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THE EFFECTS OF THE ANNUAL CYCLE ON *UROMASTYXACANTHINURA* (BELL, 1825)UTERUS STRUCTURE AND FUNCTIONS.

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Abstract

This study describes the effects of the annual cycle on the structure and functions of uterus of *Uromastyx acanthinura* (Bell, 1825) (hibernation and activity seasons). The results revealed that, histological sections in uterus of *U. acanthinura* during hibernation season showed considerable atrophy and degeneration of the glandular region, comparedto nearly normal structure and less atrophied glandular mucosa after hibernation. The expression of protein bands and their concentration markedly decreased during hibernation season and increased during activity season. Genomic DNA showed apparent separation during hibernation. Also, caspase3 and caspase7 activity reached high levels in the uterus tissue during hibernation compared to activity season.

Keywords: Histology of uterus, protein expression, DNA fragmentation, hibernation, caspase 3 and caspase 7.

Introduction

Vertebrate reproduction is highly complex and diverse, and variation in reproductive morphology and the tempo and mode of reproductive cycles are thought to be important contributors to speciation (Lewis,1969 and Templeton,1981).

Reptiles exhibit a variety of reproductive strategies as response to different environmental conditions. In species with seasonal reproduction, gonadal cycle phases (recrudescence, climax and gonadal quiescence) appear temporarily organized according to their thermic and energy demands, and possibly their duration (Saint Girons, 1984).

Temperature plays an important role in various aspects of the life history, ecology, and physiology of reptiles and other ectotherms (Angilletta*et al*., 2002). Growth rates (Arnold and Peterson, 1989; Avery, 1994 and Litzgus and Brooks, 1998a), reproduction (Schwarzkopf and Shine, 1991; Litzgus and Brooks, 1998b and Rock and Cree, 2003), seasonal activity patterns, habitat use (Webb and Shine, 1998; Whitaker and Shine, 2002) and geographic distribution (Castonguay *et al*., 1999) are all influenced by environmental temperatures. Physiological processes

such as metabolic rate generally increase with temperature (Gatten, 1974; Bennett and Dawson, 1976; Beaupre *et al*., 1993; Karasov and Anderson, 1998 and Mc Nab, 2002); however, a few reptile species are known to have plateaus of temperature-independent metabolism (Waldschmidt *et al*., 1987).

The present study aims to illustrate the effects of the annual cycle on the uterus structure and function of *Uromastyx acanthinura* (Bell, 1825).

Material and Methods

A total of thirty female mature individuals of *U. acanthinura*were caught from south Libya. They were brought directly to the laboratory from their natural habitats. Specimens were divided into two groups based on theannual cycle (seasons), the activity season (summer: late June, to mid of July) and hibernation season (winter: late November, to mid of January).

Light microscopic investigations

During the annual cycle, the collected specimens were dissected and the uteri were immediately washed in 10% normal saline. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraplast 58-60oC. Serial 5µm thick histological sections were cut, stained in Mayer's hematoxylin and eosin and processed for investigation under bright field light microscope and photographed.

Molecular Examinations

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE):

Fresh samples of whole uteri of animals captured during hibernation season and activity season were examined by SDS-PAGE according to the method of Andrew (1986). Electrophoresis was carried out at a constant 200 V. The separated proteins were placed on polyacrylamide gels stained with Coomassie blue R-250 (60 mg/L) in an acidic medium for the generation of an electrostatic attraction between the dye molecules and the amino groups of proteins.

DNA Fragmentation Assay

DNA fragmentation was assayed by a modification of the method of Arends *et al*. (1990) and Bortner *et al*. (1995). Freshly isolated specimens were washed twice with ice-cold PBS and suspended in 100 ml of lyses buffer (10 mM Tris HCl/10 mM EDTA/0.5% Triton X-100, pH 8.0), vortex-mixed, sonicated, and incubated on ice for 20 min. After centrifugation for 20 min at 4°C 14,000 rpm the supernatant containing fragmented (soluble) DNA was transferred to another tube. Lyses buffer

(100 ml) was added to the pellet containing insoluble DNA. Both samples were treated with RNase A (0.5 mg/ml) for 1 hr at 37°C and then with proteinase K (Sigma, 0.4 mg/ml) for 1 hr at 37°C. After adding 20 ml of 5M NaCl and 120 ml of isopropanol, the samples were incubated overnight at 220°C, and the DNA concentrations were determined. Fragmented DNA was calculated as 100% X soluble DNA/ (soluble+insoluble DNA). The soluble fraction of DNA was determined by electrophoresis on 1.5% agarose gel and has a ladder-like appearance.

