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Spermatogenesis without spermiogenesis in *Laudakia caucasia* (Reptilia: Agamidae): the role of hibernation

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During hibernation period from Mid October to Late March, we removed testis of *Laudakia caucasia*. Based on histological and statistical analyses we obtained two phases in spermatogenesis in this lizard as follows: (a) from October to December, in which the spermatogenesis is inactive and (b) from January to March, in which the spermatogenesis is active. Spermatogenesis during late hibernation occurred without spermiogenesis. An evidence for this is that the first and secondary spermatocytes were produced but lumena of the seminiferous tubules were without spermatozoa.

Key words: Spermatogenesis, Spermiogenesis, Hibernation, *Laudakia caucasia*, Iran

INTRODUCTION

The production of spermatocytes and spermatozoa from spermatogonia is called spermatogenesis, and production of spermatids and spermatozoa from secondary spermatocytes as well as formation of the tail of sperm is called spermiogenesis (Lebonde and Clermont, 1952; Vieira et al, 2001; Gribbins et al, 2005). Spermatogenesis and spermiogenesis in lizards inhabiting tropical regions (Vial and Stewart, 1985; Vieira et al, 2001; Hernandez-Gallegos et al, 2002) occur year-round. Nevertheless, the studied region is limited to the ITCZ (Inter Tropic Convergence Zone) region (Torki, 2006, in press). In contrast, outside of the ITCZ region, spermatogenesis activity and spermiogenesis occur during well-defined periods (Wilhoft and Reiter, 1965; Fitch, 1970; Marion and Sexton, 1971; Torki, 2006).

In the western Iranian Plateau, the process of spermatogenesis is in intervals, and in many lizards this process occurs during the post-hibernation period (Torki, 2005, 2006, in press). In contrast, during the pre-hibernation period, the spermatogenesis process is inactive (e.g., Torki, 2006). Spermatogenesis in lizards of western Iran during biological activity is divided into three phases, active, inactive and silent (Torki, 2006). The first report of spermatogenesis activation in Iranian lizards was described by Torki (in press) in the agamid *Trapelus lessonae*. Based on Torki's description (in press), during spermatogenesis activation, spermiogenesis is inactive. Therefore, during the spermatogenesis activation, spermatozoa are not found in the lumena of the seminiferous tubules. This paper is the second report of spermatogenesis activation during the hibernation period in the lizards of the Iranian Plateau.

MATERIAL AND METHODS

We collected 23 adult male specimens of *Laudakia caucasia* (SVL: 124.42±5.96mm), in the highlands of the Zagros Mountains, western Iran. The geographic position approximates 34˚05N, 47˚55E and the elevation is between 1900 to 2200 m. In the studied area, the hibernation period of *L. caucasia*

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occurred from October to March (six months), this is similar to *Laudakia nupta* in this region (Torki, in press). All the specimens captured in their natural habitat (in pre-hibernation period), then were placed in a special cage. The cage was of aluminum sheeting with the dimensions 1x3x3 meter, the same as the other studies carried out in nature during a cool season (e.g., Grenot et al, 2000; Torki, in press). All the specimens started the hibernation period; therefore, during each month, two specimens were treated and their testes removed and the H&E technique was used following Torki (2006; in press). The testes were photographed with an Axiophoto Zeiss microscope. For data collecting, the mean of 45 transversally oriented tubules at the same section, next to the core of the testes were used. For this study five parameters of testicular tissue were used as follows: testis volume (TV), diameter of germinative layer of seminiferous tubules (GS), diameter of lumen of seminiferous tubules (LS), primary spermatocytes (PS), secondary spermatocytes (SS). Tukey HSD test (α=0.05), and Discriminant Functions analysis (DF) were employed for comparison among parameters during hibernation. During dormancy, two specimens died and 21 specimens were treated for this study.

