

Spermatogenesis timing durability in lizards: *Ophisops elegans* (Sauria: Lacertidae) in Iran

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As a model for study of spermatogenesis durability in lizards, 75 specimens of the snake-eyed lizard, *Ophisops elegans*, were collected from western Iranian Plateau. The testes of each specimen were removed, during four years from March to October. Based on histological and statistical analyses, three phases were observed in *Ophisops elegans* as follows: (I) active phase that occurs from March to May, (II) transitional phase that occurs from June to July and finally (III) resting phase that occurs from August to October. Based on this study, spermatogenesis durability in *O. elegans* is less than five months. Spermatogenesis durability of *O. elegans* occurred in post-hibernation period and stopped in pre-hibernation.

Key words: Spermatogenesis durability, Hibernation, Snake-eyed lizard, *Ophisops elegans*, Western Iran

INTRODUCTION

The reproduction cycle is divided into three types; continuous, associated and dissociated (Pough et al,1998). In the continuous reproductive type, reproduction in male and female occurs year-round (Somma and Brooks, 1976; Jenssen and Nunez, 1994; Hernandez-Gallegos et al, 2002), but in both associated and dissociated reproduction is seasonal (Guillette and Sullivan, 1985; Van Wyk, 1995). In the dissociated reproduction strategy, sperm storage occurs both in male and female, but in the associated strategy there is no sperm storage. In both associated and dissociated reproduction activity timing in male and female is asynchronous because spermatogenesis activity and mating timing are asynchronous with fertilization timing (e.g. Conner and Crews, 1980; Sever and Hamlett, 2002; Taylor, 2004; Sever et al, 2004; Torki, 2006), but in the continuous mode of reproduction, the male and female reproduction activity timing is synchronic because spermatogenesis activity and mating times are synchronic with fertilization (e.g., Sherbrooke, 1975; Jenssen and Nunez, 1994; Vences et al, 2004). Spermatogenesis timing in lizards is controlled by environmental, physiological and genetic factors (Shine, 1977; Kind and Duvall, 1990; Schuett, 1992; Amey and Whittier 2000) and in the tropical regions climatic conditions (continuous) during the four seasons are suitable for spermatogenesis, but in the temperate regions (dissociated and associated) climatic conditions during the year are different, hence spermatogenesis solely occurs in suitable conditions (e.g., Radder et al, 2001; Taylor, 2004; Torki, 2006). The spermatogenesis cycles of some lizards in the Iranian Plateau have already been, briefly, reported (e.g., Amini et al, 2005; Torki, 2005; Gharzi et al, 2006, Heidari, 2006; Torki, 2006). Our objective here is to study the spermatogenesis timing durability in the snake-eyed lizard *Ophisops elegans* (Lacertidae).

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TABLE 1. The monthly means of four studied parameters with snout-vent length (SVL) during four years (2002-2005). N= number of lizards, SEM= Standard error of the mean

Month		SVL	TV	GS	LS	S
Mar	N	9	9	9	9	9
	Mean	43.20	212.07	56.55	79.88	1.00
	SEM	0.90	1.54	1.27	1.61	0.00
Apr	N	10	10	10	10	10
	Mean	42.93	157.83	48.50	63.60	1.00
	SEM	0.744	3.27	1.50	4.59	0.00
May	N	10	10	10	10	10
	Mean	42.05	127.37	40.00	45.60	1.00
	SEM	0.758	3.28	2.54	2.47	0.00
Jun	N	9	9	9	9	9
	Mean	41.77	73.72	24.22	39.22	0.67
	SEM	0.60	4.00	0.98	1.30	0.16
Jul	N	10	10	10	10	10
	Mean	43.19	52.81	23.20	32.00	0.40
	SEM	0.73	3.33	1.21	0.97	0.16
Aug	N	8	8	8	8	8
	Mean	44.20	39.85	12.50	7.50	0.00
	SEM	0.83	2.07	2.06	0.90	0.00
Sep	N	10.00	10	10	10	10
	Mean	43.90	41.23	6.20	1.10	0.00
	SEM	0.66	2.46	0.24	0.48	0.00
Oct	N	9	9	9	9	9
	Mean	44.77	34.40	6.33	0.77	0.00
	SEM	0.65	1.29	0.33	0.40	0.00