Caspase 3

Caspase 3 was determed using ELISA kit of Uscn Life Science Inc. Wuhan Cat. No.: E0449Ra. Caspase-3 is a member of the caspase (cysteine aspartate protease) family of proteins, and has been shown to be an executioner protein of apoptosis. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis.

Caspase 7

It is determined colorimetrically using Stressgen Kit (catalogue No. 907-013). Cells that are suspected to or have been induced to undergo apoptosis are first lysed to collect their intracellular contents. The cell lysate can then be tested for protease activity by the addition of a caspase-specific peptide that is conjugated to the color reporter molecule p-nitroaniline (pNA). The cleavage of the peptide by the caspase releases the chromophorep NA, which can be quantitated spectrophotometrically at a wavelength of 405 nm. The level of caspase enzymatic activity in the cell lysate is directly proportional to the color reaction.

Statistical analysis

Data were presented as means ± standard error (SE). The statistical analysis was performed with multi-variant analysis of variance (MANOVA) using SPSS (version 13) software package for Windows comparing the multivariations between the groups. *F-test* was calculated and considered statistically significant at $p < 0.05$.

Results

Histological observation of the uterus

By light microscopy, the histological sections of the uterus in the activity season showed normal structure and less atrophied glandular mucosae. On the other hand, sections of the uterus showed atrophy of the glandular mucosa and degeneration of the glandular region in hibernation season(Hx-E)(Fig.1).

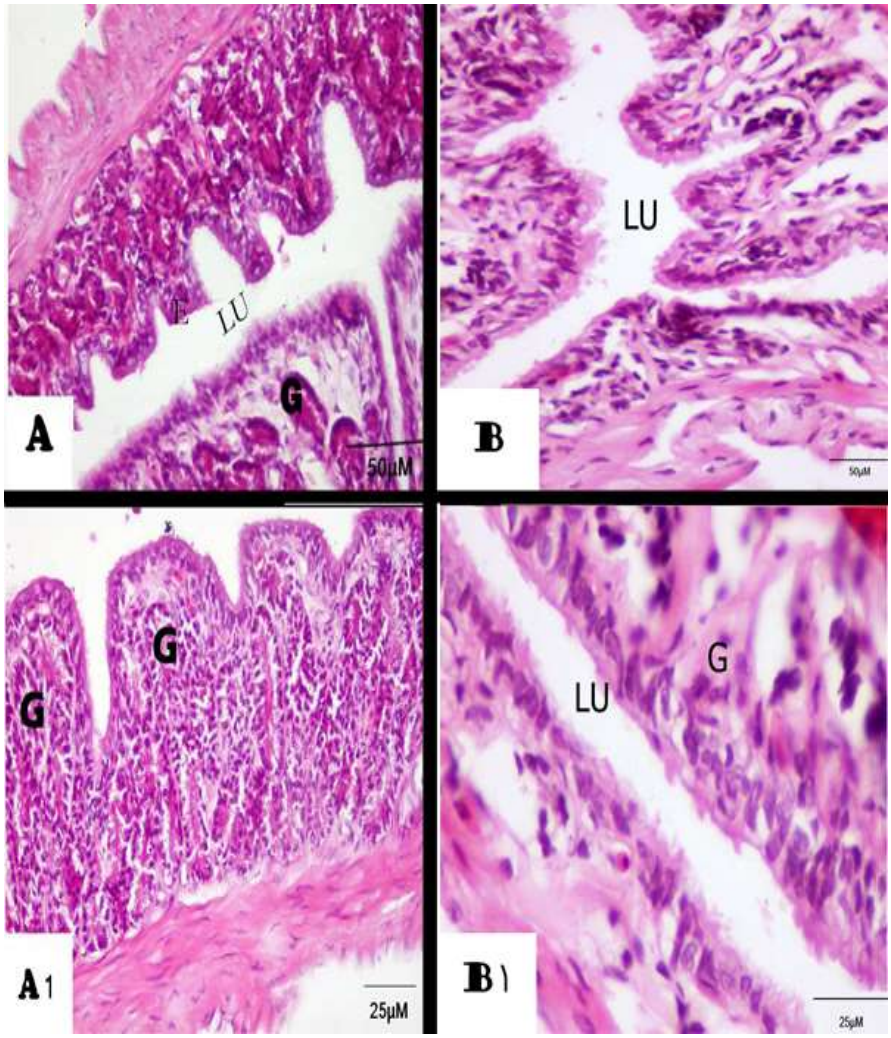

Fig.(1): Photomicrographs of histological sections of *U. acanthinura*uterus during annual cycle, in two seasons, activity season, (A&A1), showing normal structure and lessatrophied glandular mucosa. Hibernation season (B&B1) atrophy of the glandular mucosa and degeneration of the glandular region. AbbreviationsE, epithelium, G, glandular mucosa, LU, lumen (Hx-E, stain).

