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Variation in Testicular Histology of the Spiny Tailed Lizard *Uromastyx aegyptius microlepis* During Hibernation and Active Periods

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Abstract: The testicular histology of the lizard, *Uromastyx aegyptius microlepis* were varied considerably during hibernation and activity periods, reaching maximum values in summer. The onset of winter induces testicular regression, reduced testis size, testis diameter, diameter and epithelial height of the seminiferous tubules and epididymal epithelial height. Animals exhibit testicular recrudescence during late winter (February and March) and maximum testicular volume occurred during June. The period of maximal testicular volume was positively correlated with increasing ambient temperature. The summer season induces the testicular activity in contrast with the winter season in which the activity decreased and the testes were collapsed. Spermatocytogenesis is active in early spring and the major portion of the seminiferous epithelium comprises spermatids in various stages of maturation and their luminae were filled with spermatozoa. The size of the interstitial cells is direct correlated with changes occurring in epididymis and seminiferous epithelium. Most interstitial cell nuclei show clear regression when spermatocytogenesis were diminishes.

Key words: *Uromastyx aegyptius microlepis*, testicular histology, hibernation

INTRODUCTION

Recent investigations and reviews concerned with reptilian reproductive cycle and seasonal variations in their testes have been studied by many authors (Droge *et al.*, 1982; Elliotte, 1985; Said and Hussein, 1992; Bhagyashri *et al.*, 2000; Licht, 2005; Nora and Bertona, 2006).

In reading much of the literature about hibernation one gets the impression that ectotherms become dormant in winter simply because they are unable to maintain normal metabolic rates at reduced ambient temperatures occurring at that time of the year (El-Masry and Hussein, 2001). The utilization of endogenous energy reserves by reptiles under hibernation conditions has received little attention despite the fact that these reserves in certain reptiles undergo pronounced changes throughout the year (Gillet and Dacruz, 1981; Said and Hussein, 1992; Oswaldo *et al.*, 2002). A number of biologists have investigated lizard reproductive cycles but until quite recently, relatively little has been written about reproductive activity during hibernation and activity periods (El-Ghazaly *et al.*, 1987; Said and Hussein, 1992; Madkour *et al.*, 1999; Bhagyashri *et al.*, 2000).

The purpose of this study is to show that hibernation is a rather complex phenomenon in the agamid lizard, *Uromastyx aegyptius microlepis*. It presents information dealing with testicular activity and discuss one environmental phenomenon that tends to modify that activity. To do this, we investigated variations in testicular histology. Such histological components gives a measure of the state of reproductive activity of the animal.

MATERIALS AND METHODS

Animals: *Uromastyx aegyptius microlepis* (Reptilia, Lacertilia, Agamidae) is generally found in arid and semi-arid localities of the northern Saudi Arabia and extend to the south; it also occurs in the dry localities of the sea face in the western coast. This lizard is a herbivorous animal, constructs its burrow in hard substratum and hangs about near the burrow to avoid its enemies, besides being armed by strong tail. This lizard is active during the day, during midday it retreats to its deep burrow to avoid high dangerous temperatures. It is noticed that this lizard is active only through March to November and hibernate in winter (Leviton *et al.*, 1992).

Methods and measurements: Adult male lizards were collected from different locations near Jeddah, Saudi Arabia. Lizards were captured twice in the year; in the active season (July, 2005) and during hibernation (January, 2006). Ten animals were picked during summer and ten animals during winter (snout-vent length: 175- 215 mm; tail: 75-125 mm; total length: 260-335 mm; mass: 256-269 g).

The animals were transported to the laboratory on the day of capture. The captured animals were dissected, the right testes and epididymides were extracted weighted and immediately fixed in Bouin's solution, dehydrated in series of alcohol and embedded in paraffin wax. They were serially sectioned at 5 µ and stained with Ehrlich's acid alum haematoxylin and counter stained with eosin.

A number of measurements were undertaken including the following:

- Weight, volume and diameter of the testis
- Diameter and epithelial height of the seminiferous tubules
- Epididymal epithelial height
- Interstitial (Leydig) cell nuclear diameter

All measurements were made by using an ocular micrometer (20 mm diameter, 10 mm divisions into 200 parts).

Statistical analysis: Comparisons between the mean different measurements in hibernation and active periods were made using the Analysis of Variance (ANOVA). The rejection level of statistical significance adopted was $p>0.01$ (Sokal, 1981).

RESULTS

The testis measurements varied considerably during hibernation and activity seasons ($p<0.01$) (Table 1). In winter, lizards remain sequeterd in their burrows and become dormant because they are unable to maintain normal metabolic rate at the reduced ambient temperatures occurring at that time of the year. In this period, the testes were much smaller than in summer (Table 1, Fig. 1a).The seminiferous tubules tend to collapse and loose the characteristic circular cross section when compared with those seen during the period of activity in the summer season. The diameter and epithelial height decreased highly during this dormant period. Seminiferous tubules with large luminae and empty from any spermatozoa. Specimens sacrificed in summer had larger tubule diameters (Fig. 1b). The walls of the tubules consisted of larger cell layers, the epithelial heights increase (Table 1), all spermatogenic stages were clearly represented, spermiogenesis (transformation of spermatids into spermatozoa) apparently occurs at an increased rate, as indicated in most of their luminae being filled with spermatozoa (Fig. 1b).