RESULTS

Descriptive statistics of the four studied characters are shown in Table 1. The GS from early hibernation to December has slightly increased, but based on Tukey HSD test (α=0.05) it is not significant. In contrast, the GS from January to March has greatly increased, and doesn't show a significant relationship among three months of phase (b). Tukey HSD test (α=0.05) shows significant relationship between the two phases (a) and (b). Theses relations are true for PS and SS as well as TV. Increasing in four characters is unexpected and started from January (Figs. 1a, b). In contrast, LS does not show a significant relationship between the two phases (Fig. 1b). Based on Discriminant Function Analysis (Fig 2) the process of spermatogenesis during hibernation is divided into two phases as follows: (a) from October to December and (b) from January to March. Based on histological study, lumena of the seminiferous tubules in phase (a) were without spermatocytes and spermatozoa (Fig 3a). In contrast, lumena of the seminiferous tubules in phase (b) bore primary and secondary spermatocytes (Fig 3b).

DISCUSSION

Based on Torki's definition (in press), the above-mentioned phase (a)was called silent phase and phase (b) activation phase. Histological changes in two phases are similar to *Trapelus lessonae* (Torki, 2006). Nevertheless, activation phase in *L. caucasia* is one month longer than *T. lessonae*. This occurred due to dormancy, because the hibernation period in *L. caucasia* is longer than in *T. lessonae*. During activation phase spermatogenesis is active, due to production of spermatocytes. Also GS has been increased, but the spermatids and spermatozoa are not found in seminiferous tubules. The production of sperm in *T. lessonae* occurred during post hibernation (Torki, in press), this may be true for *L. caucasia* as well. In contrast, the production of sperm in *Laudakia nupta* occurred after post-hibernation or during late spring and early summer (Torki, in press). Spermatogenesis of *L. nupta* described by Torki (in press) is in the central Zagros, an area of sympatry for *L. nupta* with *L. caucasia*. We think the process of sperm production (or spermiogenesis) of *L. caucasia* is similar to that of *L. nupta* and is not similar to *T. lessonae*. We present here the effects of three factors of this hypothesis as follows: (1) the hibernation period of *L. nupta* and *L. caucasia* is similar, (2) the body size of the two species of *Laudakia* is nearest in comparison with T. *lessonae*, (3) phylogenetically, *L. caucasia* is closer to *L. nupta* than to *T. lessonae*.