MATERIAL AND METHODS

We collected 75 adult specimens (Mean of SVL: 43.22 mm) of the snake-eyed lizard *Ophisops elegans*, during a four-year time period (2002: 16 specimens; 2003: 19 specimens; 2004: 23 specimens; and 2005: 17 specimens), in western regions of the Iranian Plateau, western slopes of the Mid-Zagros Mountains, north of Lorestan Province. Exact geographical location of the studied area is 34°05N, 47°55E with the elevation up to 1900 m.. The climate type of the studied area is very similar to climatic conditions in highlands of the western Iranian Plateau, being cool-temperate (Bobek, 1968; Ganji, 1968; Nazemosadat, 2000; Evans et al, 2004; Sharifi et al, 2004; Heidari, 2006; Toriki, 2006). From March to October the specimens of *O. elegans* were collected. The left testis of each specimen was removed by dissection. In each specimen maximum length and width of the left testis were measured (with digital calipers to the nearest 0.01 mm) and Testis Volume (TV) using the ellipsoid formula; $v = 4/3\pi abc$, was estimated where v is volume, a and c are equal to half the testicular height, and b is half the testicular length (e.g., Vieira et al., 2001; Taylor, 2004; Heidari, 2006; Toriki, 2006). For histological analysis, we fixed testes in 3.7% formalin dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin. Histological sections were prepared at 5-7 μm , in hematoxylin followed by an eosin counterstain (H&E). Then photographs were taken with an Axiophoto Zeiss microscope. Sections of testes were examined to determine the stage of the testicular cycle. Under light microscopy, the sections were examined and the following factors in seminiferous tubules were measured: diameter of the tubules (LS) and thickness of the germinative layer (GS). In order to validate our data, we used various statistical tests including: descriptive statistic, Tukey HSD test, Pearson correlation and Canonical Discriminant Functions Analysis (DFA) (e.g., Vieira et al., 2001; Toriki, 2006).

RESULTS

Table (1) shows means of four parameters of testicular and SVL during four years studies in each month (from 2002 to 2005). During this period, there is no significant relation in SVL ($F=0.22$, $P=0.88$) ($p>0.05$), because we have chosen adult specimens during all the stages of this study. Based on the ANOVA no significant changes were observed in GS ($F=0.15$, $p=0.92$), LS ($F=0.25$, $p=0.85$), TV ($F=0.18$, $p=0.90$) and spermatozoa in lumen ($F=0.63$, $p=0.63$), from 2002 to 2005. Also, Tukey HSD shows a single subset for $\alpha=0.05$ during the four studied years from 2002 to 2005, in four parameters. The four testicular parameters (TV, GS, LS, and Spermatozoa) from March to October are decreasing (Fig 1), during all the four years studied. Based on the Discriminant Function Analysis (Fig 2) and Tukey HSD test ($\alpha=0.05$) the three phases are significant ($p<0.05$) in the four studied parameters of testicular cycle: the phase (I) from March to May, phase (II) from June to July and phase (III) from August to October. Histological studies confirm the presence of three phases as follows: in phase (I) spermatogenesis activity occurs in all the specimens and spermatozoa are found in LS (Fig. 3), phase (II) spermatogenesis activity occurs in most of the specimens, and finally in the phase (III) spermatogenesis activity in all the specimens stopped and there were no spermatozoa in LS.