The seminiferous tubules open in the epididymis which is an enclosed tubule, lined throughout its most length with cuboidal epithelial cells. These cells are often extensively vacuolated and have prominent basal nuclei. The height of the epithelial lining of the ductus epididymis were significantly varied ($p<0.01$) during

Table 1: Changes in testicular parameters of *Uromastyx aegyptius microlepis* during hibernation and activity periods

	Season			
Measurements	Summer	Winter	Changes (%)	p-value
Testis weight (g)	1.64	1.02	23.05	<0.01
Testis volume ($mm^3 \times 10^3$)	9.81	6.33	36.16	<0.001
Testis diameter (µ)	1404.00	864.00	27.39	<0.01
Seminiferous tubule diameter (µ)	196.80	143.20	13.83	<0.01
Tubule epithelial height (µ)	47.90	30.12	37.11	<0.001
Epididymal epithelial height (µ)	15.20	11.64	15.40	<0.01
Leydig cell nuclear diameter (µ)	4.11	1.33	16.45	<0.01

ANOVA test: $p<0.01$, significant difference; $p<0.001$, highly significant. Data are expressed as mean±SE of ten animals

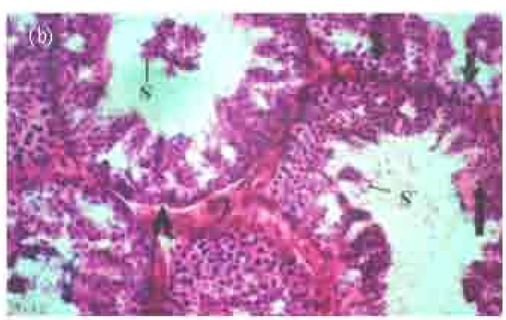

Fig. 1: (a) TS in the testis of the adult lizard, *Uromastyx aegyptius microlepis* during hibernation. Seminiferous tubules with large luminae most of them are empty except from few spermatozoa (S) with low epithelial height and collapsed intercellular space (arrows) x300 and (b) TS in the testis of the adult lizard, *U.a. microlepis* during activity period. The seminiferous tubule lumina are filled with mature spermatozoa (S), note increased epithelial height with active cell division in the nuclei of spermatogonia and spermatocytes (arrows) x500

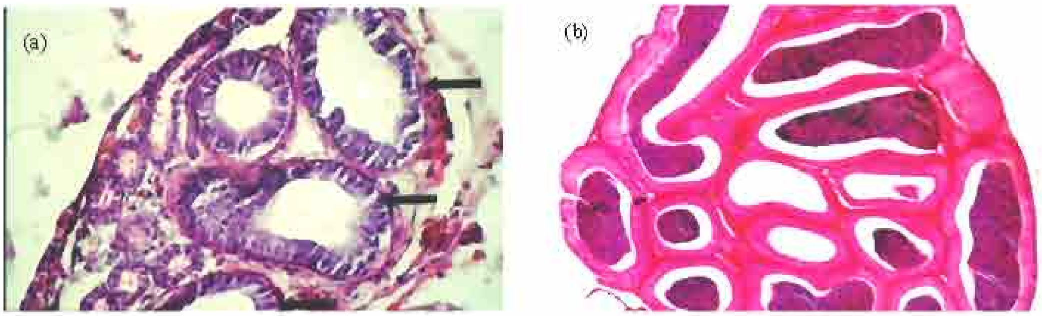

Fig. 2: (a) TS in the epididymis of the adult lizard, *U.a. microlepis* during hibernation small tubule are visible with empty luminae arrows pointing low epithelial heights x200 and (b) TS in the epididymis of the adult lizard, *U.a. microlepis* during activity period demonstrating increased epithelial height. The luminae are filled with aggregated spermatozoa (S) x200