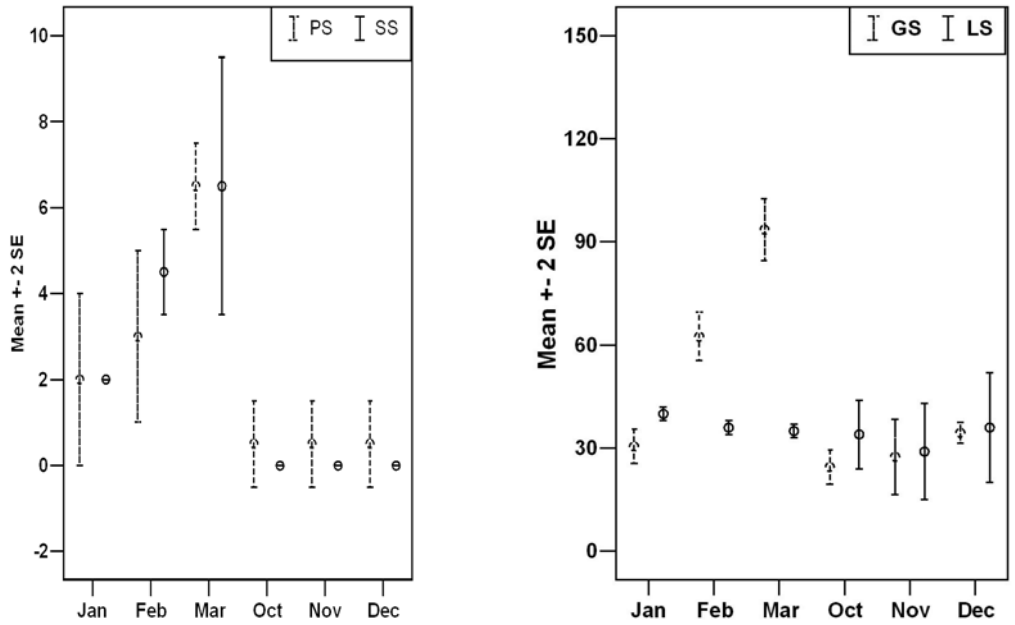


FIG.1.- Ranges of changes (Mean±SE) of (a) PS and SS, and (b) GS and LS, during hibernation period from October to March.

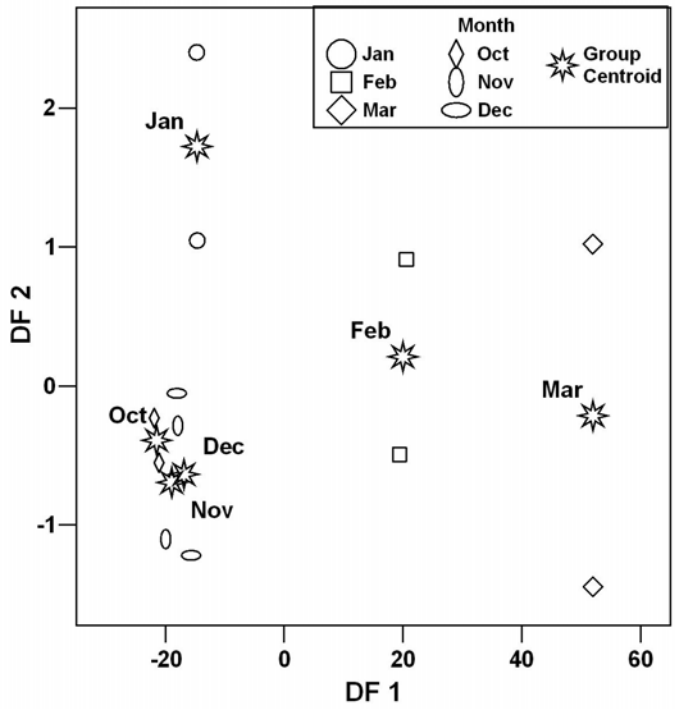
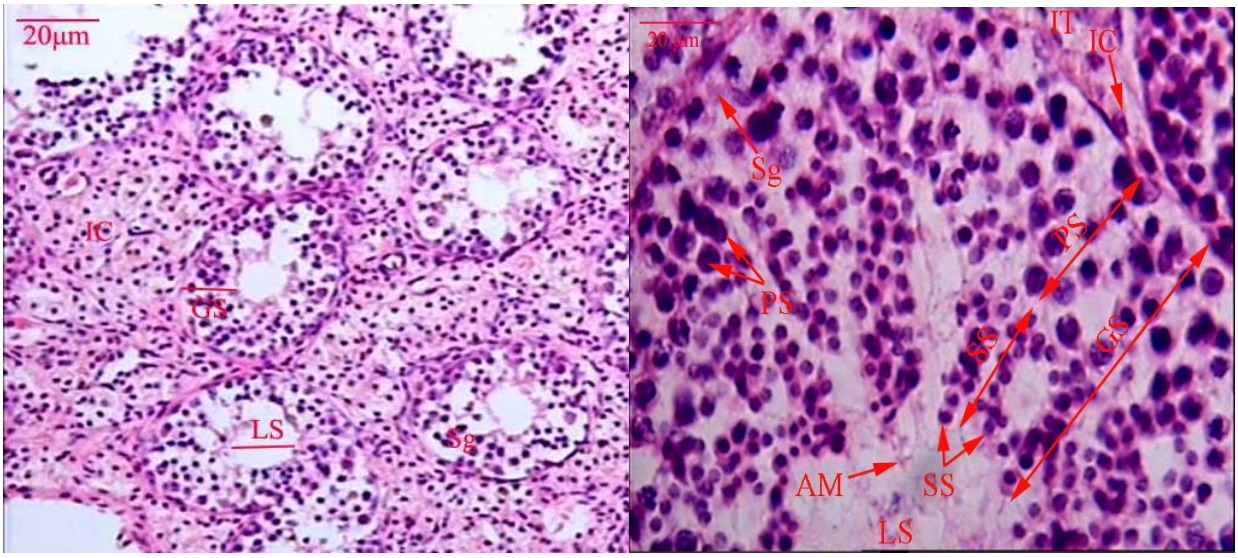