DISCUSSION

Based on our findings, there were no significant changes in the four testicular parameters from March to October. Therefore, spermatogenesis durability in *Ophisops elegans* in this latitude has been stabilized. So, based on our results most of the spermatogenesis activity occurred during phase (I) and slightly in the phase (II), but in the phase (III) spermatogenesis has been stopped. Spermatogenesis in *Ophisops elegans* is similar to the other lizards of the Iranian Plateau in that it is seasonal (e.g., Torki, 2005; Heidari, 2006; Gharzi et al, 2006; Torki, 2006). In the Zagros Mountains (western Iran), lizards are hibernating during the winter (Torki, 2005; Heidari, 2006; Torki, 2006). During the hibernation period, testicular tissue is renewed (e.g., Torki, 2005; Heidari, 2006; Torki, 2006) and the spermatogenesis activity in *O. elegans* occurs during the post-hibernation period the same as in the agamids *Trapelus lessonae*, *Laudakia nupta* and *L. caucasia* (Heidari, 2006; Torki, 2006). As well, the pre-hibernation spermatogenesis activity is stopped in *O. elegans* the same as in *T. lessonae* (Torki, 2006). The spermatogenesis stability of *T. lessonae* is less than five months (Torki, 2006), in *L. nupta* it is two months and in *L. caucasia* almost one month (Gharzi and Heidari, 2006, Heidari, 2006). Spermatogenesis in *L. nupta* and *L. caucasia* shows three phases: pre-active, active and resting phase (Heidari, 2006). The Pre-active phase occurred in the post-hibernation period and resting phase occurred in the pre-hibernation period and between the two phases spermatogenesis activity occurred, but in *T. lessonae* and *Ophisops elegans* the pre-active phase doesn't occur in post-hibernation period (Torki, 2006).

Therefore, with regards to climatic conditions (e.g. Bobek, 1968; Ganji, 1968; Ghobadian, 1990; Nasrallah, 1995; Nazemosadat, 2000; Evans et al, 2004; Sharifi et al, 2004; Torki, 2006), our results and other studies of spermatogenesis of lizards in the Iranian Plateau (e.g., Amini et al., 2005; Torki, 2005; Heidari, 2006; Heidari and Gharzi, 2006; Gharzi et al, 2006; Gharzi and Torki, 2005a-b; Torki and Rastegar-Pouyani, 2006a-b; Torki, 2006), we consider four important rules for spermatogenesis timing and durability in lizards of the Iranian plateau as follows: 1- Seasonal spermatogenesis of lizards is pronounced. 2- Spermatogenesis activity occurs during post-hibernation period and stops in pre-hibernation. 3- The hibernation period may be necessary for renewal of spermatogenesis activity. 4- The pronounced spermatogenesis durability is less than five months.

