., 1996) and in the bulbourethral gland of the castrated mouse (Parr et al., 1993). The hypertrophy of the stroma consecutive to the epithelial regression is also found in the majority of the hormone dependent organs such as the mammary gland (Green and Lund, 2005; Watson, 2006) and shows the reciprocal interactions between the stroma and epithelium in the maintenance of viability and differentiation of the epithelium (Parmar and Cunha, 2004). The impact of extracellular matrix on the epithelial fraction is the subject of many investigations employing recent technology such as the tissue recombination and transgenic and gene knockout models (Barclay et al., 2005). Indeed, it was shown that the hormones do not act directly on the epithelium in spite of the presence of their receptor, but the hormones act forward using the paracrine action of surrounding stroma in the male and female reproductive organs (Cooke et al., 1997; Cunha et al., 2004).

During regression, the seminal vesicle presents a decrease of weight and an epithelial involution accompanied with the reorganization of tissues. The gland with predominance of epithelium during the active phase is filled with secretion. During the period of quiescence, when the seminal vesicle is deprived of secretion, the connective and fibro-muscular tissue become predominant. These observations clearly show the remodelling of seminal vesicle during the reproduction. This phenomenon was also underlined in the prostate of the Guinea-pig after treatment with estradiol (Scarano et al., 2005) and in the male gerbil *Meriones unguiculatus* after inhibition of 5-α-reductase, an enzyme converting testosterone into dihydrotestostérone (DHT) in which a change of phenotype of the muscular cells during this remodelling was also observed in this same species (Corradi et al., 2004). These contractile cells would be transformed into synthetic cells contributing to the biosynthesis of the stroma. Vilamaior et al. (2005) indicated the de-differentiation of the smooth muscular cells of the ventral prostate in castrated Rat, with a synthetic phenotype preserving their differentiated state. Examining the cells with scanning electron microscope, Antonioli et al. (2004) observed the folding up of the plasma membrane, due probably to an alteration of the cytoskeleton. A morphological change of the smooth muscular cells was also found on the ventral prostate of the castrated rat (Vilamaior et al., 2000). The phenomenon of cells transdifferentiation was also found in mammary gland in which alveolar epithelial cells can transdifferentiate into white adipocytes during involution and that white adipocytes can transdifferentiate into alveolar epithelial cells during pregnancy (Faraldo et al., 2002; Morroni et al., 2004).

The immunohistochemical studies of collagen in the extracellular matrix of the seminal vesicle of *Meriones libycus* revealed the presence of both type I and III collagen, in the interstitial space and in the axis of the epithelial folds. The immunolabelling of these collagen fibers was located near the basal portion of the epithelium and dispersed in the interstitial compartment. The immunoreactivity of the two collagen types was weak and occupied a narrow space during the active period. The collagen fibres appeared fine, elongated and rectilinear. During the resting period the immunolabelling of collagen fibers was more intense and spread out over an important surface area. These collagen fibers were gathered in thick and undulated beams placed in interstitial compartment and around the epithelium. Some comparable results were observed in the ventral prostate of the guinea-pig after treatment with estradiol (Scarano et al., 2005) and in that of the castrated rat (Ilio et al., 2000; Vilamaior et al., 2000). These authors observed an intimate association between the undulated fibers of collagen and the smooth muscular cells. So, they stipulate that those would contribute very actively to the reorganization of the extracellular matrix during the process of regression caused by castration. Müntzing (1981) established a relation between the quantity of collagen and the growth of the prostate in the rat, proposing that collagen would limit the growth of the prostate. This hypothesis was also advanced by Izumiya and Nakada (1997) and Nemeth et al. (1997). The origin of collagen accumulation in the extracellular matrix of the seminal vesicle of *Meriones libycus* during the resting period and after castration remains still unknown. It could be due to biosynthesis, reduction of degradation or the association of both the two processes. On the other hand, such a similar proliferation in the prostate of the rat is the consequence of the combined effects of increase in synthesis and reduction of degradation (Nakada et al., 1994).

Conclusion

The exocrine activity of the seminal vesicle of *Meriones libycus* varies according to the climatic conditions. In spring and in the beginning of summer, when water and food are available, and temperatures lenient, the seminal vesicle shows a developed structure with the predominance of secreting epithelial cells and a scattered and dispersed connective fraction, the whole is surrounded with a thin fibro-muscular wall. From the end of the summer until the winter, the trophic and hydrous resources are rare and the temperatures become extreme, consequently, the seminal vesicle regress actively by losing a great part of the secreting epithelium and reorganize to become a gland characterized by a strong growth of the connective and fibro-muscular fractions. This structural plasticity makes it possible for the *Meriones libycus* to support the difficult conditions of their arid Saharan habitat.

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