FIG. 2.- Discriminant Function Analysis of five characters from October to March.



(a) (b)

FIG.3. -Histological structure of seminiferous tubules during a: phase (a) and b: phase (b). Abbreviations: GS: Germinative layer of seminiferous; LS: Lumen of seminiferous; Sg: Spermatogonia; SP: Spermatocyte; PS: Primary spermatocyte; SS: Secondary spermatocyte; St: Spermatids; Sz: Spermatozoa; IC: Interstitial cells; IT: Interstitial tissue; AM: Amorphous materials.

In temperate regions spermatogenesis of lizards is divided into two types, degeneration and regeneration (Fitch 1970, Lofts 1987, Castilla & Bauwens 1990); this is true in the Zagros Mountains lizards as well (Torki, 2006, in press). During regeneration period the testicular tissue has been renewed (e.g., Torki, in press) and activity of spermatogenesis and spermiogenesis occurred during the early degeneration period (Torki, 2006). In contrast, during the degeneration period the seminiferous tubules have been reduced, and this is contrary to the other tissues of the testis (Torki, in press). Therefore, the regeneration period of spermatogenesis in *T. lessonae* (Torki, in press) and *L. caucasia* occurred during the mid-hibernation period and lasted until the activation phase of spermatogenesis. For renovation of testicular tissue, adipose tissue has an important role and is the only resourse to renew testicular tissue (Torki, 2005, 2006, in press). Therefore, the activation phase is completed in relation to the adipose tissue or brown fat (Torki, in press). Because, in the beginning of activation, the habitat of *L. caucasia* is snowy, temperature is very low (see Torki, in press), and almost no food material is available. At the beginning of the activation period, the steroid hormones and gene expression have important roles (Crews, 1975; Allen et al, 1997; Hud et al, 1994 Marques et al, 2004). These two factors are related to climatic conditions (Torki, in press). Perhaps, the activation phase in *L. caucasia* and *T. lessonae* is due to the effects of natural selection.

And finally, we may conclude that the spermatogenesis without spermiogenesis is an important adaptation during dormancy and is crucial in the survival and demography of *L. caucasia* and other hibernating lizards occurring in cool-temperate areas such as the Zagros Mountains, western Iran.

Table 1. Descriptive statistics of five characters during hibernation period (Abbreviations are shown in material and methods). N= Number, SEM= Standard Error of Mean.

Month		GS	LS	TV	PS	SS
Jan	N	2	2	2	2	2
	Mean	30.50	40.00	67.00	2.00	2.00
	SEM	2.500	1.000	8.000	1.000	.000
Feb	N	2	2	2	2	2
	Mean	62.50	36.00	139.00	3.00	4.50
	SEM	3.500	1.000	41.000	1.000	.500
Mar	N	2	2	2	2	2
	Mean	93.50	35.00	280.00	6.50	6.50
	SEM	4.500	1.000	40.000	.500	1.500
Oct	N	2	2	2	2	2
	Mean	24.50	34.00	66.71	.50	.00
	SEM	2.500	5.000	16.710	.500	.000
Nov	N	2	2	2	2	2
	Mean	27.50	29.00	62.50	.50	.00
	SEM	5.500	7.000	2.500	.500	.000
Dec	N	2	2	2	2	2
	Mean	34.50	36.00	47.50	.50	.00
	SEM	1.500	8.000	4.500	.500	.000

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