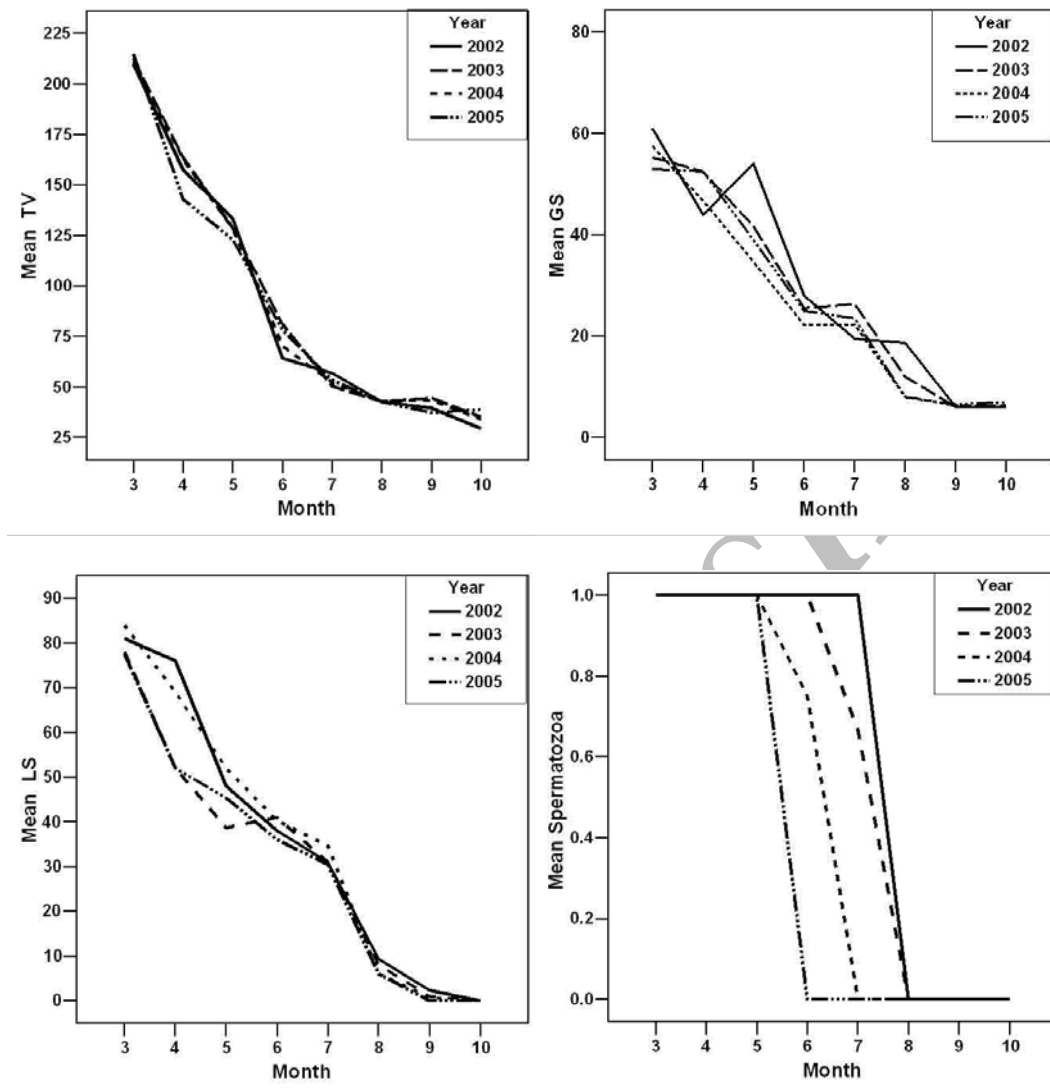


Fig 1.- The means of four parameters pertaining to spermatogenesis during four years from March to October. (a) TV (testis volume), (b) GS (thickness of the germinative layer), (c) LS (diameter of the tubules), (d) Spermatozoa.

SPERMATOGENESIS DURABILITY IN LIZARDS

The spermatogenesis durability in lizards is strongly correlated to climatic conditions, as well as geographic location (e.g., Heideman, 1995; Vences et al, 2004; Gharzi et al, 2006; Torki, 2006), and also a variety of spermatogenesis durability in all climate types are found. In many lizards inhabiting tropic regions especially ITCZ (Inter Tropic Center Zone), spermatogenesis activity occurs year-round and production of spermatozoa occurs during all seasons (Sherbrooke 1975; Vial and Stewart, 1985; Hernandez-Gallegos et al, 2002). Lizards in temperate regions are different from the tropic regions and do not show spermatogenesis activity year-round and the production of spermatozoa occurred in some months of the year (e.g., Radder et al, 2001; Taylor 2004; Torki, 2005; Heidari, 2006; Torki 2006). On the other hand, in many wet-dry tropic and other tropic regions or neotropic regions, spermatogenesis activity occurs almost year-round and production of spermatozoa has been shown to occur almost during all the four seasons (e.g., Colli, 1991; Van Sluys, 1993; Jensen and Nunez, 1994; Vieira et al, 2001).