Annual cycle of uterus protein electrophoresis (SDS-PAGE)

Table (1) and figure (2) illustrate the annual cycleof uterus protein electrophoresis of *U. acanthinura* during two seasons. Expression of protein bands and their concentrations were markedly decreased during hibernation season. On the other hand, increase of bands during activity season was revealed.

Table (1): Annual cycleof uterus protein electrophoresis of *U. acanthinura.*

Lane	Marker		Lane (1) active season		Lane (2) hibernation season	
Bands	KDa	%	KDa	%	KDa	%
1	205	4.2	128.94	2.0	84.21	11.2
2	166	7.0	98.96	9.7		
3	97	8.3	83.76	2.0	70.71	12.7
4	80	9.8	67.42	7.8	60.01	19.2
5	66	8.6	54.41	1.9	54.22	10.3
6	55	6.3	51.47	0.6		
7	45	8.0	39.50	4.2	28.45	26.9
8	30	15.8	30.97	33.7	24.47	12.6
9	21	6.1	24.60	25.9	14.68	1.5
10	14	11.1	20.28	11.0	11.66	5.6
11	6.5	14.8	11.75	1.2		
12						
13						
14						
15						
Total bands	11		11		9	

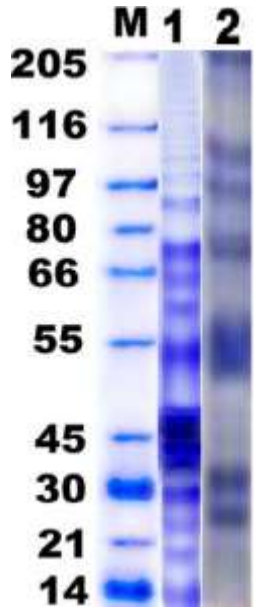

Fig.(2):Annual cycle in uterus of *U. acanthinura*, SDS-PAGE protein expression, Lane (M) Marker, Lane (1) represents active season and Lane (2) hibernation season.

Uterus DNA fragmentation

Table (2) and figure (3) illustrate the uterus DNA fragmentation of *U. acanthinura* during the annual cycle. The genomic expression of the degree of laddering (total DNA fragmented) increased and was more expressed during hibernation, showing highest degree of genomic DNA fragmentation. There was no detected genomic DNA damage during the activity season.

Table (2): Illustrates the uterus DNA fragmentation of *U. acanthinura* during the annual cycle.

Lanes	Ladder Lane(1)		Lane (1) active season		Lane (2) hibernation season	
Bands	B. P.	%	B. P.	%	B. P.	%
1	1000	18.1	1249.92	97.1	1260.00	90.1
2	900	12.5	1155.43	2.9	1199.02	6.8
3	800	11.1				
4	700	9.9				
5	600	18.6				
6	500	15.7			730.74	2.3
7	400	4.1			654.66	0.8
8	300	4.2				
9	200	3.2				
10	100	2.6				
Total Bands			2		4	
In Lane		100		100		100

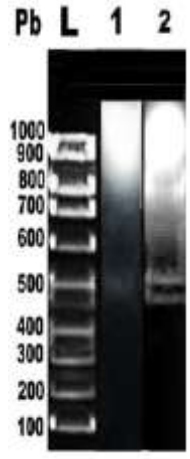

Fig.(3):Annual cycle in uterus DNA fragmentation of *U. acanthinura*, Lane (L) Lader, Lane (1) Active season, Lane (2) represents hibernation, Genomic DNA shows apparent separation during hibernation.

Table (3) and figures (4&5) illustrate the annual cycle of uterus caspases of *U.acanthinura*. The assayed caspases enzymes were markedly increased reaching highest level during hibernation season. However, during activity season there was a marked decline of the enzyme activities. There were no wide variations of the enzyme activities between caspase 3 and caspase 7.

Table (3): shows changes of uterus caspases of *U. acanthinura* during annual cycle (Mean±SE).

Caspases		
	CAS-3	CAS-7
Activity Season	0.21±0.08	0.23±0.12
Hibernation Season	0.39±0.11	0.54±0.20
F-test	7.25	14.34
p≤0.05	S.	S.