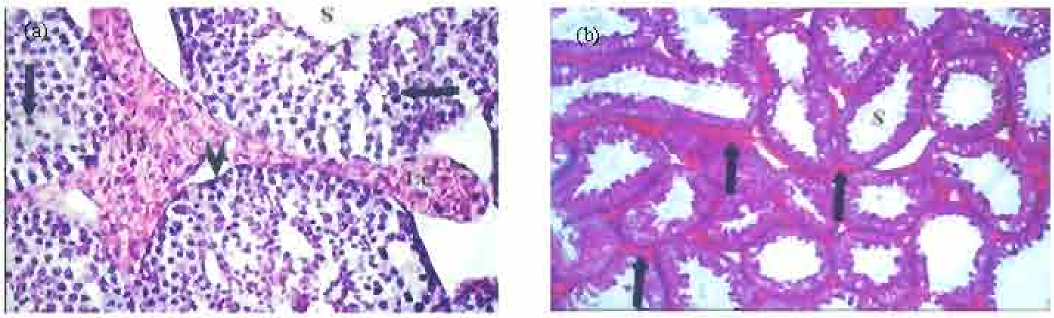

Fig. 3: (a) TS in the testis of the adult lizard, *U.a. microlepis* during hibernation showing seminiferous epithelial (long arrow) is much reduced with few spermatozoa (S), Leyding Cells (LC) are collapsed (arrow head) with small rouclei x500 and (b) TS in the epididymis of the adult lizard, *U.a. microlepis* during activity period revealing cellular deformation in germ cells (arrows) and hypertrophid Leyding Cells (LC) in large intercellular space (arrow head) x500

hibernation and activity periods (Table 1). Considerable changed were noted in the activity of the epididymis ranged from small tubules with decreased epithelial heights with empty luminae throughout hibernation (Fig. 2a) to the increase in the thickness of the epididymal epithelia associated with the time of testicular activity in summer with tubule luminae filled with mature spermatozoa (Fig. 2b).

The leydig cells are located between the seminiferous tubules and appear as vacuolated cells with spherical nuclei. The recorded data of the nuclear diameter, indicated significant decrease ($p<0.01$) during winter with disrupted peritubular tissue (Table 1, Fig. 3a). On the other hand, the nuclear diameter was hypertrophid reaching maximum values in summer with the increase of testicular activity (Fig. 3b).

DISCUSSION

The ectotherms become dormant in winter simply because they are unable to maintain normal metabolic rates at the reduced ambient temperatures occurring at that time of the year. The annual reproductive cycles in temperate zone squamate reptiles appear to be controlled by environmental stimuli with temperature as playing the major role. The majority of studies on the environmental control of the sexual cycles of squamate reptiles have been done on lizards and snakes (Hawley and Aleksiuk, 1976; Van Wyk, 1990; Jennifer *et al*., 2003; Licht, 2005). According to their conclusions, exposure of individuals to low temperatures prevents completion of spermatogenesis while exposure to high temperatures permits or stimulates complete testicular recrudescence.

The pattern of gonadal activity of *Uromastyx aegyptius microlepis* during activity and hibernation was synchronous an similar to other studied lizards. It appears to follow that of *Anolis carolinensis* (Fox, 1958); *Sceloporus orcutti* (Mayhew, 1963); *Urosaurus ornatus* (Asplund and Low, 1964), *Chalcides ocellatus* (El-Ghazaly *et al*., 1987); *Scincus officinalis* (Said and Hussein, 1992); *Podarcis sicula* (Ciarcia *et al*., 1994); *Hemidactylus brooki* (Bhagyashri *et al*., 2000);

Crotophytus collaris (Jennifer *et al*., 2003) and *Boa occidentalis* (Nora and Bertona, 2006). Winter induces testicular regression chracterized by reduced testis size, reduced seminiferous tubules diameter and epithelial height and reduced epididymal activity. However, the type of seasonal change in *U. amicrolepis* differ considerably from that found in many species of lizard so far. For example, *Sceloparus undulates* (Altland, 1941); *Sceloporus occidentalis* (Wilhoft and Quay, 1961); *Uta Stansburiana* (Asplund and Low, 1964); *Cordylus polzonus* (Van Wyk, 1990) and *Lacerta vivipara* (Gavaud, 2005) emerge from hibernation with testes almost at maximum size. The gonads of these species become greatly reduced in size during summer season, followed by tremendous increase just before hibernation. These pattern were not found in *U.a. microlepis*.

It is of interest to compare, the seasonal changes in the epididymal epithelial heights of the lizard, *U.a. microlepis* (moderate zone) with the lizards in other zones. Elliott (1985) on *Eumeces obsoletus* and Jennifer *et al.* (2003) on *Crotophylus collaris*, concluded that there are seasonal changes in activity and epithelial heights of the ductus epididymis.

The most striking finding is that the size of the Leydig cells is correlated with seasonal changes occurring in the epididymis and seminiferous epithelium, i.e., when spermatocytogenesis diminishes some Leydig cell nuclei also show regression. This parallelism, according to Nora and Bertona (2006), does not necessarily means that the Leydig cell activity stimulate spermatogenesis but probably reflects the response that both the seminiferous epithelium and interstitial cells are stimulated by pituitary hormones.

It is therefore, possible to conclude that the regulation of the testicular cycle depends on the interaction between some exogenous and an endogenous cues. The pronounced exogenous temperature sensitivity of the gonads and accessory structures induced the endogenous gonadotropins which may influence the rate of testicular recrudescence and discuss the role of environmental temperatures in the initiation and control of testicular activity.

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