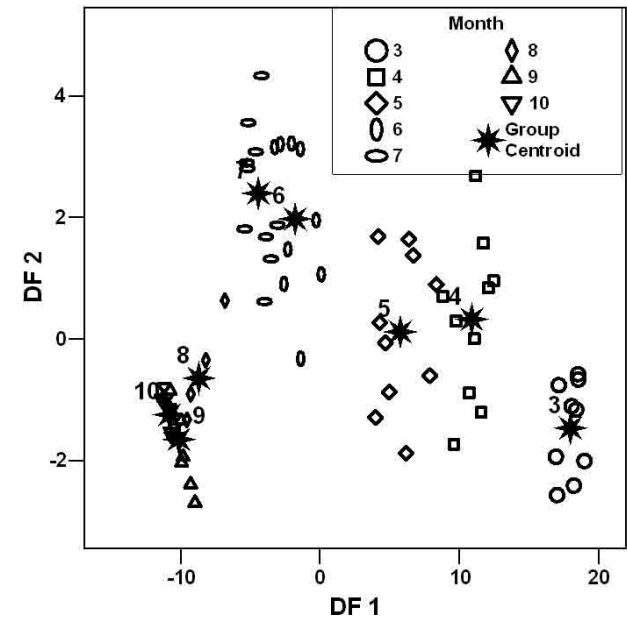


FIG. 2.- Three phases of testicular parameters, based on Discriminant Function Analysis (DFA), during the year from March to October.

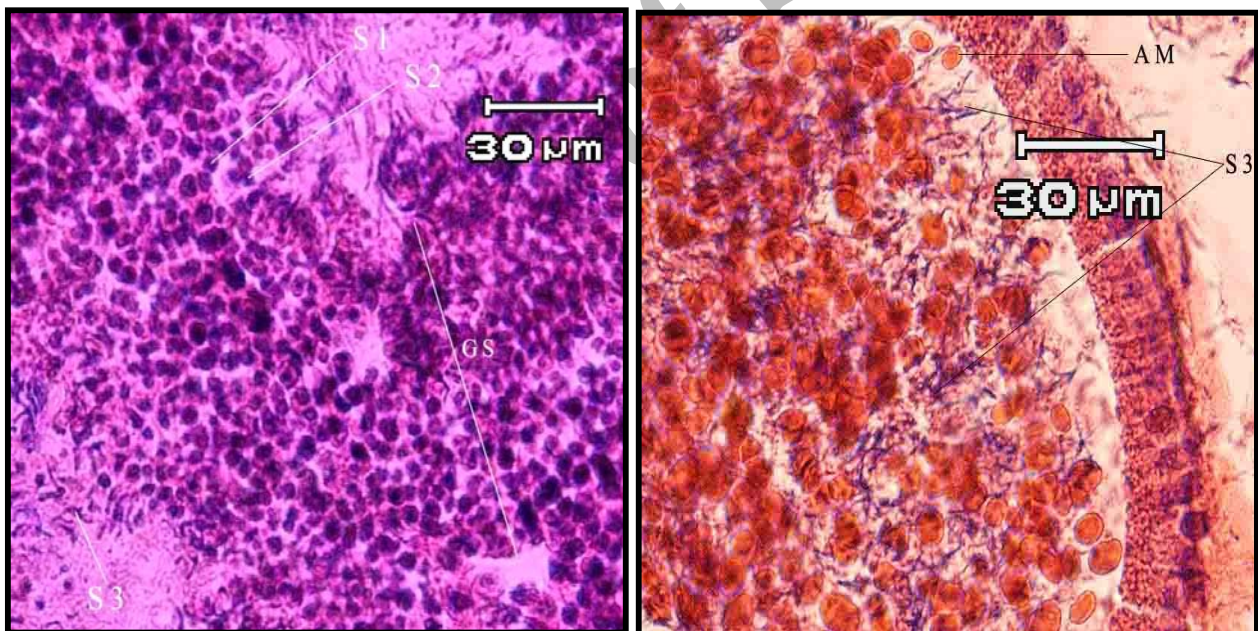


FIG.3.- The seminiferous tubules during active phase, (a) spermatozoa are found in LS, and (b) in LE. S1: primary spermatocyte; S2: secondary spermatocyte; S3: spermatozoa; LS: diameter of the tubule; GS: thickness of Germinative layer; LE: Lumen of Epididymis.

In summary, based on the spermatogenesis durability studied in lizards, three types of spermatogenesis durability are presented as follows: (I) Continuous spermatogenesis that occurs year-round and the spermatozoa are found in the lumen of seminiferous tubules during all seasons. (II) Weak seasonal spermatogenesis activity occurs almost year-round except for one season or spermatogenesis activity stops during one or two months during unsuitable climatic conditions. (III) Seasonal spermatogenesis activity limited to one season and doesn't occur year-round.

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