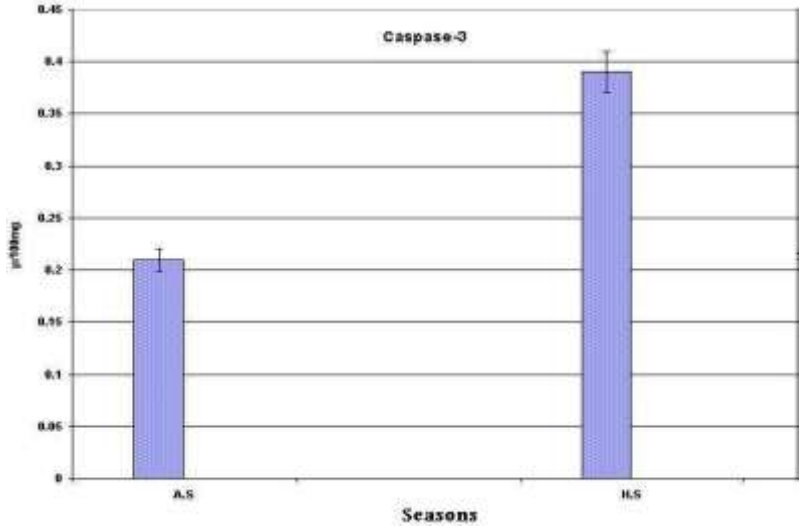

Fig.(4):Annual cycle of uterus caspases-3 of *U. acanthinura* (Abbreviations: A.S, Activity season; H.S, hibernation season)

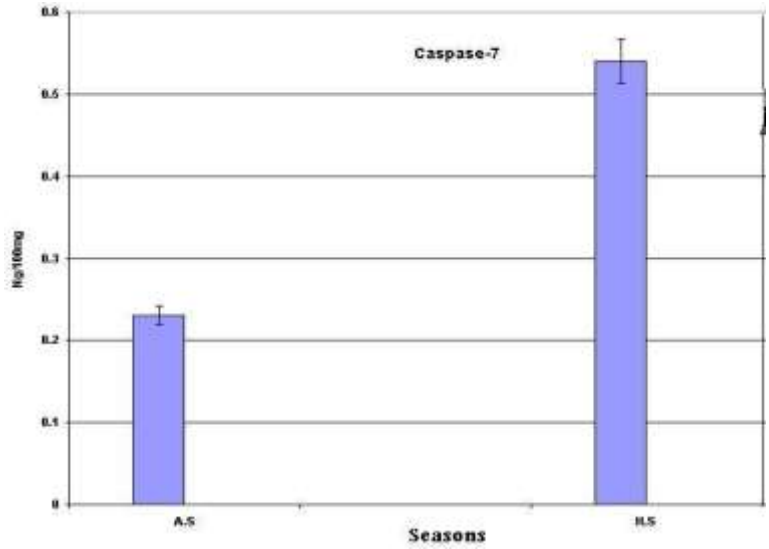

Fig.(5):Annual cycle of uterus caspases-7 of *U. acanthinura* (Abbreviations: A.S, Activity season; H.S, hibernation season)

Discussion

U. acanthinura , often known as the North African Spiny-tailed Lizard, is a medium–sized lizard occurring in desert habitats of north-western Africa, and the northern part of western Libyan Desert.

The ecology and physiology of *U. acanthinura* in Libya are still little studied, although the amount of information on the subject has increased considerably within the last ten years. This lack of knowledge hampers understanding of how ecological and physiological differences may arise as a result of the environmental changes in terms of seasonal variation.

The present work examines the effects of the annual cycle on the structure and functions of the uterus of *U. acanthinura*. The results revealed that histological sections of the uterus during hibernation season showed considerable atrophy and degeneration of the glandular region, compared to nearly normal structure and less atrophied glandular mucosa after the hibernation. Expression protein bands and their concentration markedly decreased during hibernation season and increased in activity season. During hibernation, a highest degree of genomic DNA fragmentation was exhibited, whereas no detected genomic DNA damage was shown during the activity season. On the other hand, caspase 3 and caspase 7 activity reached a high level in the uterus tissue during hibernation compared to activity season.

Frerichs*et al.*(1994)defined hibernation in lizards as an evolutionary adaptation to harsh environmental conditions, such as cold weather and starvation, in

temperate-zone squamates exhibit seasonal reproductive activity. Frerichs*et al.*(1995) also said: the decrease in body temperature is associated with profound reductions of blood flow, oxygen delivery and glucose utilization in body organs and in particular the brain and liver.

In temperate areas lizard reproduction is seasonal with mating and egg-laying often occurring from spring to summer (Fitch, 1970). Some species mate in autumn and females of some of these species can store sperms over winter (Fox, 1963; Conner and Crews, 1980 and K wait and Gist, 1987). However, tropical lizard species reproduce continuously in some areas (Inger and Greenberg, 1966; Fitch, 1982; James and Shine, 1985;Vitt, 1986 and Patterson, 1990) and seasonally in other ones where rainfall is seasonal (Fitch, 1982; James and Shine, 1985;Patchelland Shine, 1986;Clerkeand Alford, 1993 and Vrcibradic and Rocha, 1998).

The histological changes of the uterus structure during annual cycle reflected the reduced metabolic activity of *U. acanthinura*. In addition, these changes illustrated the drastic damage and degeneration of the glandular region especially the epithelial cells in the uterus and comparatively the reduction of the epithelial cell thickness during hibernation. The observed SDS-SPAGE of uterus protein expression reflected the interference of low temperature during hibernation in decreasing protein biosynthesis and metabolism. This is illustrated by protein expression and loss of particular protein bands compared to the activity season. The observed uterus cell damage was confirmed by assayed segregation of double helical DNA fragmentation during hibernation as well as increase of caspase 3 & 7 activities.

Similar observations were previously reported on the snake *Eryx colubrinus* and the lizard *Eumeces schneideri* by Abdel-Raheem *et al*.(1989 a) and on the ground squirrel *Spermophilus tridecemlineatus* by Squire and Andrews (2003). The maintenance of a minimal DNA content in liver and kidney during the hibernating cycle might be responsible for retardation of protein biosynthesis in such organs. These results run in agreement with the previous studies of Abdel-Raheem *et al*. (1989b).It might be also responsible for declining the mitotic index of cells. Studies performed by Kruman *et al*. (1986) indicated that hibernation induced adecline in DNA synthesis in intestinal crypt cells of ground squirrels. Similarly, Abdel-Raheem *et al*. (1989 a) reported that decreases in DNA content were observed in different tissues of two reptilian species, *E. colubrinus* and *E. schneideri* during the hibernating season.

According to Abdalhafid *et al*.(2012), there were a high vascularisation and hypertrophy of the glandular portion in the uterus of *L. stellio* which are markedly increased in size during spring and summer and a marked reduction of the luminal cavity was noticed in autumn. A considerable atrophy of the mucosa associated with collapsing and non-secretory function of its glands and a massive hyaline degeneration of the glandular mucosa was detected during winter season. For protein

expression, Abdalhafid*et al*.(2012) also found an increase of bands protein during spring and summer in brain, serum and gonads of *L. Stellio* and a decrease during winter and autumn.

Similar findings were reported by Abdalhafid (2013) who pointed out that, in the liver of *U. acanthinura*, genomic DNA showed apparent separation during hibernation and caspase 9 and caspase 12 activity reached a high level in the liver tissue during hibernation compared to the activity season.

Finally, we can conclude that, the annual cycle and climate changes might lead to biological changes of the body temperature during two seasons, then influence on the biological structure and function of uterus which in turn affect its biological activity through caspases secretion, protein synthesis, and DNA biosynthesis.

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الملخص العربي

دراسة تأثير الدورة السنوية في تركيب ووظيفة الرحم *Uromastyxacanthinura*(Bell, 1825) في الضب

يوسف خليفة عبدالقادر عبدالحفيظ
قسم علم الحيوان كلية العلوم جامعة عمر المختار –البيضاء ليبيا

وصفت هذه الدراسة تأثيرات الدورة السنوية في تركيب ووظيفة رحم الضب *Uromastyxacanthinura*(Bell,1825)في فصلي النشاط والسبات وأظهرت النتائج ضمورا كبيراوتحلل المنطقة الغدية في المقاطع النسيجية للرحم في فصل السبات، بينما أظهرت تركيبا طبيعيا وأقل ضمور في فصل النشاط و بعد موسم السبات.

وقد أوضحت النتائج أيضا ظهور نقص كبير في تعبير وتركيز البروتين في فصل السبات وزيادة في فصل النشاط، كذلك حدث ظهور انفصال وتجزؤ للمادة الوراثية وزيادة إنزيمات الموت الخلوي (7,3) أثناء فصل السبات مقارنة بفصل النشاط .